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Note
Veterinary knowledge is constantly changing. As new information becomes available, changes in treatment, procedures, equipment and the use of drugs become necessary. The editors/authors/ contributors and the publishers have, as far as it is possible, taken care to ensure that the information given in this text is accurate and up to date. However, readers are strongly advised to confirm that the information, especially with regard to drug usage, complies with the latest legislation and standards of practice.

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Foreword

It is with a sense of pride and fulfillment that I respond to my fellow authors' to have the eighth edition of "Veterinary Reproduction and Obstetrics" entitled to me.

It was in 1962 that the late Professor J. G. Wright invited me to take over his "Benesh and Wright Veterinary Obstetrics", 2nd edition. In agreeing to his request, it was my intention to revise and greatly enlarge his text for undergraduate students of veterinary science in English-speaking countries and for it to serve as a reference source for practising veterinary surgeons, as well as for scientists of allied disciplines. These objectives have been followed since my 1964 third, to the present eighth, edition.

The original format of the book and the divisional layout of its contents into appropriate sections and chapters have undergone progressive development but the only major changes in subject titles have been the addition of chapters on "Exotic Species", "Small Mammals" and "Embryo Transfer", together with a vocational appendix of the materials used in the diagnosis and treatment of reproductive disorders. The advent of ultrasonic imaging has become established as a most useful addition to the veterinary surgeons' armoury; it is appropriately incorporated in the relevant chapters of the last three editions.

The favourable literary reviews of our book and its wide use overseas, together with translation into French, Italian, Spanish, Portuguese and Japanese, have provided welcome encouragement to me and fellow authors to continue to update the book.

The need to make comprehensive reviews for student use from the vast volume of new literature, particularly in the fields of reproductive endocrinology, gonadal physiology and genetics, is now a heavy task. In the context of stimulus to further effort in authorship is the inspiration from the work of prominent contributors to one's particular discipline. The period of my 60 year career in clinical veterinary science has been notable for the contemporary lives of outstanding workers in my field, from whose publications I have benefited substantially. It is a pleasure to record my deep gratitude to the following gentlemen:- Professor W. Williams (Cornell), F. Benesch (Vienna), N. Lagerlof (Stockholm), J. G. Wright (London), E. Amoroso (London), M. Vanderplassche (Gent) and S. J. Roberts (Cornell).

I will close by expressing my warmest thanks to all who have contributed to previous and present editions of our book. My special thanks go to Professor David Noakes, who has played a leading role in his work for the last three editions.

It is extremely sad to refer to the death of Professor Harold Pearson since the publication of the seventh edition. He is still greatly missed by all who knew him as a brilliant clinician and teacher.

Geoffrey H Arthur
2001

Preface

It is with great pleasure that we dedicate the 8th edition of Veterinary Reproduction and Obstetrics to Professor Geoffrey Arthur, initially the sole, and subsequently senior author who successfully husbanded five previous editions of the book from 'conception to parturition'. It is in recognition of the outstanding contributions that he has made to the subject over the last 50 years. He has influenced, either directly or indirectly, the professional lives of all of the contributors. Although this will have been primarily in his role as a dedicated and stimulating clinical teacher, it is also because he was one of the first clinical researchers in veterinary reproduction; this will be recognised by readers of the book by the frequent reference to his published work.

We are sad that the late Professor Harold Pearson was unable to contribute to this 8th Edition. He was one of the outstanding clinical teachers of the last 40 years, his writings were based on his vast experience as a clinician working at the Bristol Veterinary School as shown by his substantial contributions to the literature including previous editions of this book.

It is intended that this new edition should still cater primarily for the undergraduate, although others will find it to be a useful reference source. We have added just one additional chapter, and that is by Mr David Whittaker who covers normal reproduction and reproductive disorders in rabbits and rodents, which as well as still having a major role as laboratory animals in research are also being kept in increasing numbers as pets. We have also deleted the chapter devoted specifically to Retained Fetal Membranes and included this material in the chapters devoted specifically to infertility in the specific species. We welcome the contributions from four new authors: Dr Nazir Ahmad , Professor Marzook Al-Eknah , Mr Martin Sheldon and Dr Keith Smith who revised the chapters on Buffalo, Camels, the caesarian operation in large animals and infertility in the ewe and goat, respectively. We also thank those contributors to the 7th Edition, who have painstakingly revised their chapters for the 8th edition: Mr Will Christie, embryo transfer; Dr Christianne Glossop, infertility in the sow; Dr Susan Long, abnormal development of the conceptus and its consequences, and Dr Jonathon Pycock, infertility in the mare.

In addition, we would also like to express our thanks to Wendy Lee who did an excellent job as Copy Editor, and Sheila Black and Scott Millar of Harcourt Publishers for their hard work and courteous professionalism in producing the 8th Edition of the book.

Inevitably, as we complete the revision of the 8th Edition, thought must be given to a 9th Edition in the not-to-distant-future. With the vast expansion in the knowledge base in the subject, and bearing in mind that the principal target for the book is the veterinary undergraduate, we will need to decide whether we can continue to enlarge both the length and the scope of the book or whether we must review its format.

David Noakes,
Tim Parkinson
Gary England
2001

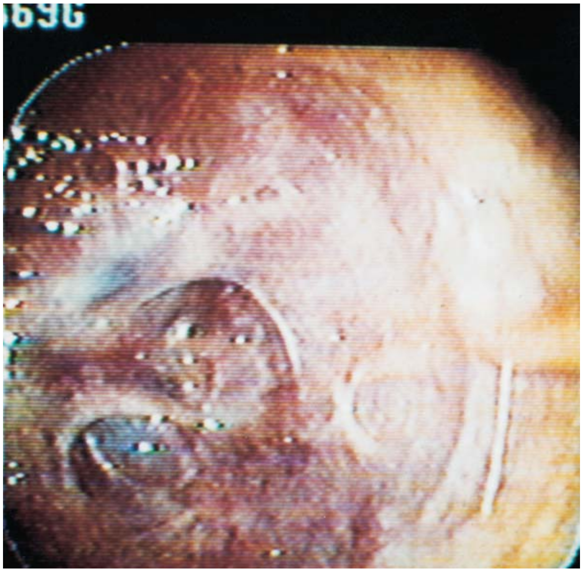


Plate 1 Adhesion almost completely obstructing the left uterine horn.

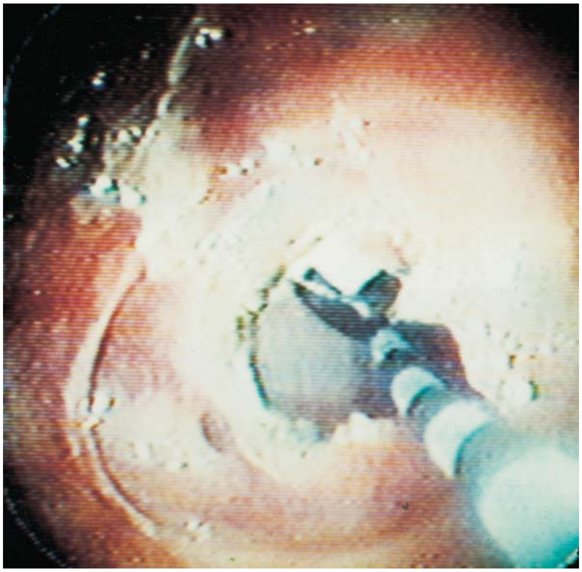
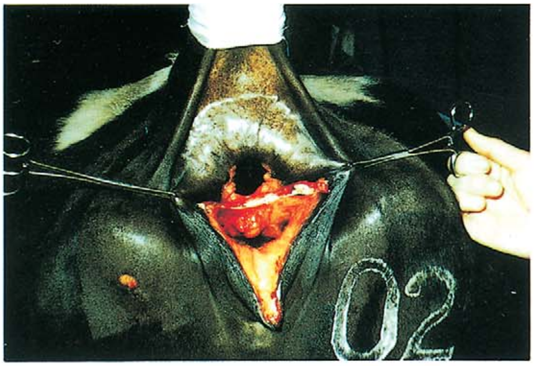


Plate 2 Removal of the obstruction by endoscopic cauterisation.



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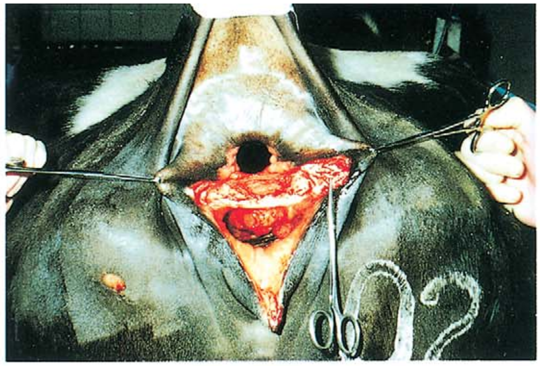

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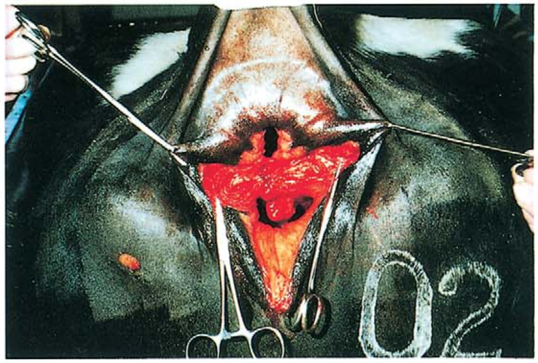

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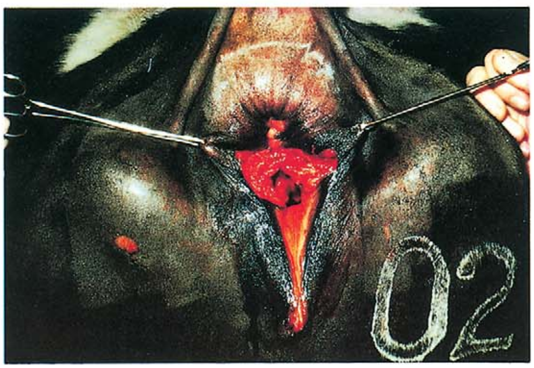

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Plate 3 Repair of a third degree perineal laceration in a Friesian cow. (a) The perineal laceration exposed with cow under caudal epidural anaesthesia. (b) Commencement of dissection of vaginal mucosa. (c) Completion of dissection of vaginal mucosa; note exposed tissue ready for suturing. (d) Commencement of closure. (e) Closure almost completed. (f) Restoration of a complete shelf of tissue between rectum and vagina, the dorsal commissure of the vulva is subsequently repaired (see Figure 18.5).

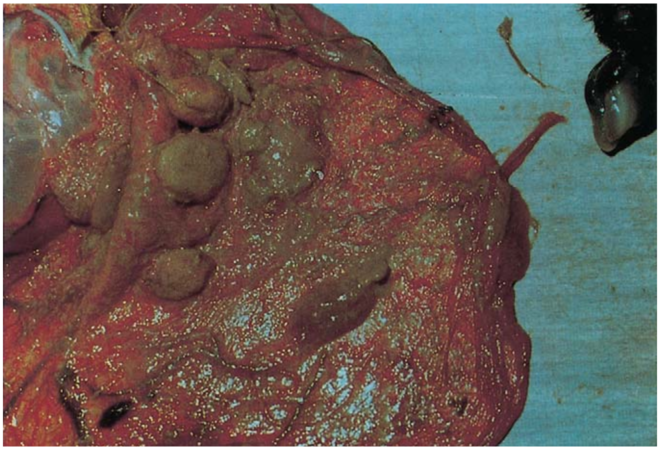


Plate 4 Placenta of lamb that had aborted due to infection with *C. psittaci (ovis).* Note cotyledons covered with a light-brown deposit and similar material on the surface of the intercotyledonary chorionic surface. (Courtesy of A. J. Wilsmore).

Plate 5 Placenta of lamb that had aborted due to infection with *T. gondii.* (Courtesy of A. J. Wilsmore).

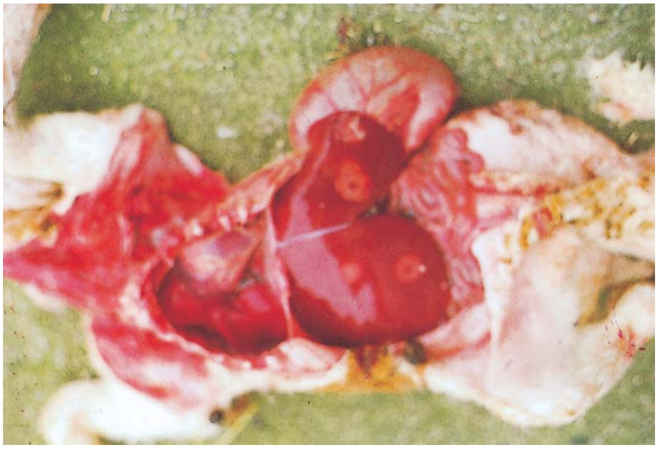
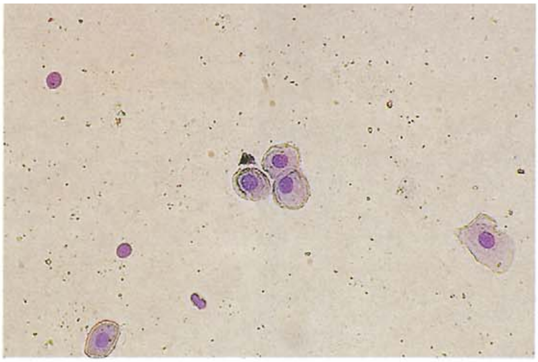


Plate 6 Aborted fetus showing characteristic lesion on the liver of *C. fetus* infection.



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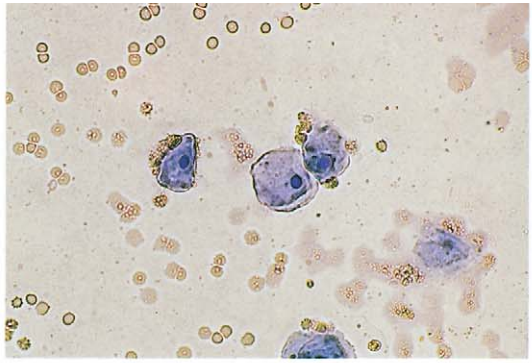

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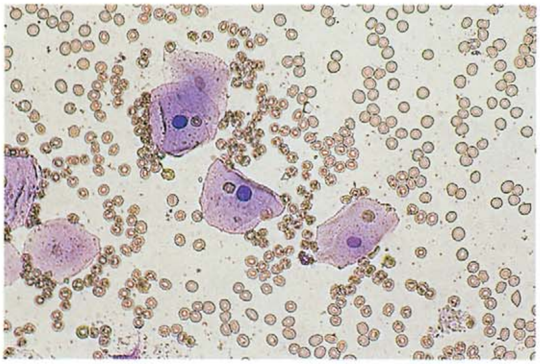

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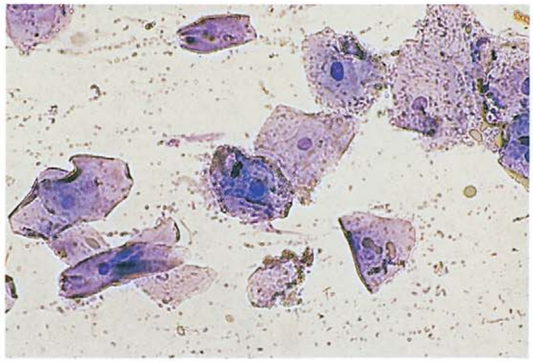

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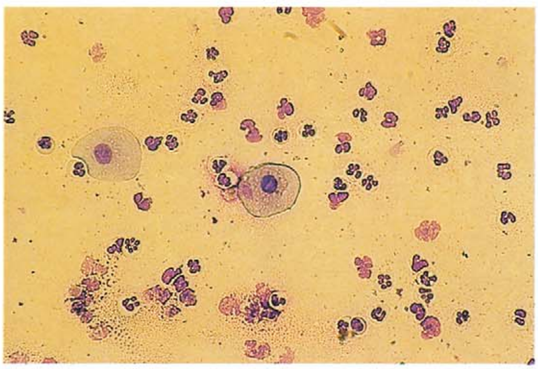

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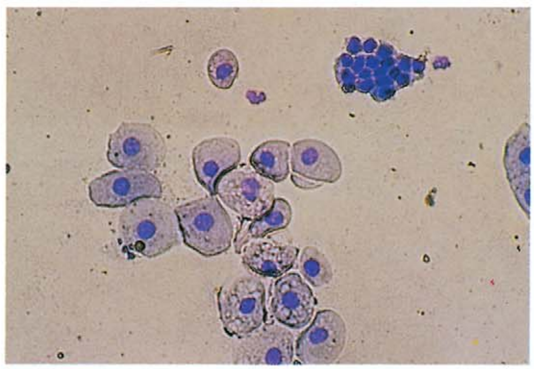


(f)

Plate 7 Photomicrographs of exfoliative vagina cells during various stages of the reproductive cycle. The smears have been stained with a modified Wright–Giemsa stain. (a) Anoestrus: parabasal epithelial cells and small intermediate epithelial cells. (b) Pro-oestrus: small intermediate epithelial cells, large intermediate epithelial cells and erythrocytes. Polymorphonuclear leucocytes are also found in low numbers during this stage of the cycle but are not demonstrated here. (c) Early oestrus: large intermediate epithelial cells, anuclear epithelial cells and erythrocytes. Polymorphonuclear leucocytes are generally absent during this stage of the cycle. (d) Oestrus: anuclear epithelial cells, large intermediate epithelial cells and erythrocytes. The percentage of anuclear cells is high. (e) Metoestrus (higher magnification than a–d): small intermediate epithelial cells and large numbers of polymorphonuclear leucocytes. During early metoestrus large intermediate epithelial cells may be present, and later numbers of parabasal epithelial cells increase. There is often a large amount of background debris. (f) Late metoestrus (higher magnification than a–d): parabasal epithelial cells and small vacuolated intermediate epithelial cells are typical of this stage of the cycle but may also be found during anoestrus and more rarely during pro-oestrus.

1 Endogenous and exogenous control of ovarian cyclicity

In nature, it is the general rule that animals breed once annually and parturition occurs in the spring, the time most favourable to the progeny in that they grow up during the period of increasing light and warmth, and also at the time when food for the mother is most abundant to ensure adequate lactation. Under the conditions of feeding and housing provided by domestication the breeding season tends to be lengthened, and some of our species, particularly the cattle, may breed at any time during the year; all domesticated animals, however, show a constant tendency to revert to the natural breeding season.

For an animal to breed, it must be mated and hence must attract the male and be sexually receptive (in heat or in oestrus). All domestic species show recurring periods of sexual receptivity, or oestrous cycles, which are associated with the ripening in the ovaries of one or more Graafian follicles and culminate in the shedding of one or more ova. If a fertile mating occurs then pregnancy may ensue.

PUBERTY AND THE ONSET OF CYCLIC ACTIVITY

The young female animal shows no evidence of recurring or cyclic periods of sexual receptivity. The onset of such changes when the female becomes sexually mature and able to reproduce is referred to as puberty. Amongst females of the domestic species, puberty precedes the development of physical maturity and, although they become capable of reproducing, their efficiency, particularly with respect to their fecundity, has not reached its maximum.

The initiation of puberty is largely a function of the animal's age and maturity since the female is born with a genetic potential for cyclic reproductive activity. Provided the environmental influences are favourable at this time, then once the 'biological clock' is started it will continue for as long as the environment remains favourable. In none of our domestic species is there a physiological change comparable with the menopause of women.

Amongst non-seasonal polycyclic animals, such as the cow and sow, the recurring cyclic activity is interrupted by pregnancy, lactation and pathological conditions. In those species which are seasonally polycyclic, the mare, ewe, doe (or nanny) goat and cat, or monocyclic like the bitch, there are periods of sexual quiescence or anoestrus.

When the female reaches puberty the genital organs increase in size. During the prepubertal period the growth of the genital organs is very similar to that of other organ systems, but at puberty their growth rate is accelerated, a point well illustrated in the gilt, where the mean length of the uterine horns is increased by 58%, the mean weight of the uterus by 72% and the mean weight of the ovaries by 32% between 169 and 186 days of age (Lasley, 1968). Females of domestic species reach the age of puberty at the following times:

- mare: 1–2 years
- cow: 7–18 months
- ewe: 6–15 months
- doe or nanny goat: 4–8 months
- sow: 6–8 months
- bitch: 6–20 months
- queen cat: 7–12 months

The changes that occur at puberty depend directly upon the activity of the ovaries, which have two functions: the production of the female gametes and the synthesis of hormones. Let us consider the changes that occur in the ovary of the young heifer calf. At birth, each ovary may contain up to 150 000 primary or primordial follicles; each consists of an oocyte surrounded by a single layer of epithelial cells, but there are no thecal cells. Soon after birth, the ovaries start to develop and produce growing follicles which consist of an oocyte with two or more layers of granulosa cells and a basement membrane. The stimulus for the development of these follicles is intra-ovarian, and until the heifer reaches the age of puberty they will develop only to the stage where they have a theca interna and then start to undergo atresia. Further development of these follicles to produce mature Graafian or antral follicles, of which there are about 200 growing follicles at puberty in the heifer, is dependent upon the stimulus of gonadotrophic hormones. Despite the absence of oestrous cycles, there is follicular growth as has been shown using transrectal ultrasonography in calves from 2 weeks of age. It was seen that there were follicular waves in response to follicle-stimulating hormone (FSH) secretion that were similar to those of the adult, and that individual follicular development was characterised by growing, static and regressing phases (Adams, 1994).

The sheep has been used extensively for studying many of the mechanisms involved in the initiation of puberty; however, it must be stressed that seasonality will exert an overriding influence in this species (see below). The onset of puberty is signalled by either the occurrence of the first oestrus or the first ovulation; in the ewe lamb these do not occur simultaneously because the first ovulation is not preceded by behavioural oestrus. A similar response is seen in sexually mature ewes at the onset of the normal breeding season.

The hormone that is primarily responsible for the onset of ovarian activity, and hence puberty, is luteinising hormone (LH). In adult ewes during the normal breeding season, basal LH concentrations increase together with the LH pulse frequency to one per hour during the period of maximum follicular growth. This results in the development of follicles to the preovulatory stage, and their secretion of oestradiol, which activates the LH surge causing ovulation and corpus luteum formation. In the prepubertal ewe lamb, LH pulses occur at similar amplitudes but much lower frequencies (one every 2–3 hours). As a consequence, follicular growth is insufficient to activate the LH surge necessary for final follicular maturation and ovulation.

Experimental evidence in prepubertal ewe lambs has shown that ovarian follicles are capable of responding to exogenous gonadotrophin stimulation, and the pituitary is capable of secreting LH at a frequency to stimulate ovulation. The failure of the prepubertal ewe lamb to undergo ovulation and exhibit oestrus is due to the high threshold for the positive-feedback effect of oestradiol, and thus there is no LH surge. At puberty, the threshold is lowered, thus allowing the pituitary to respond. This is sometimes referred to as the 'gonadostat' theory.

Other factors are also involved. The frequency of LH secretion is dependent upon gonadotrophin-releasing hormone (GnRH) from the hypothalamus, which is controlled by an area in the hypothalamus referred to as the neural GnRH pulse generator. Age-related changes in brain morphology and neuronal cytoarchitecture may also be important, since extrapolation from studies performed in rats, for example, has shown an increase in the number of GnRH cells with spine-like processes on the soma and dendrites. In addition, the inhibitory effect of opioid peptides on LH secretion is reduced with age, which may provide a neurochemical explanation for the changes in pituitary sensitivity to oestradiol feedback that occur at puberty (Bhanot and Wilkinson, 1983; Wray and Hoffman-Small, 1986).

The reason for the 'silent' first oestrus of the pubertal animal is believed to be because the central nervous system requires to be primed with progesterone before it will respond and the animal will show behavioural signs of heat. The first ovulatory cycle has been shown to be short in pubertal heifers (7.7 +/– 0.2 days), and the first corpus luteum (CL) not only has a shorter than normal life span but is also smaller in size. One explanation for this is that the dominant follicle, from which the first ovulation arises, had already entered the static phase of growth. The subsequent interovulatory interval was normal (Adams 1999).

External factors influencing the time of onset of puberty

The time of onset of puberty is determined by the individual's genotype, with smaller breeds of animal tending to be slightly more precocious. However, this inherent timing is influenced by a number of external factors.

Nutrition. There is good evidence that in most domestic species, the age of puberty is closely related to body weight; therefore, it is not surprising that nutrition is an important factor. Animals that are well fed with good growth rates reach puberty before those that are poorly fed with slow growth rates. However, unless the animal is severely malnourished, the onset of cyclical activity will eventually occur.

Season of the year. In those species which are seasonal breeders, such as the ewe, mare and queen cat, the age at which puberty occurs will be influenced by the effect of season of the year. For instance, a filly born early in the year, i.e. January or February, may have her first oestrus in the May or June of the following year, i.e. when she is 16 or 17 months old. A filly foal born late in the year, July or August, may not have her first oestrus until she is 21 or 22 months old. The same is true of ewes which, depending upon the time of year at which they are born, may reach puberty as early as 6 months or as late as 18 months old.

Proximity of the male. Studies in sheep and pigs have shown that exposure to the male of the species will advance the timing of the onset of puberty. This so-called 'ram or boar effect' is probably mediated by pheromonal and other sensory cues influencing hypothalamic GnRH secretion.

Climate. Anthropomorphic extrapolation has assumed that animals living in the tropics reach puberty at an earlier age than those in temperate climates. Studies carried out in Zambia have shown that in cattle this is not true.

Disease. Any disease which can influence the growth rate, either directly or because of interference with feeding and utilisation of nutrients, will delay the onset of puberty.

THE OESTROUS CYCLE AND ITS PHASES

Traditionally, the oestrous cycle is divided into a number of phases.

Pro-oestrus. The phase immediately preceding oestrus. It is characterised by a marked increase in activity of the reproductive system. There is follicular growth and regression of the corpus luteum of the previous cycle (in polycyclic species). The uterus enlarges very slightly; the endometrium becomes congested and oedematous, and its glands show evidence of increased secretory activity. The vaginal mucosa becomes hyperaemic; the number of cell layers of the epithelium starts to increase, and the superficial layers become cornified. The bitch shows external evidence of pro-oestrus with vulval oedema, hyperaemia and a sanguineous vulval discharge.

Oestrus. The period of acceptance of the male. The onset and end of the phase are the only accurately measurable points in the oestrous cycle, and hence are used as the baseline for determining cycle length. The animal usually seeks out the male and 'stands' for him to mate her. The uterine, cervical and vaginal glands secrete increased amounts of mucus; the vaginal epithelium and endometrium become hyperaemic and congested; the cervix is relaxed.

Ovulation occurs during this phase of the cycle in all domestic species with the exception of the cow, where it occurs about 12 hours after the end of oestrus. Ovulation is a spontaneous process in all domestic species with the exception of the cat, rabbit and camel, in which it is induced by the act of coitus.

During pro-oestrus and oestrus there is follicular growth in the absence of functional corpora lutea, the main ovarian hormones produced being oestrogens. Pro-oestrus and oestrus are frequently referred to collectively as the follicular phase of the cycle.

Metoestrus. The phase succeeding oestrus. The granulosa cells of the ovulated follicle give rise to lutein cells which are responsible for the formation of the corpus luteum. There is a reduction in the amount of secretion from the uterine, cervical and vaginal glands.

Dioestrus. The period of the corpus luteum. The uterine glands undergo hyperplasia and hypertrophy, the cervix becomes constricted and the secretions of the genital tract are scant and sticky; the vaginal mucosa becomes pale. The corpus luteum is fully functional during this phase, and is secreting large amounts of progesterone.

The period of the oestrous cycle when there is a functional corpus luteum is sometimes referred to as the luteal phase of the cycle, to differentiate it from the follicular phase.

Since in most of our domestic species oestrus is the only readily identifiable phase of the oestrous cycle, there is some merit, in polyoestrous species, in dividing the cycle into oestrus and interoestrus, the latter including pro-oestrus, metoestrus and dioestrus. Another alternative division can be into follicular and luteal phases.

Anoestrus. The prolonged period of sexual rest during which the genital system is mainly quiescent. Follicular development is minimal; the corpora lutea, although identifiable, have regressed and are non-functional. Secretions are scanty and tenacious, the cervix is constricted, and the vaginal mucosa is pale.

Natural regulation of cyclical activity

Regulation of cyclical activity in the female is a complex process. With the development of new techniques, particularly those of hormone assays, and the application of new molecular biological techniques, there is a continual advance in the knowledge and understanding of the mechanisms involved. Although much of the early work was done on laboratory animals – notably the rat and guinea pig – there is now much more information about domestic species, although there are still areas, particularly in the bitch, which are not fully understood.

Regulation of cyclical activity is mainly under the control of the hypothalamic–pituitary–ovarian axis. At one end of this axis there is the influence of the extrahypothalamic areas – the cerebral cortex, thalamus and mid-brain – and the role played by stimuli such as light, olfaction and touch (Ellendorff, 1978), whilst at the other end is the influence of the uterus upon the ovary.

The pineal gland appears to have an important role in controlling reproduction in seasonal breeding species and also in the timing of puberty by influencing the release of FSH, LH and prolactin. Although much of the interest has been in the action of the indoleamine melatonin, there is increasing interest in the other pineal peptide hormones, namely arginine vasotocin, gonadotrophin and prolactin-releasing and inhibitory hormones. There is some suggestion that melatonin may not act directly upon the hypothalamus/anterior pituitary, but indirectly via the other pineal peptide hormones.

Melatonin drives the reproductive response of the ewe to inductive photoperiods (Bittman et al., 1983). Rhythmic administration of melatonin to adult ewes exerts a similar effect to increased hours of darkness by inducing the onset of the breeding season (Arendt et al., 1983) and causes changes in prolactin concentrations in the plasma that are similar to those following exposure to short days (Kennaway et al., 1983).

In sheep, an intact pineal gland is required for a normal photoperiodic response to altered daylight patterns; however, other seasonal environmental cues are important, since pinealectomised ewes still show seasonal breeding (Lincoln, 1985).

The mare is a seasonal breeder, but is 'switched on' by increasing day length. The pineal gland is involved, since if it is removed the mare does not show a normal response to changes in photoperiod. In intact mares, melatonin concentrations increase during hours of darkness (Grubaugh et al., 1982). There is some evidence that foals are conditioned at an early age and develop a pattern of melatonin secretion from about 7 weeks of age (Kilmer et al., 1982).

The hypothalamus is responsible for the control of release of gonadotrophins from the anterior pituitary by the action of specific releasing and inhibitory substances. These are secreted by the hypothalamic neurons, and are carried from the median eminence of the hypothalamus by the hypothalamic–hypophyseal portal system. In 1971 the molecular structure of porcine GnRH was determined (Matsuo et al., 1971) as being a decapeptide, and subsequently synthesised (Geiger et al., 1971). Opinion is divided as to whether GnRH is responsible in vivo for the release of both FSH and LH (Lamming et al., 1979), although the injection of GnRH stimulates the release of both FSH and LH in domestic species. As yet, no specific inhibitory factor such as that for prolactin has been identified for gonadotrophins.

Specific neurotransmitter substances are involved in the regulation of the release of pituitary hormones. The role of three monoamines has now been fairly well established (Kordon, 1978). Noradrenaline stimulates the release of FSH and LH; the inhibition of the conversion of dopamine to noradrenaline blocks the 'oestradiol-induced' release of LH, which is responsible for ovulation; whilst serotonin inhibits the basal secretion of LH and regulates other neurosecretory systems. Dopamine also has an important role in the control of prolactin release.

There is good evidence that in domestic species the secretion of FSH and LH is controlled by two functionally separate, but superimposable, systems. These are the episodic/tonic system, which is responsible for the continuous basal secretion of gonadotrophin and stimulates the growth of both germinal and endocrine components of the ovary, and the surge system, which controls the short-lived massive secretion of gonadotrophin, particularly LH, responsible for ovulation. There are two hypothalamic centres that are involved in controlling these two systems (Figure 1.1).

With the exception of the cat, rabbit and camel, all domestic species are spontaneous ovulators. However, in these three species ovulation is induced by the stimulation of sensory receptors in the vagina and cervix at coitus. This initiates a neuro-endocrine reflex ultimately resulting in the activation of GnRH neurons in the surge centre and release of a surge of LH.

Not only does the anterior pituitary have a direct effect upon ovarian functions by stimulating folliculogenesis, follicular maturation, ovulation and corpus luteum formation, but the ovary has an effect upon the hypothalamus and anterior pituitary. This is mediated by oestradiol, produced by the maturing follicle, and by progesterone, produced by the corpus luteum. The episodic/tonic hypothalamic release centre is influenced by the negative-feedback effect of oestradiol and progesterone. Low levels of progesterone also have a modulating influence on this centre, which appears to be particularly important in ruminants (Lamming et al., 1979). In the cow, ewe and sow (and probably in other domestic species) FSH secretion is also controlled by a number of ovarian-derived peptide hormones. The first that has been characterised, inhibin, is produced by the granulosa cells of large antral follicles, and can be isolated from follicular fluid. It has also been isolated from the testis and seminal plasma (see Chapter 29). Inhibin and oestradiol act in concert in suppressing FSH secretion. Inhibin, which is produced by all antral follicles, has a longer half-life, and sets the overall level of negative feedback, whereas oestradiol, which is produced only by those antral follicles that have the potential for ovulation, is responsible for the day-to-day fluctuations (Baird et al., 1991). Two other peptide hormones have been isolated from ovarian follicular fluid; these have been designated activin, which stimulates, and follistatin, which suppresses, FSH secretion. Their roles in controlling and regulating follicular growth are not known.

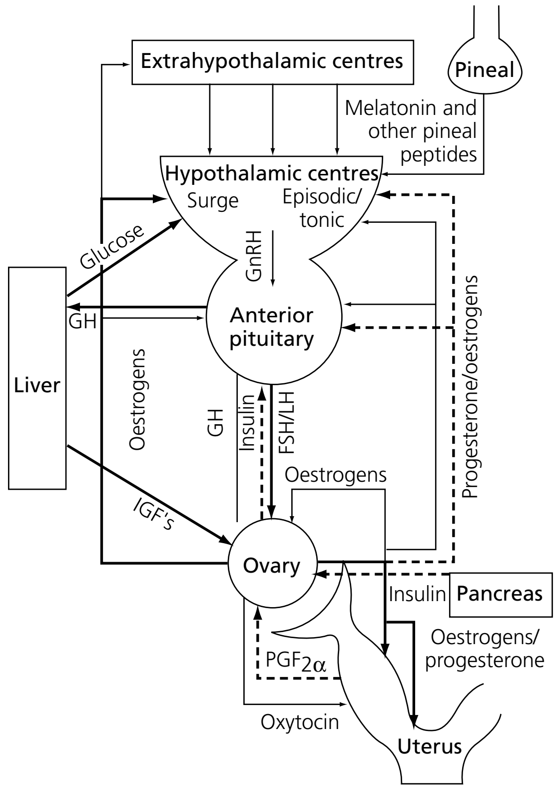


Fig. 1.1 Endocrine control of cyclical reproductive activity. ——, stimulation; - - -, inhibition; $PGF_{2\alpha}$, prostaglandin$_{2\alpha}$; IGFs, insulin-like growth factors; GH, growth hormone. (Adapted from Lamming et al., 1979.)

The positive-feedback effect of oestradiol on hypothalamic–pituitary function is well demonstrated in farm animals, since the preovulatory surge of oestradiol stimulates the release of LH, which is so necessary for the process of ovulation and corpus luteum formation. The response of the anterior pituitary to GnRH is influenced by the levels of ovarian steroids so that there is increased responsiveness shortly after the level of progesterone declines and that of oestradiol rises (Lamming et al., 1979). There are probably self-regulatory mechanisms controlling gonadotrophin secretion acting locally within the anterior pituitary and hypothalamus.

Tonic release of gonadotrophins, especially LH, does not occur at a steady rate but in a pulsatile fashion in response to a similar release of GnRH from the hypothalamus. The negative feedback of progesterone is mediated via a reduction in pulse frequency of gonadotrophin release, whereas oestradiol exerts its effect via a reduced pulse amplitude. The onset of cyclical activity after parturition (see Chapter 7), at puberty or at the start of the breeding season is associated with increased pulse frequency of tonic gonadotrophin secretion. When the ram is placed in contact with ewes before the start of the breeding season, there is increased frequency of pulsatile LH release, which stimulates the onset of cyclical activity (Karsch 1984).

Progesterone appears to play a critically important role in the inhibition of the tonic mode of LH secretion in the ewe (Karsch et al., 1978). Progesterone is thus the main regulatory hormone which controls the oestrous cycle of the sheep and probably of other species too. Thus when the concentration of progesterone in the circulation falls, associated with the regression of the corpus luteum, there is release of LH from the anterior pituitary. The rise in LH triggers the secretion of oestradiol; this sudden rise stimulates the surge centre for the LH release and, as a result of this sudden increase, ovulation of the mature follicle occurs (Karsch et al., 1978).

In some species, notably the cow (see Figure 1.28 later), there is also a concomitant surge in FSH; although its significance is unclear it may be part of the 'ovulation-inducing' hormone complex. For this reason it is probably incorrect to assign a separate and specific physiological role for the two pituitary gonadotrophins. Thus, although ovulation and steroidogenesis can be initiated by both FSH and LH, it would appear that only FSH can induce early follicular growth, so that when the granulosa cells have matured and are able to respond to endogenous LH, the formation of a fully developed vesicular follicle occurs. Large amounts of a peptide similar to the hormone inhibin, produced by the Sertoli cells of the testis, have been found in bovine and porcine follicular fluid and granulosa cells. This hormone probably selectively inhibits FSH release from the anterior pituitary but it may also have a local role in controlling ovarian function; it has been shown to inhibit the binding of FSH to granulosa cells in the cow (Sato et al., 1982). Recently, the roles of insulin-like growth factors (IGFs), notably IGF-1, and their associated binding proteins have been shown to exert an influence on ovarian function at the level of the granulosa, thecal and luteal cells, probably by acting synergistically with gonadotrophins (Lucy et al., 1999). Furthermore, there is also good evidence in the cow that growth hormone (GH) also has a role in regulating ovarian function either directly or by stimulating the synthesis and secretion of IGF-1 by the liver (Lucy et al., 1999). These findings are of considerable practical interest, because it is likely that they may be important in mediating the effects of nutrition on reproduction.

Throughout the oestrous cycle, during pregnancy and other reproductive stages, there is dynamic follicular activity with growth and atresia; only about 1% of antral follicles subsequently ovulate. There appear to be two different patterns of follicular growth in mammals (Fortune, 1994). Thus in cattle, sheep and horses the development of antral follicles to sizes close to that at ovulation occurs throughout the oestrous cycle including the luteal phase, whereas in the pig and rat the development of preovulatory-size follicles only occurs in the follicular phases of the cycle in the absence of a functional corpus luteum (CL). Follicular development occurs in stages, the following terminology for which is now generally accepted (Webb et al., 1999):

- *recruitment* – gonadotrophin stimulation of a pool of rapidly growing follicles
- *selection* – a process whereby one or more of the recruited follicles are selected to develop further

- *dominance* – the mechanism whereby one (the dominant follicle), or several follicles, undergo rapid development in an environment where the growth and development of other follicles is suppressed.

The pattern of follicular dynamics has been summarised, particularly in ruminant species, by Adams (1999), and it is appropriate to quote this as follows: '(1) follicles grow in a wave-like fashion; (2) periodic surges in circulating FSH are associated with follicular wave emergence; (3) selection of a dominant follicle involves the decline in FSH and acquisition of LH responsiveness; (4) periodic anovulatory follicular waves continue to emerge until the occurrence of an LH surge; (5) within species, there is a positive relationship between the duration of the oestrous cycle and the number of follicular waves; (6) progesterone is suppressive to LH secretion and the growth of the dominant follicle; (7) the duration of the interwave interval is a function of follicular dominance, and is negatively correlated with circulating FSH; (8) follicular dominance in all species is more pronounced during the first and last follicular waves of the oestrous cycle; (9) pregnancy, the prepubertal period and seasonal anoestrus are characterised by regular, periodic surges of FSH and emergence of anovulatory follicular waves.'

The CL is rapidly formed from the Graafian follicle after ovulation primarily from the granulosa and the thecal cells; in the ewe, for example, its mass increases 20-fold over 12 days (Reynolds and Redmer, 1999). For some time it was assumed that, once formed, it remained a relatively static structure but now it has been shown that when it is functionally mature there is rapid cellular turnover, although there is little change in size. The fully formed CL consists of a number of different cell types: the steroid-secreting large and small luteal cells, fibroblasts, smooth muscle, pericytes and endothelial cells. It has the greatest blood supply per unit tissue of any organ (Reynolds and Redmer, 1999). In the ewe, based on volume, the large luteal cells comprise 25–35%, the small luteal cells 12–18% and vascular elements 11% (Rodgers et al., 1984). Although the CL develops as a result of ovulation, in some species, notably the bitch, there are early signs of luteinisation of the follicle before it has ovulated. The stimulus for the formation and maintenance of the CL probably varies within species. The hormones which are most likely to be involved are prolactin and LH, but there is some evidence that they are involved together, perhaps in association with FSH. Although all three hormones are probably involved in the induction of luteinisation of granulosa cells, the available evidence suggests that FSH is probably not required for the maintenance of luteal function. The difference between species is well illustrated by the observation that LH will prolong luteal function in the sow, but prolactin will not (Denamur et al., 1966; Anderson et al., 1967). However, in the ewe prolactin appears to be more important as a luteotrophic agent, since LH will exert an effect only if infused from day 10 to day 12 of the oestrous cycle.

The role of prolactin in the control of reproduction in many domestic species is still largely speculative, and in many cases it is only possible to extrapolate from studies in the traditional laboratory species. Unlike other anterior pituitary hormones that require hypothalamic stimulation, it appears that prolactin secretion is spontaneous, and that it is largely controlled by inhibition by hypothalamically-derived prolactin inhibitory factor (PIF), which is believed to be dopamine. There is some evidence to suggest that dopamine may have a dual role as a stimulant of prolactin secretion, rather like a prolactin-releasing factor (PRF).

Much interest has been directed towards the role of certain endogenous peptides with opioid activity such as β-endorphin and met-enkephalin. These substances have been found in high concentrations in hypothalamic–hypophyseal portal blood. The administration of exogenous opioid peptides inhibits the secretion of FSH and LH whilst stimulating the secretion of prolactin. If an opiate antagonist such as naloxone is infused, there is an increase in mean concentrations of gonadotrophins in the plasma and the frequency of episodic gonadotrophin secretion. The effect of opioids appears to be influenced by the steroid environment of the animal; for example, in ewes, naloxone increased the mean plasma concentration of LH and the episodic frequency in a high-progesterone environment. However, in ovariectomised ewes or those with oestradiol implants, naloxone had no effect (Brooks et al., 1986). It is possible that the negative feedback of progesterone on LH release (see below) may be mediated via opioids (Brooks et al., 1986).

The presence of a functional CL, by virtue of its production of progesterone, inhibits the return to oestrus by exerting a negative feedback effect upon the anterior pituitary; this is most obvious during pregnancy (see Chapter 3). In the normal, non-pregnant female, oestrus and ovulation occur at fairly regular intervals; the main control of this cyclical activity would appear to be the CL. There is also evidence that the CL also exerts a positive intra-ovarian effect by increasing the number of small antral follicles in that ovary (Pierson and Ginther, 1987).

Although it has been known for nearly 80 years that in certain species of animal the uterus influences ovarian function (Loeb, 1923) the mechanism has been fully understood only in recent years.

It has been demonstrated that in many species removal of part or all of the uterus will result in the prolongation of the life span of the CL (du Mesnil du Buisson, 1961; Rowson and Moor, 1967); these species include the cow, mare, ewe, goat and sow. In the human, dog and cat the normal life span of the CL is unaltered in the absence of the uterus. In the cow, ewe and goat the 'luteolytic' action of the uterine horn is directed exclusively to the CL on the adjacent ovary (Ginther, 1974). Thus, if one of the uterine horns is surgically removed on the side adjacent to the ovary with a CL then the latter will persist. If the contralateral horn is removed, then the CL will regress at the normal time. It appears that in these species the luteolytic substance is transported directly from the uterus to the ovary. In the ewe it has been shown experimentally that the most likely route for transport of the substance is the middle uterine vein, since when all other structures between the ovary and uterus are severed there is still normal regression of the CL (Baird and Land, 1973).

In the mare no local effect can be demonstrated, since if the ovary is transplanted outside the pelvic cavity, luteal regression still occurs (Ginther and First, 1971). It is generally assumed that in this species the luteolysin is transported throughout the systemic circulation.

In the pig the luteolytic substance is transported locally (du Mesnil du Buisson, 1961) but not exclusively to the adjacent ovary. It has been shown that, following surgical ablation of parts of the uterine horns, provided at least the cranial quarter of the uterine horn is left, regression of the CLs occurs in both ovaries. If more than three-quarters of the horn is excised, then regression of the CLs occurs only in the ovary adjacent to the intact horn. In the bitch, the mechanisms of control of the life span of the CLs are not fully understood, and in this species even in the absence of pregnancy there is always a prolonged luteal phase traditionally called metoestrus.

Although the importance of the middle uterine vein in the transfer of the luteolytic substance has been demonstrated, the mechanisms whereby the luteolytic substance passes to the ovary have not been conclusively shown in all species, although they have been fairly well evaluated in the ewe and cow. In the former species, it appears that the close proximity of the ovarian artery and utero-ovarian vein is important, particularly since at their points of approximation the walls of the two vessels are thinnest; there is no anastomosis (Coudert et al., 1974). This allows the leakage of the luteolytic substance from the uterine vein into the ovarian artery and thus to the ovary, by a form of counter-current exchange through the walls of the vessels. It has been suggested (Ginther, 1974) that the variation in the response to partial or total hysterectomy in different species is probably due to differences in the relationships between the vasculature of the uterus and ovaries.

It was not until 1969 that the substance responsible for luteolysis was identified, when the duration of pseudo-pregnancy in the rat was shortened by the injection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). This same substance has subsequently been shown to have potent luteolytic activity in the ewe, doe, cow, sow and mare. Although it has been proved only in ruminants and the guinea pig that it is the natural luteolysin, it is likely that it is also true for the other species listed.

$PGF_{2\alpha}$ is a derivative of the unsaturated hydroxy acids linolenic and arachidonic acids. It derived its name because it was first isolated from fresh semen and it was assumed to be produced in the prostate gland. It is synthesised in the endometrium of a number of species (Horton and Poyser, 1976), and in the ewe it has been demonstrated in increasing amounts at and around the time of luteal regression (Bland et al., 1971).

Luteal regression can be viewed from two aspects. Firstly, functional regression is rapid, so that the secretion of progesterone declines rapidly. Secondly, as regards structural regression when the CL is reduced in size, the latter process takes longer than the former. In ruminants, luteal regression is caused by episodic release of $PGF_{2\alpha}$ from the uterus at intervals of about 6 hours. This is induced by oxytocin secreted by the CL; thus, each episode of $PGF_{2\alpha}$ release is accompanied by an episode of oxytocin release. Furthermore, $PGF_{2\alpha}$ stimulates further secretion of oxytocin from the ovary. It has been postulated that the abundant, non-steroidogenic endothelial cells of the CL may mediate the actions of $PGF_{2\alpha}$, and that its physical demise is due to the action of invading macrophages which may secrete cytokines, such as tumour necrosis factor $(TNF)_{\alpha}$ (Meidan et al., 1999).

The sensitivity of the uterus to oxytocin is determined by the concentration of endometrial oxytocin receptors. At the time of luteal regression in sheep they rise approximately 500-fold (Flint et al., 1992). Their concentration is determined by the effects of progesterone and oestradiol. Thus, the high concentrations of progesterone which occur after the formation of the CL reduce the number of receptors, so that in the normal oestrous cycle of the ewe they start to increase from about day 12. Exogenous oestradiol causes premature induction of oxytocin receptors, resulting in premature luteolysis (Flint et al., 1992).

In non-ruminant species, much less is known about the mechanisms of luteolysis.

The CL becomes more sensitive to the leuteolytic effect of $PGF_{2\alpha}$ as it ages. The early CL is unresponsive to $PGF_{2\alpha}$.

THE MARE

Cyclic periodicity

Fillies are often seen in oestrus during their second spring and summer (when they are yearlings), but under natural conditions it is unusual for them to foal until they are over 3 years old. The mare is normally a seasonal breeder, with cyclic activity occurring from spring to autumn; during the winter she will normally become anoestrous. However, it has been observed that some mares, especially those of native pony breeds, will cycle regularly throughout the year. This tendency can be enhanced if the mares are housed and given supplementary food when the weather is cold and inclement, and if additional lighting is provided when the hours of daylight are short.

Horse breeding has been influenced by the demands of thoroughbred racing, because in the northern hemisphere foals are aged from 1 January, irrespective of their actual birth date. As a result, the breeding season for mares has been, for over a century, determined by the authorities as running from 15 February to 1 July. Since the natural breeding season does not commence until about the middle of April, and maximum ovarian activity is not reached until July, it is obvious that a large number of thoroughbred mares are bred at a time when their fertility is suboptimal (see Chapter 26).

The winter anoestrus is followed by a period of transition to regular cyclic activity. During this transition, the duration of oestrus may be irregular or very long, sometimes more than a month. The manifestations of heat during the transitional phase are often atypical and make it difficult for the observer to be certain of the mare's reproductive status. Also, before the first ovulation, there is poor correlation between sexual behaviour and ovarian activity; it is common for the early heats to be unaccompanied by the presence of palpable follicles, and some long spring heats are anovulatory. However, once ovulation has occurred, regular cycles usually follow.

The average length of the equine cycle is 20–23 days; the cycles are longer in spring and shortest from June to September. Typically, oestrus lasts 6 days and dioestrus 15 days. Ovulation occurs on the penultimate or last day of heat, and this relationship to the end of heat is fairly constant and irrespective of the duration of the cycle or the length of oestrus; Hammond (1938) found that manual rupture of the ripe follicle resulted in termination of oestrus within 24 hours. The diameter of the ripe follicle is 3–7 cm. During the last day before ovulation, the tension in the follicle usually subsides, and the palpable presence of a large fluctuating follicle is a sure sign of imminent ovulation.

The onset of heat after foaling occurs on the fifth to 10th day. This foal heat is sometimes rather short, 2–4 days. It is traditional to cover a mare on the ninth day after foaling. The first two post-parturient cycles are a few days longer than subsequent ones.

During oestrus, a single egg is usually released, and there is a slight preponderance of ovulations from the left ovary. Assessing the functional activity of the two ovaries on the basis of post-mortem counts of CLs in 792 equine genitalia, Arthur (1958) recorded an incidence of 52.2% of ovulations from the left ovary. Twin ovulation commonly occurs in mares; Burkhardt (1948), in a study of June–July slaughterhouse specimens, saw 27% of double ovulations, and Arthur (1958) found an overall frequency of 18.5%, with a summer peak of 37.5%. However, there is a strong breed influence on twin ovulation; thoroughbreds are prone to it, but pony mares rarely show it. A fascinating finding by Van Niekerk and Gernaeke (1966) was that only fertilised eggs pass into the uterus; non-fertilised eggs remain for months in the uterine tubes, where they slowly disintegrate. All equine ovulations occur from the ovulation fossa; only at the ovarian hilus may occasional protrusions of corpora lutea be seen, but because of the curvature of the ovary and the presence of the adjacent substantial fimbriae these protusions cannot be identified by rectal palpation.

Day (1939) has given a clear picture of the changes which occur in the equine ovary during an oestrous cycle. Figures 1.2–1.6 are diagrams of ovaries of the mare, half natural size, at different stages of the oestrous cycle, whereas Figures 1.7–1.12 show examples of whole ovaries, cross-sections and B-mode ultrasound images. Just before the onset of heat, several follicles enlarge to a size of 1–3 cm. By the first day of oestrus one follicle is generally considerably larger than the

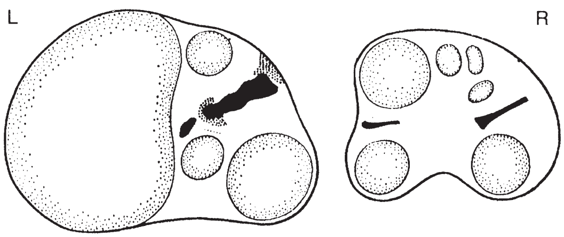


Fig. 1.2 The ovaries of a 5-year-old farm mare in oestrus. Specimens obtained in May. Regressing corpus luteum in left ovary, bright yellow ochre in colour.

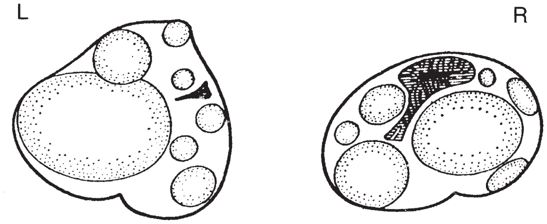


Fig. 1.3 Ovaries of a 9-year-old farm mare in dioestrus. Corpus luteum in right ovary, orange in colour; pleats loose.

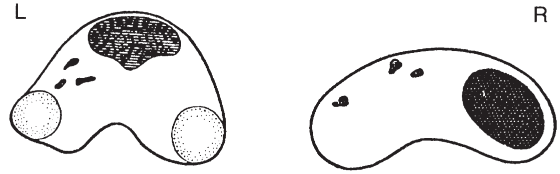


Fig. 1.4 Ovaries of a 4-year-old shire mare in dioestrus. Corpus luteum in left ovary, brownish-red in colour; pleats distinct; central cavity containing blood clot. Right ovary contains a follicle filled with blood.

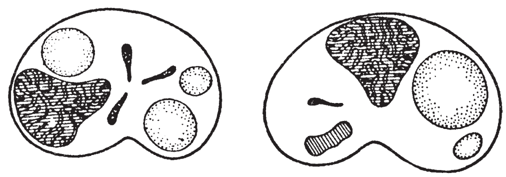


Fig. 1.5 Ovaries of a 6-year-old farm mare in dioestrus. A corpus luteum in each ovary, orange-yellow in colour; pleats distinct.

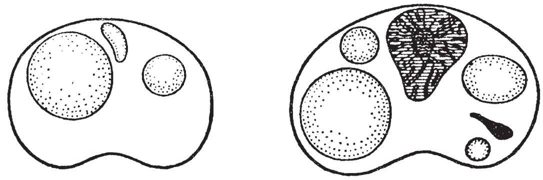


Fig. 1.6 Ovaries of a 6-year-old hunter mare in dioestrus. Corpus luteum in right ovary, pale yellow in colour; pleats distinct. Central cavity.

remainder, having a diameter of 2.5–3.5 cm. During oestrus, this follicle matures and ruptures when it has attained a diameter of 3–7 cm. After ovulation, the other follicles regress, until, during the first 4–9 days of the ensuing dioestrus, no follicles larger than 1 cm are likely to be present. Several hours before ovulation the ripe follicle becomes much less tense. The collapsed follicle is recognised by an indentation on the ovarian surface; there is usually some haemorrhage into the follicle, and the coagulum hardens within the next 24 hours. Quite frequently the mare shows evidence of discomfort when the ovary is palpated soon after ovulation. Unless sequential transrectal palpation or ultrasonic examinations are performed, it is sometimes possible to confuse a mature follicle with the early corpus haemorrhagicum, since before ovulation the follicular antrum is filled with follicular fluid and then soon after ovulation it becomes filled with blood. For this reason mares are sometimes incorrectly diagnosed as having failed to ovulate. For the next 3 days the

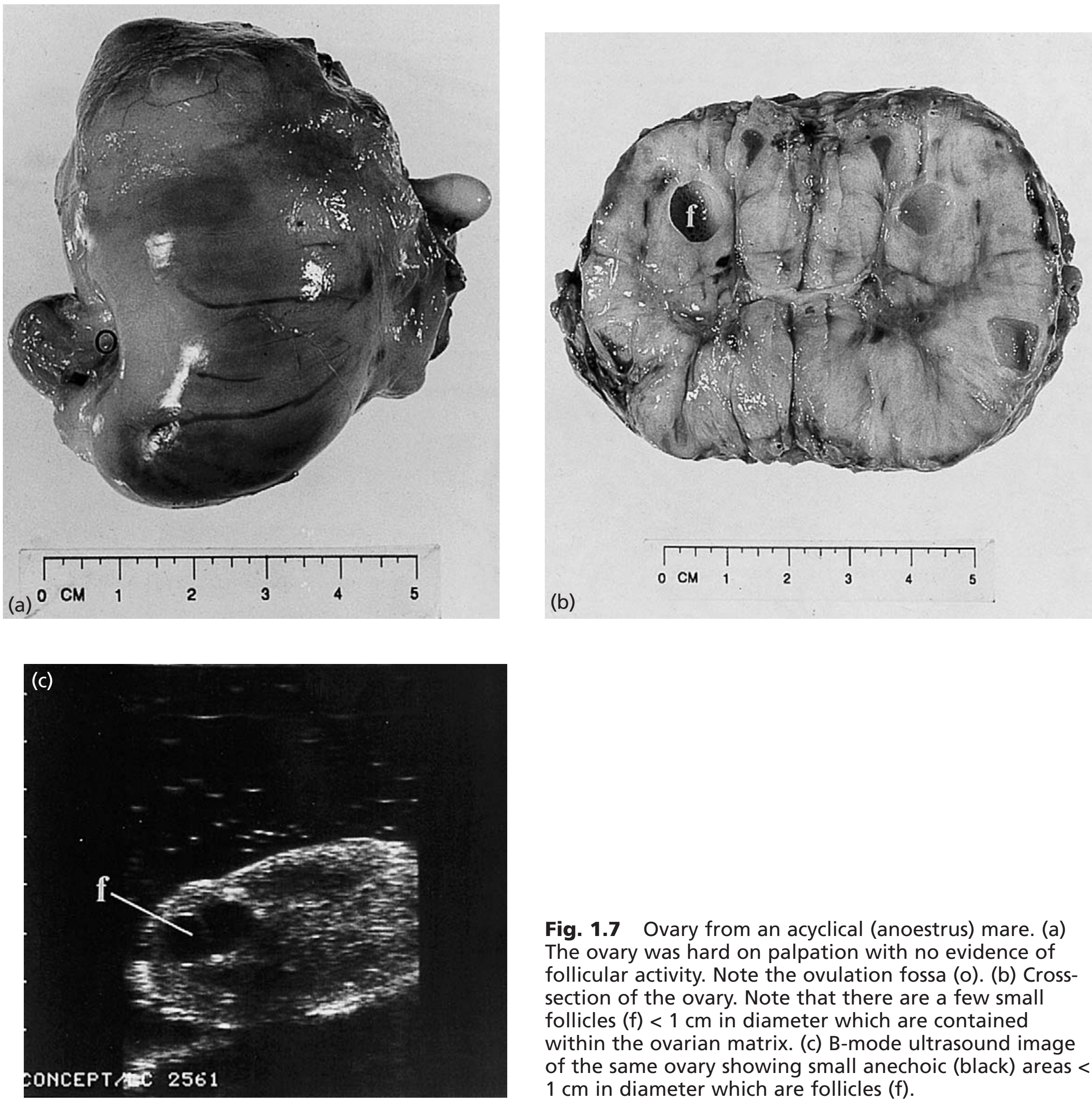
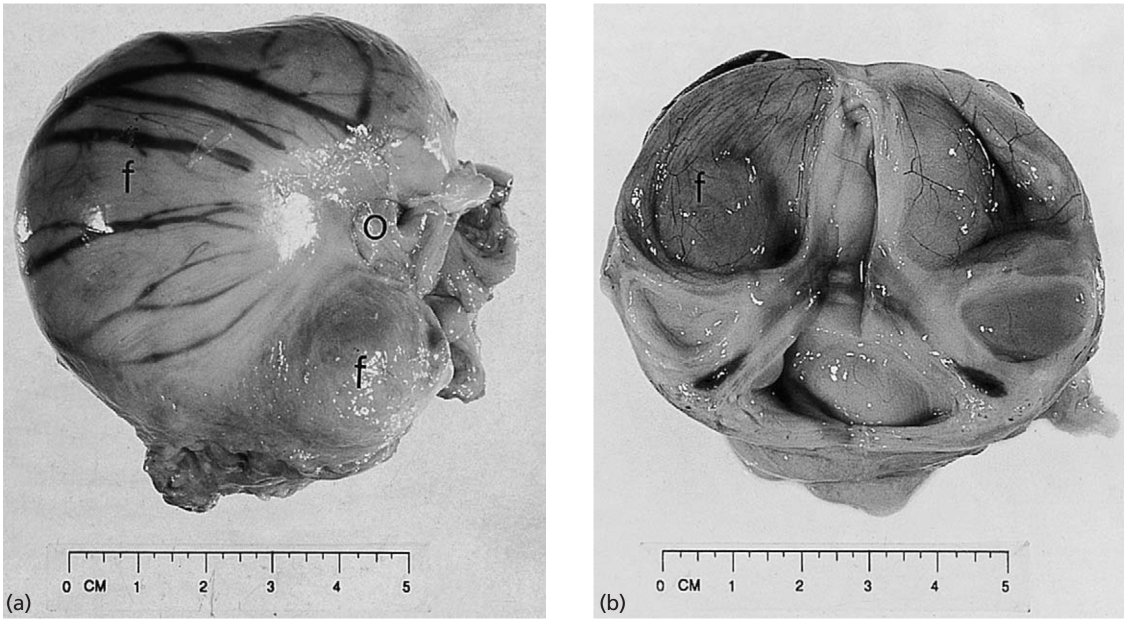


Fig. 1.7 Ovary from an acyclical (anoestrus) mare. (a) The ovary was hard on palpation with no evidence of follicular activity. Note the ovulation fossa (o). (b) Cross-section of the ovary. Note that there are a few small follicles (f) < 1 cm in diameter which are contained within the ovarian matrix. (c) B-mode ultrasound image of the same ovary showing small anechoic (black) areas < 1 cm in diameter which are follicles (f).



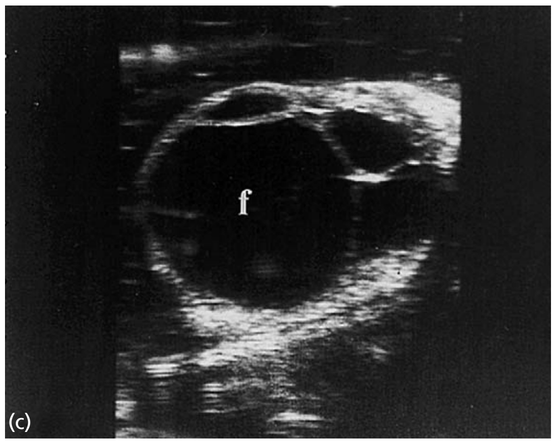
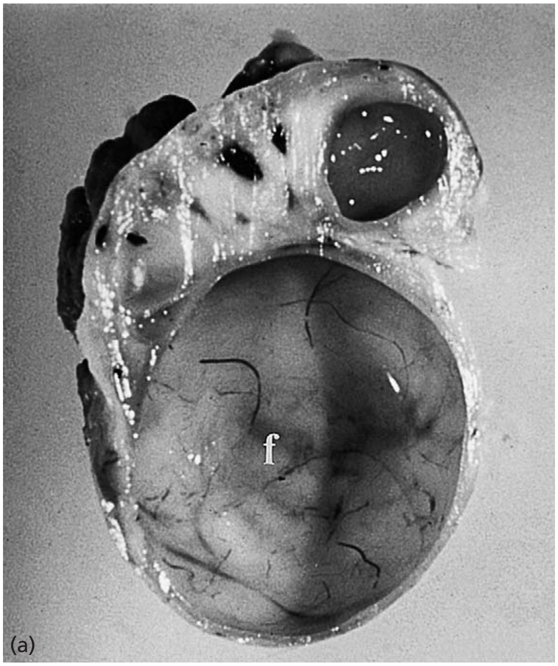


Fig. 1.8 Ovary from a mare in the early follicular phase. (a) The ovary was soft on palpation with evidence of large follicles near the surface of the ovary (f). Note the ovulation fossa (o). (b) Cross-section of the ovary. Note three follicles are at least 2 cm in diameter. (c) B-mode ultrasound image of the same ovary showing one large anechoic (black) area about 3.5 cm in diameter which is a follicle (f), together with three smaller ones.

luteinising mass can be felt as a resilient focus, but later it tends to have the same texture as the remainder of the ovary. In pony mares, however, of known history from daily examinations, Allen (1974) reports that it is possible to follow the growth of the CL by palpation because in ponies it forms a relatively large body in a small ovary. The CL attains maximum size at 4–5 days, but it does not protrude from the ovarian surface. On section of the ovary it is brown and later yellow and of a triangular or conical shape, with the narrower end impinging on the ovulation fossa. Its centre is commonly occupied by a variable amount of dark-brown fibrin. The cyclical CL begins to regress at about the 12th day of the cycle, when there is a parallel fall in the blood progesterone concentration. From this day onwards the events previously described recur. Ovulation, with the subsequent formation of a CL, does not always occur; the follicle may regress or sometimes undergo luteinisation (see Figure 1.10(b)).

B-mode ultrasound imaging with a rectal transducer has been used to visualise follicles (see Figures 1.7–1.12). This is particularly useful in



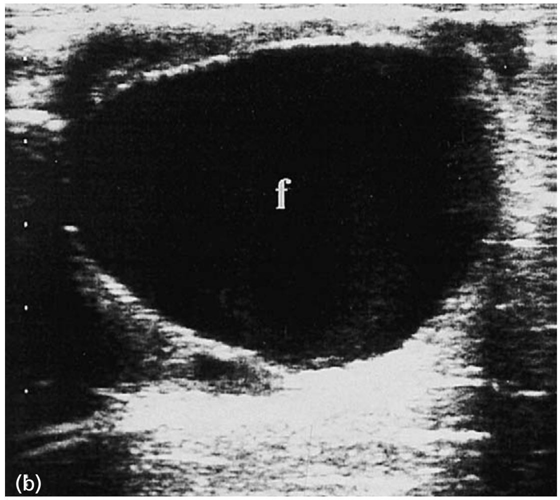


Fig. 1.9 Ovary of a mare with a single large preovulatory follicle. (a) Section of the ovary showing a 4 cm follicle (f). (b) B-mode ultrasound image of a different ovary showing a 4–5 cm preovulatory follicle (f) as a large anechoic (black) area.

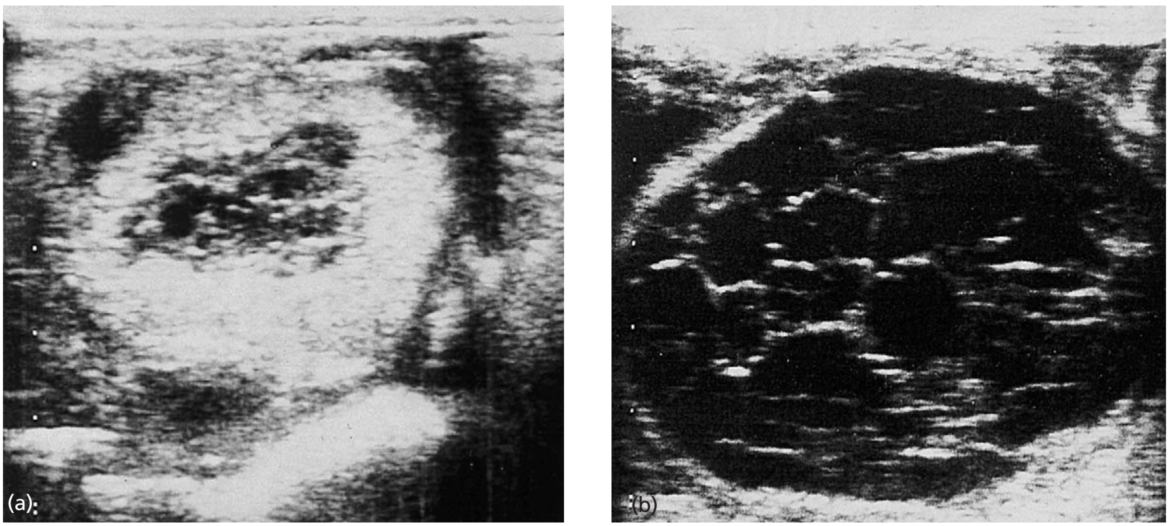
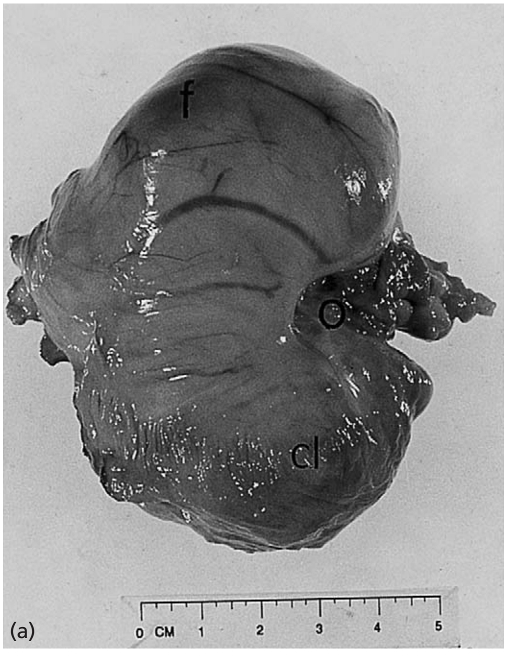
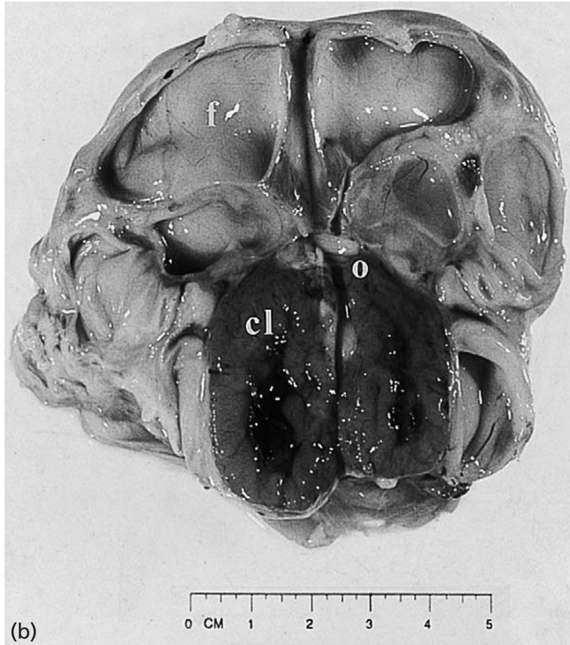


Fig. 1.10 (a) B-mode ultrasound image of an ovary showing the corpus haemorrhagicum. (b) B-mode ultrasound image of a 5 cm anovulatory follicle that is undergoing luteinisation.

detecting the possibility of twin ovulations and also in determing the timing of ovulation. Ginther (1986) observed that in the preovulatory period there was a change in the shape of the follicle and a thickening of the follicular wall, which, together with the assessment of the size of the follicle, could be used to predict the time of ovulation. The same author has used this technique to assess corpora lutea. He identified differences in the echogenic properties of the CL depending upon the persistence of the corpus haemorrhagicum; this he identified in about 50%.





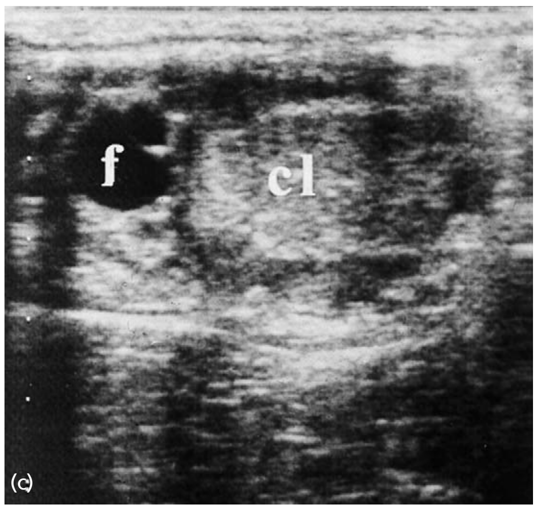


Fig. 1.11 Ovary of a mare in early dioestrus. (a) The corpus luteum (cl), although present, could not be palpated externally, whereas a follicle (f) could be identified. Note the ovulation fossa (o). (b) Section of the same ovary. Note that the corpus luteum (cl), still with a central blood clot, impinges on the ovulation fossa (o) where ovulation occurred. Also, one large follicle (f) and several smaller ones can be identified. (c) B-mode ultrasound image of a different ovary showing the corpus luteum (cl) and follicles (f).

During winter anoestrus, both ovaries are typically small and bean-shaped, common dimensions being 6 cm from pole to pole, 4 cm from the hilus to the free border and 3 cm from side to side. Not uncommonly, however, in early spring or late autumn, the anoestrous ovaries are of medium or large size and knobbly due to the content of numerous follicles of 1–1.5 cm diameter. During the cycle, there are large variations in the ovarian size depending on the number and size of the follicles. During oestrus the ovary of the thoroughbred mare may contain two or even three follicles, each of 4–7 cm, and these, with other subsidiary follicles, combine to give it a huge size. During dioestrus, however, with an active CL and only atretic follicles the ovary may be little larger than in anoestrus.

By visual examination of the vagina and the cervix using an illuminated speculum, it is possible to detect the preovulation period. In dioestrus, the

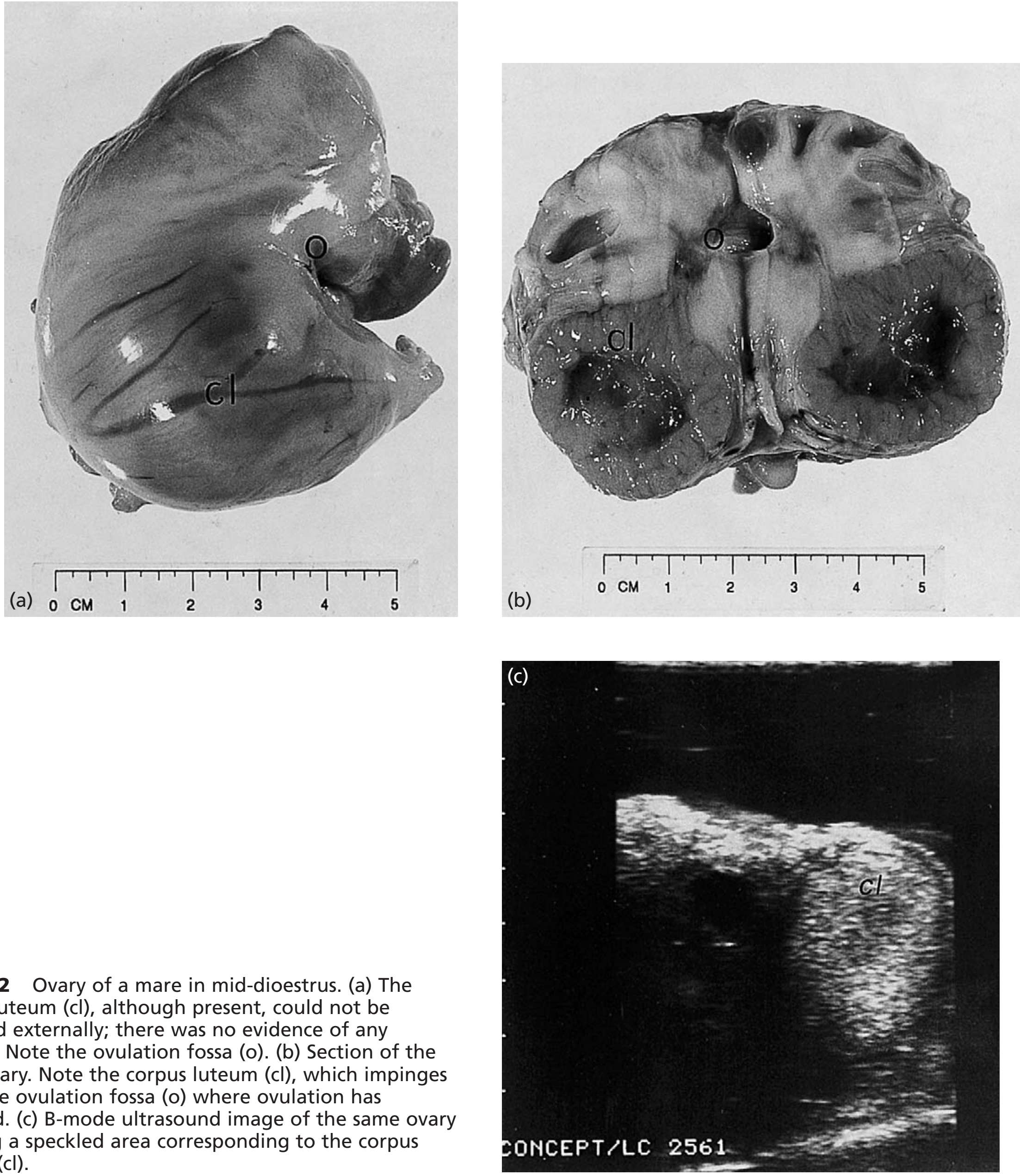


Fig. 1.12 Ovary of a mare in mid-dioestrus. (a) The corpus luteum (cl), although present, could not be palpated externally; there was no evidence of any follicles. Note the ovulation fossa (o). (b) Section of the same ovary. Note the corpus luteum (cl), which impinges upon the ovulation fossa (o) where ovulation has occurred. (c) B-mode ultrasound image of the same ovary showing a speckled area corresponding to the corpus luteum (cl).

cervix is small, constricted and firm; it and the vagina are pale pink, while mucus is scanty and sticky. During oestrus, there is a gradual increase in the vascularity of the genital tract and relaxation of the cervix with dilatation of the os. As oestrus advances and ovulation time approaches, the cervix becomes very relaxed and its protrusion can be seen lying on the vaginal floor, with its folds oedematous; the vaginal walls are glistening with clear lubricant mucus. After ovulation there is a gradual reversion to the dioestrous appearance.

During anoestrus, as in pregnancy, both the vagina and cervix are blanched; the cervix is constricted and generally turned away from the midline, the external os being filled with tenacious mucus.

On palpating the uterus per rectum, cyclic changes can be detected. With the development of the CL the uterus increases in tone and thickness, but these features diminish when the CL regresses. At oestrus there is no increase of tone. During anoestrus and for the first few days after ovulation the uterus is flaccid.

During dioestrus, pregnancy and pseudopregnancy the cervix is identified on rectal palpation as a narrow firm tubular structure; at oestrus it is soft and broad. A temporary pneumovagina assists in this examination (Allen, 1978).

Signs of oestrus

The mare becomes restless and irritable; she frequently adopts the micturition posture and voids urine with repeated exposure of the clitoris (Figure 1.13). When introduced to a stallion or teaser, these postures are accentuated; the mare raises the tail to one side and leans her hindquarters. The vulva is slightly oedematous, and there is a variable amount of mucoid discharge. A mare which is not in oestrus will usually violently oppose the advances of a stallion, and for this reason when 'trying' mares at stud it should be done over a gate, box-door or stout fence. If the mare is in oestrus the stallion usually exhibits 'flehmen'. Good stud management requires that a mare is accustomed to the procedure and that, because of the interval between the end of the last oestrus and the start of the next, she is teased 15–16 days after the end of the last oestrus.

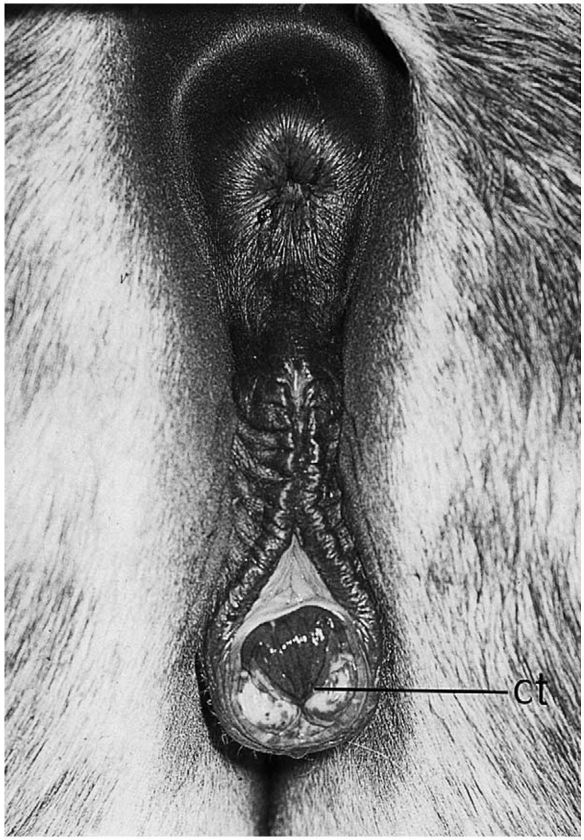


Fig. 1.13 Exposure of the clitoris (ct) in response to teasing.

Endocrine changes during the oestrous cycle

The trends in endocrine changes are shown in Figure 1.14. The secretion of FSH is biphasic with surges at approximately 10–12 day intervals. One surge occurs just after ovulation, with a second surge in mid- to late dioestrus approximately 10 days before the next ovulation. It has been suggested that this increase in FSH secretion, which is unique to the mare, is responsible for priming the development of a new generation of follicles, one of which will ovulate at the next oestrus (Evans and Irvine, 1975). The pattern of LH secretion is also unusual in this species since there is no sudden surge of this hormone but a gradual increase and persistence of elevated levels for 5–6 days on either side of ovulation. Oestrogens in the peripheral circulation reach peak values during oestrus whilst concentrations of progestrone and other progestrogens follow closely the physical changes of the CL.

THE COW

Cyclic periodicity

Under conditions of domestication, normal and well-cared-for cattle are polyoestrous throughout the year. The age at first oestrus, or puberty, is affected by nutrition and season of birth, and ranges from 7 to 18 months, with an average of 10

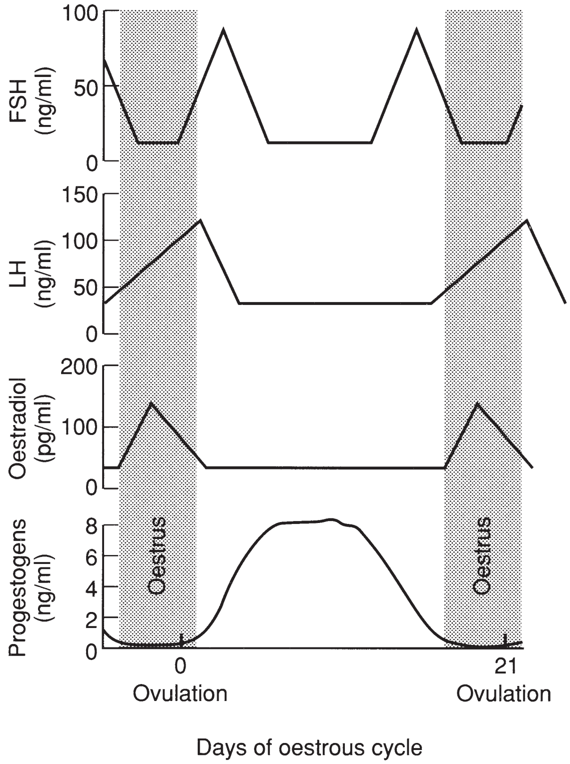


Fig. 1.14 Trends in hormonal concentrations in the peripheral circulation of the mare during the oestrous cycle.

months. A small proportion of heifers do not ovulate at the first heat, and in a majority of young cattle the oestrus associated with the first ovulation is 'silent' (Morrow et al., 1969). Poor feeding and calfhood disease delay puberty. Once puberty has been reached, cyclical activity should persist, except during pregnancy, for 3–6 weeks after calving, during high milk yield (especially if there is some evidence of dietary insufficiency), and with a number of pathological conditions (see Chapter 22). Some cows and heifers also fail to show overt signs of oestrus yet have normal cyclical activity, a condition referred to as 'silent heat' or suboestrus. This may, however, be due to failure of the herdperson to observe the signs, rather than a failure of the cow to show signs.

In heifers, the average length of the oestrous cycle is 20 days, and in cows 21 days, the normal ranges being 18–22 and 18–24 days, respectively. For many years, the average duration of oestrus has traditionally been recognised as being about 15 hours with a wide range of 2–30 hours; however, there is good evidence that in the 'modern' Holstein and Jersey cows, as compared with the heifer, the average is much shorter, perhaps an average of 8 hours. This has been shown using a radio frequency data communication system called 'Heat Watch' (Nebel et al., 1997), and is summarised in Table 1.1.

There are a number of factors which can influence the duration: breed of animal, season of year, presence of a bull, nutrition, milk yield, lactation number and, perhaps most important, the number of cows that are in oestrus at the same time (Wishart, 1972; Esslemont and Bryant, 1974; Hurnik et al., 1975). There is also good evidence that more signs of oestrus are observed during the hours of night, perhaps when the animals are least disturbed (Williamson et al., 1972; Esslemont and Bryant, 1976).

Ovulation is spontaneous, and occurs on average 12 hours after the end of oestrus.

Signs of oestrus

Where artificial insemination is used, the accurate detection of oestrus by the herdsperson is paramount in ensuring optimum fertility. Poor detection is probably the most important reason affecting delayed breeding (Wood, 1976), whilst in the USA Barr (1975) has calculated that in

Table 1.1 Duration of oestrus and number of standing events (mean and standard deviation) as determined by the 'Heat Watch' oestrus detection system (from Nebel et al., 1997)

	Nulliparous heifers		*Pluriparous cows*	
	Holstein	*Jersey*	*Holstein*	*Jersey*
No. of animals	114	46	307	128
Duration of oestrus (hours)	11.3 +/– 6.9	13.9 +/– 6.1	7.3 +/– 7.2	7.8 +/– 5.4
No. of standing events	18.8 +/– 12.8	30.4 +/– 17.3	7.2 +/– 7.2	9.6 +/– 7.4

Ohio dairy herds dairy farmers appeared to be losing twice as many days due to failure to detect heat as to conception failures.

There are great variations amongst individual cattle in the intensity of heat signs; the manifestations tend to be more marked in heifers than in cows. However, it is generally agreed that the most reliable criterion that a cow or heifer is in oestrus is that she will stand to be mounted by another (Williamson et al., 1972; Esslemont and Bryant, 1974; Foote, 1975).

The oestrous animal is restless and more active; Kiddy (1977), using pedometers, found that there was an average increase in activity of 393% at this time. More recently, Lewis and Newman (1984) found that pedometer activity was about twice as great in oestrus compared with the luteal phase of the cycle. In their study, 75% of cows showed peak pedometer readings on the day of onset of oestrus whereas 25% peaked 1 day after oestrus. There tends to be grouping of sexually active individuals; there is a reduction in the time spent eating, resting and ruminating, and frequently a reduction in milk yield. Reduced milk yield has been shown to be a reliable indicator of the onset of oestrus; there is usually a compensatory rebound at the next milking (Horrell et al., 1984). In this study of 73 dairy cows, it was found that if a cow produced 75% of her usual yield there was a 50% chance of her being in oestrus. On the rare occasions that it fell to 25%, oestrus was always present. As the cow approaches oestrus she tends to search for other cows in oestrus, and there is licking and sniffing of the perineum. During this period, during oestrus and just afterwards, the cow will attempt to mount other cows; quite often before she does this she will assess the receptivity of the other cows by resting her chin on the rump or loins. If the cow to be mounted is responsive and stands, she will mount and sometimes show evidence of pelvic thrusting (Esslemont and Bryant, 1974). If the cow that is mounted is not in oestrus she will walk away and frequently turn and butt the mounting cow. A positive mounting response lasts about 5 seconds (Hurnik et al., 1975); however, if both cows are in oestrus it will be increased to about 7.5 seconds. In a group of 60 cows, Esslemont and Bryant (1976) observed that 33 cows were mounted on average 56 times.

Sometimes there are signs of a vulval discharge of transparent mucus whose elasticity causes it to hang in complete clear strands from the vulva to the ground; it also adheres to the tail and flanks. The vulva may be slightly swollen and congested, and there is a small elevation of temperature. The tail may be slightly raised. The hair of the tail-head is often ruffled and the skin sometimes becomes excoriated due to mounting by other cows. For the same reason, the rear of the animal may be soiled with mud. At range, the oestrous cow may wander from the herd, and if isolated there will be bellowing. When she is put with a bull, the two animals lick each other and the cow often mounts the bull before standing to be mounted by him. For a short time after service, the cow stands with raised tail and arched back, and where actual service has not been seen this posture indicates that mating has occurred.

Within 2 days of service, there is an occasional yellowish-white vulval discharge of mucus containing neutrophil leucocytes from the uterus. At about 48 hours after heat, irrespective of service, heifers and many cows show a bright red sanguineous discharge, the blood coming mainly from the uterine caruncles.

The body temperature of dairy cows falls about 0.5°C the day before oestrus, increases during oestrus and falls by about 0.3°C at ovulation. The vaginal temperature, of 37.74°C, was lowest on the day before oestrus, increased by 0.1°C on the day of oestrus, and increased for the next 6 days until a plateau was reached. This was followed by a gradual decline from 7 days before oestrus (Lewis and Newman, 1984). Practical detection of this is tedious; however, the use of microwave telemetry systems may enable such measurements to be made in the future (Bobbett et al., 1977). Automated methods of measuring the related increase in milk temperature in the milking parlour have also been described (Maatie, 1976; Ball, 1977).

Vaginal pH also fluctuates throughout the oestrous cycle but is lowest, namely 7.32, on the day of oestrus (Lewis and Newman, 1984).

Cyclic changes in the vagina

The main variations are in the epithelial cells of the anterior vagina and in the secretory function of the cervical glands (Hammond, 1927; Cole, 1930). During oestrus, the anterior vaginal epithelium becomes greatly thickened due to cell division and to the growth of the tall, columnar, mucus-secreting superficial cells. During dioestrus, these cells vary from flat to low columnar. Leucocytic invasion of the vaginal mucosa is maximal 2–5 days after oestrus. Copious secretion of mucus by the cervix and anterior vagina begins a day or so before heat, increases during heat and gradually diminishes to the fourth day after heat. The mucus is transparent and flows readily.

Associated with these features of the cervical mucus are variations in its crystallisation patterns which can be seen when dried smears of mucus are examined microscopically. During oestrus, and for a few days afterwards, the crystals are disposed in a distinct aborisation pattern, while for the remainder of the cycle this pattern is absent. This phenomenon, together with the character and amount of cervical mucus, are dependent on the concentration of oestrogen. The postoestrous vaginal mucus shows floccules composed of leucocytes, and, as previously mentioned, blood is frequently present.

Hyperaemia of the mucosae of the vagina and cervix is progressive during pro-oestrus and oestrus; the vaginal protrusion of the cervix is tumefied and relaxed, so that one or two fingers can be inserted into the cervical os. During metoestrus, there is a rapid reduction in vascularity, and from 3 to 5 days after heat the mucosa is pale and quiescent and the external os is constricted while the mucus becomes scanty, sticky and pale yellow or brown. There are also cyclic variations in vaginal thermal conductance and vaginal pH, the former rising just before oestrus (Abrams et al., 1975). When pH electrodes were placed in the cervical end of the vagina the pH fell from 7.0 to 6.72 one day before the first behavioural signs of oestrus, and at the start of oestrus fell again to 6.54 (Schilling and Zust, 1968).

Cyclic changes in the uterus

During oestrus, the uterus is congested, and the endometrium is suffused with oedematous fluid; its surface is glistening. The muscularis is physiologically contractile so that when the uterus is palpated per rectum this muscular irritability, coupled with the marked vascularity, conveys a highly characteristic tonic turgidity to the palpating fingers; the horns feel erect and coiled. This tonicity is present the day before and the day after oestrus but is at its maximum during heat, and, with experience, the veterinarian can detect oestrus on this sign alone. Between 24 and 48 hours after oestrus the uterine caruncles show petechial haemorrhages, and these give rise to the postoestrous vaginal discharge of blood. In heifers there are often also associated perimetrial subserous petechiae. During dioestrus the endometrium is covered by a scanty secretion from the uterine glands.

Cyclic changes in the ovaries

Usually one follicle ovulates and one ovum is liberated after each heat, but twin ovulations occur in 4 or 5% of cows, and triplet ovulations more rarely. In dairy cattle, about 60% of ovulations are from the right ovary, although in beef cattle the functional disparity between the ovaries is not great.

The size and contour of the ovaries will depend on the phase of the cycle. It is best to begin by studying the organs of a mature unbred heifer. Post-mortem section of such ovaries will reveal the most significant structures in them to be Graafian follicles and CLs.

Follicular growth and development

Follicular growth and atresia throughout the cycle is a feature in the cow (Matton et al., 1983). In the studies of Bane and Rajakoski (1961), two waves of growth were demonstrated, with the first wave beginning on the third and fourth day, and the second starting on the 12th to 14th day of the cycle. Consequently, a normal follicle of 9–13 mm was present from the fifth to the 11th day before becoming atretic. In the second wave the ovulatory follicle developed, and was 9–13 mm between the 15th and 20th days; the ovulatory follicle is selected at about 3 days before ovulation (Pierson and Ginther, 1988). Others have observed three waves of follicular development in most oestrous cycles (Sirois and Fortune, 1988; Savio et al., 1990). The most notable feature was the regularity of the number of waves of follicular growth per oestrous cycle, which probably reflected genetic or environmental influences. Follicular growth is under the influence of FSH, with normally one follicle obtaining dominance and subsequently ovulating. The dominance does not appear to be mediated by the effect of inhibin but probably by some yet unknown intra-ovarian mechanism which does not involve the suppression of FSH secretion. In addition, other metabolic hormones such as insulin growth factor 1 (IGF-1) may also be involved in follicular growth patterns (see review by Webb et al., 1992).

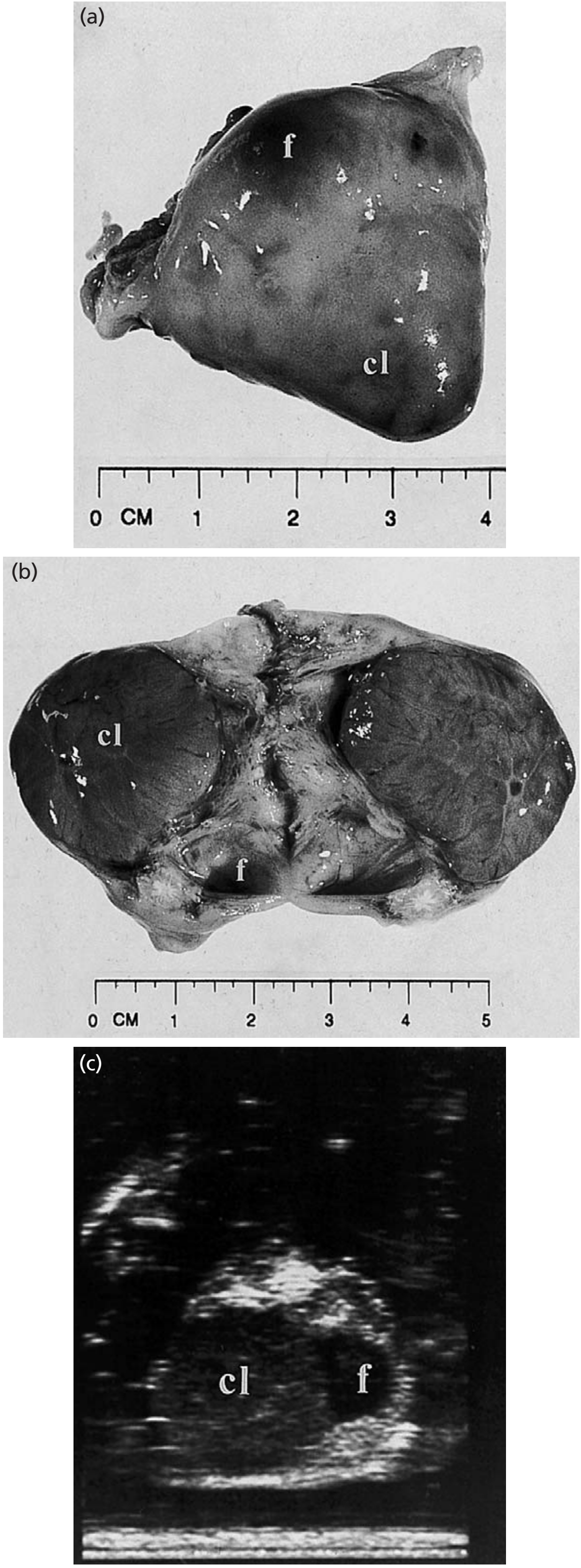
Thus, during dioestrus several large follicles will be found ranging in size up to 0.7–1.5 cm in diameter. These follicles do not alter the general oval contours of the ovaries but do cause some overall variation in gross ovarian size. The ease of palpating them rectally will depend upon the size, degree of protrusion and relationship with the corpus luteum.

During pro-oestrus and oestrus, the follicle which is soon to rupture enlarges, and ovulation occurs when it has attained a size of at least 1.9 cm (Hammond, 1927). On rectal palpation of the ovaries during heat it is usually possible to detect the ripening follicle as a slightly bulging, smooth soft area on the surface of one of them. Ovulation may occur from any aspect of the ovarian surface, and the shape of that organ subsequently when the CL develops will be chiefly influenced by this site. The point of ovulation is usually in an avascular area of the follicular wall, and consequently haemorrhage is not a feature of bovine ovulation, although there is marked post-ovulatory congestion around the rupture point, and sometimes a small blood clot is present in the centre of the new CL.

The corpus luteum of the oestrous cycle

On rupture, the ovum is expelled through a small breach in the surface of the follicle and, consequent on the escape of the greater part of its fluid, the follicle collapses. If the opportunity arose for repeatedly carrying out rectal examinations during heat and for the 24 hours succeeding it, this collapse would be detected. The ovary frequently feels flattened and soft. If such an ovary is examined post-mortem it will be seen that the surface from which ovulation has occurred is wrinkled and possibly bloodstained. The CL develops by hypertrophy and luteinisation of the granulosa cells lining the follicle. Enlargement is rapid. By the 48th hour after ovulation it has attained a diameter of about 1.4 cm. At this stage the developing CL is soft, and yields on palpation. Its colour is dull cream, and the luteinised cells can be seen in the form of loose pleats. The CL attains its maximum size by the seventh to eighth day of dioestrus (Figure 1.15). The luteinised pleats are now relatively compact, and the body comprises a more or less homogeneous mass, yellow to orange-yellow in colour. Its shape varies; the majority are oval, but some are irregularly square or rectangular. The greatest dimension of the fully developed structure varies from 2.0 to 2.5 cm; the changes in the dimensions of the CL are shown in Figure 1.16. Its weight also varies; in the authors' series, fully developed CLs have varied from 4.1 to 7.4 g. (Similar variations also occur in the weights of the CLs of pregnancy, ranging from 3.9 to 7.5 g.) Sometimes, the centre of the yellow body is occupied by a cavity (Figures 1.17 and 1.27). This has been seen in 25% of those collected by the author. The size of the cavity varies; in the majority it is small, averaging 0.4 cm in diameter, but occasionally it is large, up to 1 cm or more. It is occupied by yellow fluid. In the case just described, there is evidence of ovulation by the presence of a pin-head depression in the centre of the projection from the surface of the ovary. This serves to differentiate the CL from the abnormality of the cow's ovary: luteinisation of the walls of the follicle without ovulation. Nevertheless, it is probable that this is the condition which has been described in the past as cystic corpus luteum and regarded as pathological; the presence of a cavity is normal.

Fig. 1.15 Ovary of cow in mid-dioestrus. (a) A mature corpus luteum (cl) with ovulation papilla could be readily palpated together with a mid-cycle follicle (f). (b) Section of the same ovary showing the solid corpus luteum (cl) and mid-cycle follicle (f). (c) B-mode ultrasound image of the same ovary showing a speckled area corresponding to the corpus luteum (cl) and the mid-cycle follicle (f).



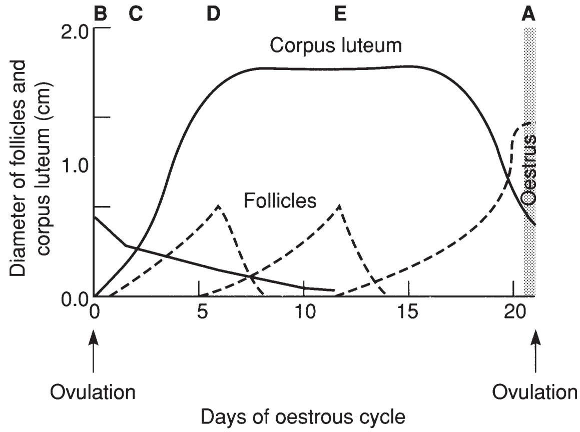


Fig. 1.16 The development of follicular waves (- - - -) and corpus luteum (——) during the oestrous cycle of the cow. (A–E refers to Figures 1.18–26.)

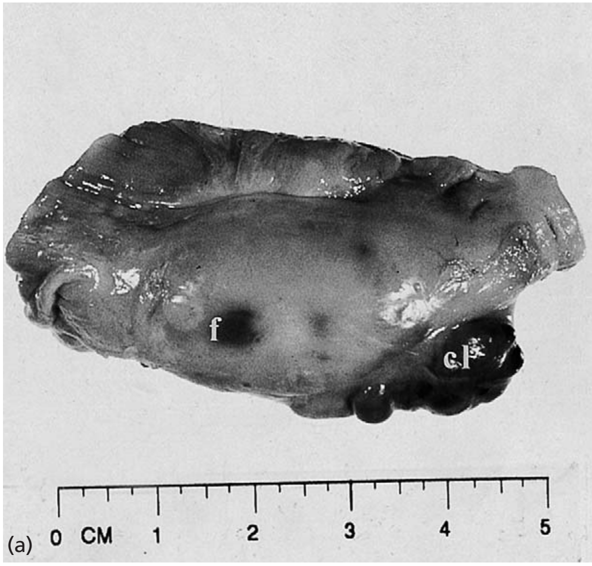
Projection of the corpus luteum from the surface of the ovary

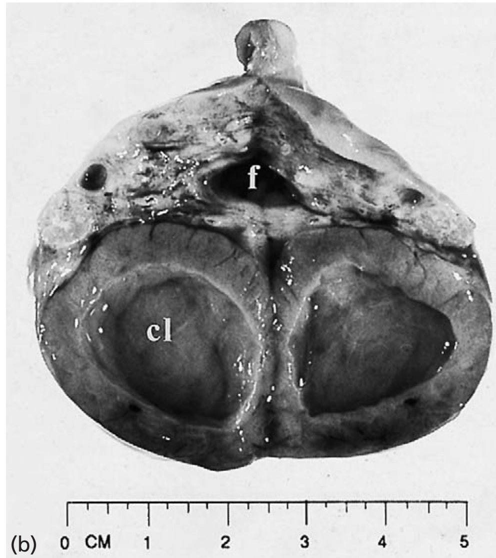
As the CL enlarges, it tends to push itself out of the ovary, stretching the surface of the latter, until by the time it attains maximum development it often forms a distinct projection. The degree and type of this projection vary. In the majority it is a distinct bulge about 1 cm in diameter with a clear-cut constriction where it joins the general contour of the ovary. In other cases it is nipple-like (Figure 1.15). In a third type the projection is indistinct but diffuse and occupies the greater part of the ovary. It would seem that the type of protrusion which develops depends on the extent of the surface of the ovary occupied by the follicle just before ovulation. Figures 1.18–1.26 show bovine ovaries* (natural size) during the oestrous cycle.

Regressing corpus luteum

The CL maintains its maximum size and remains unaltered in appearance until the onset of pro-oestrus, i.e. 24 hours or so before the onset of

*Throughout the book, sketches of bovine ovaries are of a section from the attached to the free border through the poles. In those cases in which this section did not pass through the greatest dimension of the significant corpus luteum or the largest follicle, the sketch has been made as though it did so but without materially altering the size of the ovary.





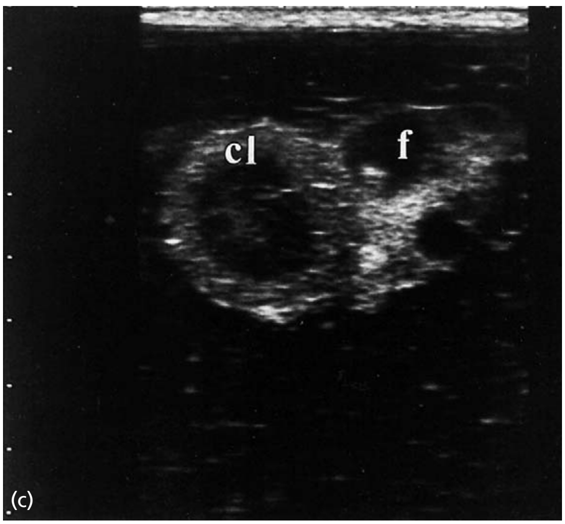


Fig. 1.17 Ovary of cow in mid-dioestrus. (a) A mature corpus luteum (cl) with prominent ovulation papilla and mid-cycle follicle (f). (b) Section of the same ovary showing the corpus luteum (cl) with a central lacuna which was filled with orange-yellow fluid, and the mid-cycle follicle (f). Note that the 'wall' of the corpus luteum comprises at least 5 mm thickness of luteal tissue. (c) B-mode ultrasound image of the same ovary showing a speckled area corresponding to the 'wall' of the corpus luteum (cl), the central lacuna, and also the mid-cycle follicle (f).

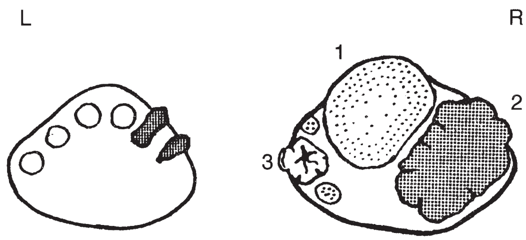


Fig. 1.18 Ovaries of a first-calf heifer in oestrus. 1, ripe follicle; 2, regressing corpus luteum, bright yellow; 3, corpus albicans. Stage A in Figure 1.16.

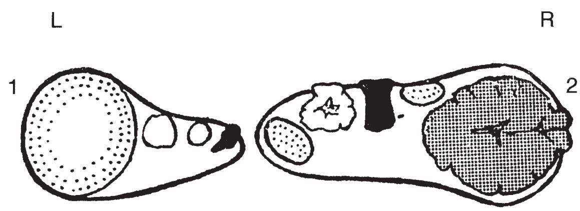


Fig. 1.19 Ovaries of a first-calf heifer in oestrus. 1, ripe follicle; 2, regressing corpus luteum, brick-brown. Stage A in Figure 1.16.

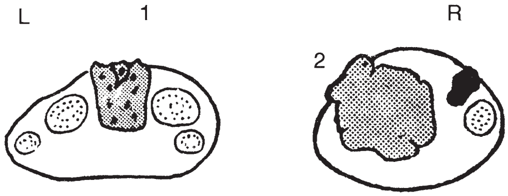


Fig. 1.20 Ovaries of a nulliparous heifer just after ovulation. 1, collapsed follicle, surface wrinkled and blood-stained petechiae in wall; 2, regressing corpus luteum, bright yellow. Stage B in Figure 1.16.

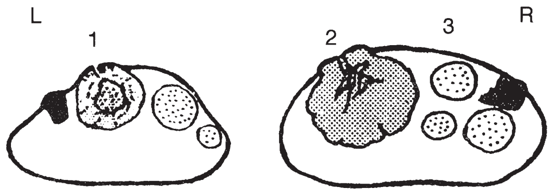


Fig. 1.21 Ovaries of a young cow 1 day after ovulation. 1, developing corpus luteum, pleats loose, colour pale cream, central cavity; 2, regressing corpus luteum, bright orange-yellow; centre filled by connective tissue; 3, corpus albicans. Stage B in Figure 1.16.

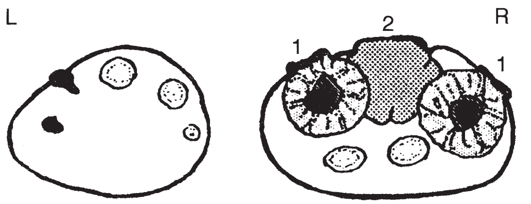


Fig. 1.22 Ovaries of a young cow 2 days after ovulation. 1, twin corpora lutea, some haemorrhage; 2, regressing corpus luteum, bright yellow. Stage C in Figure 1.16.

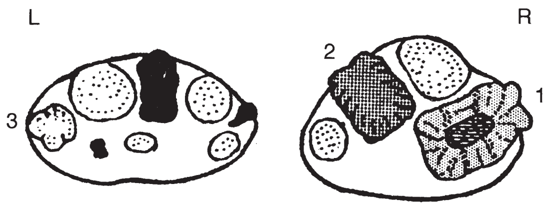


Fig. 1.23 Ovaries of a 4-year-old cow in early dioestrus. 1, active corpus luteum, pleats loose, colour orange-yellow, central cavity; 2, regressing corpus luteum, dense and brown; 3, corpus albicans. Stage C in Figure 1.16.

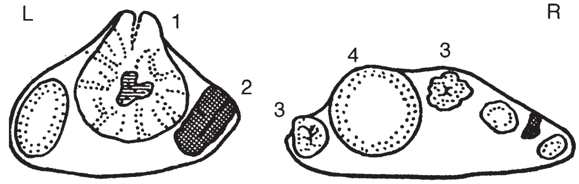


Fig. 1.24 Ovaries of a 6-year-old cow in early dioestrus. 1, active corpus luteum, orange-yellow, atypical protrusion; 2, regressing corpus luteum, small, shrunken, scarlet; 3, corpus albicans; 4, follicle. Stage D in Figure 1.16.

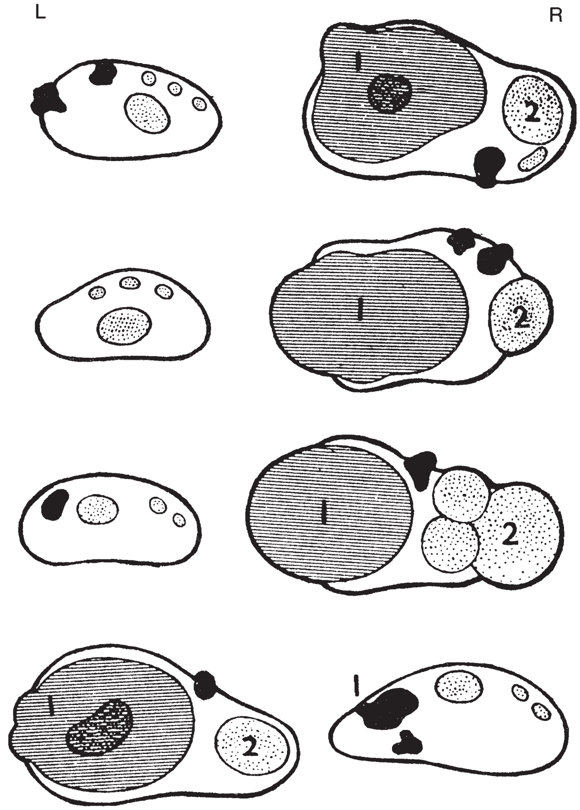


Fig. 1.25 Ovaries of nulliparous heifers in dioestrus. 1, corpus luteum 2, largest follicle. Stage E in Figure 1.16.

heat. From this point, it undergoes rapid reduction in size and changes in colour and appearance. By the middle of oestrus, its diameter is reduced to 1.5 cm and its protrusion is much smaller and less distinct, while its colour is changing to bright yellow. (This colour contrasts strikingly with that of the active body.) Its consistency is dense, and already scar tissue invasion is commencing. By the second day of dioestrus, its size is reduced to about 1 cm and its outline is becoming irregular.

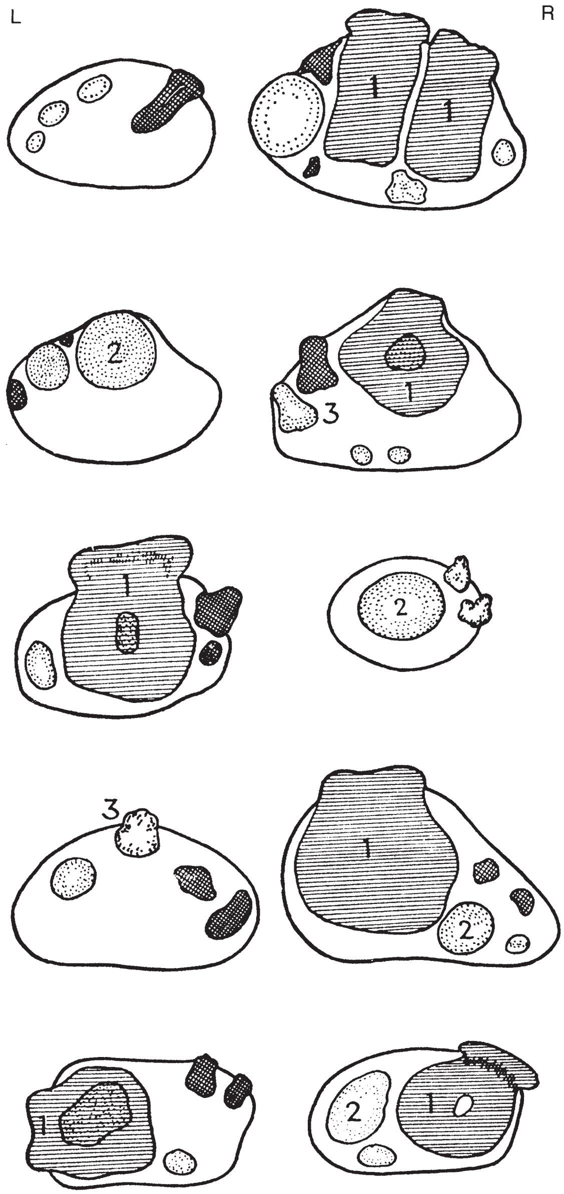


Fig. 1.26 Ovaries of parous cows in dioestrus. 1, corpus luteum; 2, largest follicle; 3, corpus albicans. Stage E in Figure 1.16.

By this time its colour is changing to brown. By the middle of dioestrus, it has shrunk to a size of about 0.5 cm, and its surface protrusion is little larger than a pin-head. As it gets older its colour tends to change to red or scarlet. Small red remnants of corpora lutea tend to persist for several months.

Size of the ovaries

From the foregoing account, it will be appreciated that the size of an ovary will depend chiefly on the period in the oestrous cycle at which it is examined and whether or not it contains an active CL. The presence of follicles does not alter the size of an ovary to anything like the same extent. In the great majority of heifers and young cows examined at any time between the sixth and 18th days of the dioestrous period, one ovary will be distinctly larger than the other. The approximate dimensions of the larger one will be 3.5 cm from pole to pole, 3 cm from the attached to the free border and 2.8 cm from side to side. (All ovarian dimensions given in this book are in this order.) From some part of its surface the CL will project. The smaller ovary will have approximate dimensions of 2.5 by 1.5 by 1.2 cm, and it will be flat from side to side. During the first 4 or 5 days of the interoestrus phase there will be relatively little variation in size, for as yet the developing CL has not attained sufficient bulk to influence the size of the ovary significantly, while the regressing CL has lost its significant bulk. During oestrus, also, there will be little difference in size. If the ovary which contains the follicle undergoing preovulation enlargement also contains the regressing CL (and this is often the case), the ovary containing the two structures will be a little larger than the other, but not strikingly so.

Ovaries of the multiparous cow

The ovaries of the normal multiparous cow do not differ greatly from those of the heifer or first calver. They tend, however, in many cases to be larger. This increase in size is due in part to the progressive deposition of scar tissue resulting from prolonged function, and in some cases also to the presence of large numbers of small but visible follicles. Not infrequently, the ovary that does not contain a CL measures 4 by 3 by 2 cm. Nevertheless, it is generally possible in mid-dioestrus to detect the CL, for, quite apart from its protrusion, the ovary containing it is plum-like, whereas the other is distinctly flattened from side to side. On section of such ovaries, the CLs, both active and regressing, and the follicles approaching maturity are identical with those described for the heifer. There is, however, an additional structure to be recognised: old scarred CLs of previous pregnancies. They generally show as a white, pin-head-sized projection on the surface of the ovary, and on section are found to comprise mainly scar tissue. They are irregular in outline, with a maximum dimension of about 0.5 cm. Their colour is white (corpus albicans) or brownish-white. The CL of pregnancy does not atrophy after parturition as quickly as does that of the oestrous cycle after it has ceased to function. It is an appreciable structure for several weeks after parturition, brown in colour and about 1 cm in diameter. It becomes progressively invaded by scar tissue and remains throughout the cow's life. On post-mortem the presence of the corpus albicans serves to distinguish the cow from the heifer and in the former a count of the corpora albicantia gives the number of calves borne.

The fully developed corpus luteum is present by the seventh day and persists unchanged until the onset of pro-oestrus at the 19th or 20th day. Figure 1.27 shows exceptional corpora lutea.

Ultrasonic appearance of the ovaries

In the previous sections of this chapter there are descriptions of the texture (as determined by palpation) and the appearance (as determined by sectioning after slaughter) of the ovaries and their contents. The advent of transrectal B-mode real-time grey scale ultrasound imaging, particularly using a 7.5 MHz transducer, has enabled detailed, accurate sequential examination of the ovaries to be made without adversely affecting the cow's health or fertility. The principles of the technique are described in Chapter 3, and for a detailed description of the echogenic appearance of the ovaries, readers are advised to consult Pierson and Ginther (1984) and Boyd and Omran (1991).

The following normal structures can be identified (see Figures 1.15 and 1.17): the ovarian stroma, antral follicles, CLs and ovarian blood vessels. In addition, pathological structures such as ovarian cysts can be seen (see Chapter 22). The ovarian stroma has a mottled echotexture. The antral follicles are readily identifiable as anechoic (black) structures of variable size, with a clearly defined line of demarcation between the follicular wall and antrum. Follicles will not always be regular and spherical in shape. CLs have a well-defined border and a mottled echogenic appearance which is less echogenic than the ovarian stroma; the presence of a fluid-filled lacuna can be readily identified as a dark, non-echogenic area in the centre. Differentiation between developing and regressing CLs can be difficult. Ovarian blood vessels, which can be confused with antral follicles, are black, non-echogenic structures. Movement of the transducer will usually demonstrate their elongated appearance.

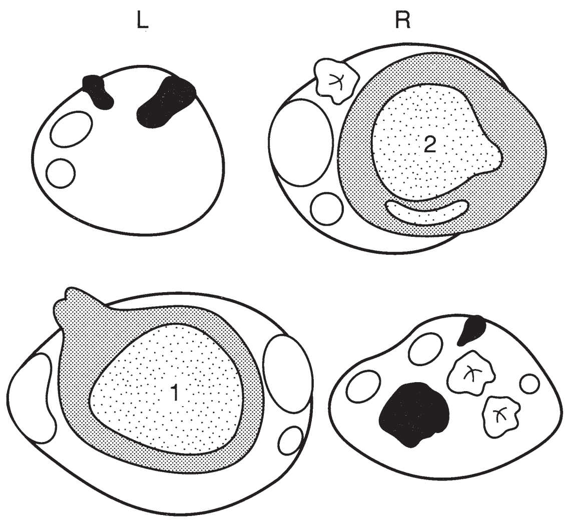


Fig. 1.27 Examples of vacuolated or cavitated bovine corpora lutea, showing single (1) and sometimes multiple (2) cavities.

Endocrine changes during the oestrous cycle

The trends in concentrations of reproductive hormones in the peripheral circulation are illustrated schematically in Figure 1.28; it is important to stress that hormones are secreted in a pulsatile manner and fluctuate considerably. An effective description is given by Peters (1985a,b). Just before the onset of behavioural oestrus, there is a sudden rise in plasma oestrogens, particularly oestradiol. Peak values occur at the beginning of oestrus with a subsequent decline to basal concentrations at the time of ovulation. During the rest of the cycle, there are fluctuations in concentrations, although there

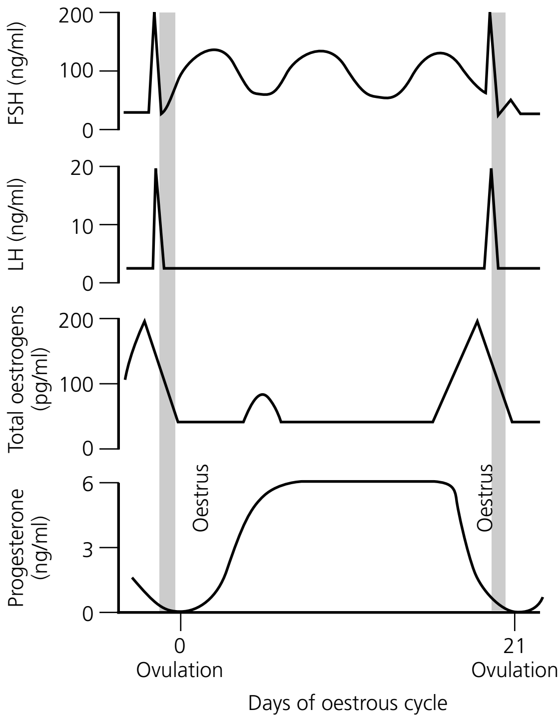


Fig. 1.28 Trends in hormone concentrations in the peripheral circulation of the cow during the oestrous cycle, with three follicular waves.

is a discrete peak around day 6 of the cycle (Glencross et al., 1973) which may be related to the first wave of follicular growth (Ireland and Roche, 1983). The pre-oestrus rise in oestrogens stimulates the surge of LH from the anterior pituitary which is necessary for follicular maturation, ovulation and CL formation. A secondary less discrete peak has been demonstrated 24 hours after the ovulatory surge of gonadotrophin (Dobson, 1978).

The changes in progesterone concentrations mimic closely the physical changes of the CL. In a number of cows there is evidence of a delay in progesterone production or secretion by the CL (Lamming et al., 1979) which does not appear to affect the fertility of the individual adversely. Peak values are reached by days 7 and 8 after ovulation, and decline fairly quickly from day 18. When progesterone values fall to fairly low basal levels the removal of the anterior pituitary block allows the sudden release of gonadotrophins. Prolactin values are frequently difficult to obtain since stress induced by restraint for venepuncture is sufficient to cause a significant rise.

THE EWE

The sexual season of most breeds of sheep in Britain is from October to February, during which time there are 8–10 recurrent oestrous cycles. The stimulus for the annual onset of sexual activity is declining length of daylight. The extent of the breeding season diminishes with increase of latitude; thus at the equator ewes may breed at any time of year, whereas in regions of high latitude – in both northern and southern hemispheres – the breeding season is restricted and distinct, with a prolonged phase of anoestrus after parturition. The breed of ewe also influences the duration of the breeding season; for example, in Dorset Horns it is distinctly longer than in other breeds – whereby three crops of lambs can be obtained in 2 years – but in hill breeds, like the Welsh Mountain and Scottish Blackface, it is shorter. Local breeds of central Europe and the Merino in Australia may not show an annual anoestrus. Also, in ‘ordinary’ breeds in Britain, like the Clun Forest, which normally show a distinct seasonal activity, isolated instances of successful mating may occur in every month of the year (Lees, 1969). Ewe-lambs and yearling ewes have shorter breeding seasons than older ewes. The seasonal onset of sexual activity can be advanced by artifical manipulation of the photoperiod and by the use of hormonal agents (described later in this chapter).

The average duration of oestrus in mature ewes of British breeds is about 30 hours, and is at least 10 hours less in immature ewes. In Merinos, heat may last 48 hours. Ovulation occurs towards the end of oestrus, and the length of the oestrous cycle averages 17 days.

Signs of oestrus and mating behaviour

Oestrous ewes are restless. They seek the ram, and together form a following ‘harem’. The ram ‘tries’ members of this group for receptivity by pawing with a forefoot, by rubbing his head along the ewe’s side and by nipping her wool. A non-receptive ewe trots away, but when in full heat she stands, waggles her tail and moves it laterally. The vulva is slightly swollen and congested, and there is often a slight discharge of clear mucus. The ram mounts and makes a series of probing pelvic thrusts and then dismounts. After variable intervals further mounts occur before intromission is achieved, and this is characterised by a deep pelvic thrust. An essential feature of successful coitus in the Najdi and Awassi breeds of the Middle East is the lateral displacement by the ram of the fat tail of the ewe. Rams of other breeds are unable to move the tail sufficiently to perform intromission.

More ovine matings occur during early morning and evening. When several rams run with a flock, a hierarchy is established and the dominant male attracts the largest harem, but, despite this, a majority of ewes mate with more than one ram. Also, ewes show a preference for rams of their own breed or for a ram of their particular group if that group is mixed with other groups of different origin (Lees and Weatherhead, 1970). The number of services received by an oestrous ewe averages a little above four. Rams may serve from eight to 38 ewes in a day.

Changes in the ovaries

The ovaries of the ewe are smaller than those of the cow, and their shape is nearer the spherical. During anoestrus their size is approximately 1.3 by 1.1 by 0.8 cm, and the largest follicles present vary from 0.2 to 0.6 cm. Studies of folliculogenesis are more difficult than in the cow or mare because transrectal ultrasonic sequential access to the ovaries is much more difficult. However, using either ultrasonography or laparoscopy evidence suggests that the ewe is similar to the cow, usually with three or four follicular waves in each oestrous cycle; if there are three then there are two during the luteal and one during the follicular phases (Noel et al., 1993; Leyva et al., 1995). There is less certainty about the mechanisms involved in the emergence of the dominant follicle(s). Even during anoestrus, folliculogenesis occurs with follicles reaching the size of those frequently present during cyclical activity (Bartlewski et al., 1995).

At the onset of oestrus, one or more follicles will have attained a size of 1 cm. Their walls are thin and transparent and the liquor folliculi appears purple in colour. Grant (1934) has observed that rupture of the follicle is preceded by the elevation of a small papilla above the general surface; ovulation occurs through rupture of this papilla about 24 hours after the onset of heat. The development of the CL is rapid, being linear from day 2 to day 12 after ovulation (Jablonka-Shariff et al., 1993); by the fifth day of dioestrus it is 0.6 cm in diameter, and it attains its maximum size of 0.9 cm when it has a central cavity (Roux, 1936). As the dioestrous period advances, its colour changes from blood red to pale pink. Its size remains constant until the onset of the next oestrus, when regression is rapid and the colour changes, first to yellow and later to brownish yellow. The luteolytic mechanism is similar to that in the cow where at the end of dioestrus, due to the effect of oestradiol and progesterone, there is an increase in the number of uterine oxytocin receptors, and at the same time luteal oxytocin stimulates uterine $PGF_{2\alpha}$ secretion and vice versa (Mann and Lamming, 1995). The first CLs formed after the first ovulations at the beginning of the breeding season have a shorter life span than subsequent ones. In twin ovulations the CLs may occupy the same or opposite ovaries. During pregnancy, the CL is 0.7–0.9 cm in diameter. Its colour is pale pink, but the central cavity has disappeared, having become filled by white tissue.

Ovulation with CL formation, but without signs of heat, may occur during the so-called anoestrous period – spurious ovulation (Grant, 1934). As to the number of ova shed at oestrus, genetic and nutritional factors play a part. Hill sheep in the UK generally have one lamb, but if they are transferred to lowland pastures where herbage is rich (before the onset of the breeding season), twins become common. With lowland breeds the general expectancy is an average of 1.5 lambs per ewe. Roux (1936) in South Africa has noted that age is also a factor in the incidence of twinning. It attains its maximum when ewes are 5–6 years old, after which it remains constant. Primiparous ewes are very much less likely to have twins than pluriparous ones. Ewes of the Border Leicester and Lleyn breeds are the most prolific of British sheep, and commonly bear triplets. The Finnish Landrace and Cambridge breeds normally produce two to four lambs per pregnancy.

Endocrine changes during the oestrous cycle

The endocrine changes are shown in Figure 1.29. Just before the onset of oestrus there is a rise in oestrogens in the peripheral circulation, particularly oestradiol-17β. This is followed by a sudden surge of LH which reaches a peak about 14 hours before ovulation; coincidental with this peak is a rise in FSH. There is also a second FSH peak 2 days after ovulation.

Progesterone concentrations follow closely the physical changes of the corpora lutea, but maximum values are lower than those of the cow, i.e. 2.5–4 ng/ml. Prolactin fluctuates throughout the oestrous cycle; however, concentrations rise during oestrus and ovulation, presumably reflecting the role of this hormone in the formation of the CLs.

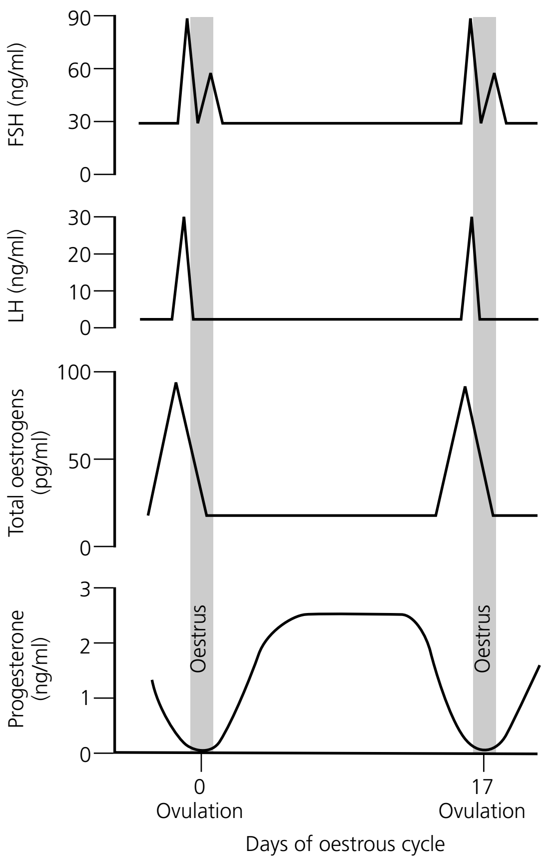


Fig. 1.29 Trends in hormone concentrations in the peripheral circulation of the ewe during the oestrous cycle.

THE DOE (NANNY) GOAT

The breeding season in Britain is from August to February with the greatest activity in the months of October, November and December. Near the equator, there is no evidence of seasonality but continuous cyclic activity. The doe is polyoestrous, with an interoestrus interval of 20–21 days, although it is rather irregular at the beginning of the breeding season. The duration of oestrus is 30–40 hours, with ovulation occurring 12–36 hours after the onset.

The detection of heat in a doe is difficult in the absence of a male goat. The vulva shows some evidence of oedema and hyperaemia; the tail is wagged from side to side and up and down (the most reliable sign according to Llewelyn et al., 1993); the doe is restless and more vocal, has a reduced appetite and milk yield, and shows mounting behaviour. The presence of the pheromones from the male goat, which can be transferred from the scent gland on to a cloth, will often intensify the signs.

The ovaries are of variable shape, depending upon the structures which are present, the longest dimension being about 2.2 cm. The largest follicles reach a maximum size of about 1.2 cm in diameter, and when they protrude from the surface often have a bluish tinge. There are few studies on follicular dynamics, however Ginther and Kot (1994), using ultrasonography, reported that there were four waves of growth, with ovulation occurring during the fourth wave. The corpora lutea are pink in colour.

The endocrine changes measured in the peripheral circulation are very similar to those of the ewe.

THE SOW

Puberty in gilts is reached at about 7 months, but diet, breed (including the degree of in-breeding) and season of birth influence its onset. At the first oestrus the number of ovulations is low, but it increases thereafter so that if mating is delayed until the third heat a larger litter will result. The cross-breeding of in-bred lines increases the ovulation rate, as does the provision of a high-energy diet for 11–14 days before the expected oestrus; continuing such a diet after mating, however, increases embryonic loss. Fecundity is best from the fourth to seventh gestations.

Although the domestic sow is generally considered to be polyoestrous, the wild pig is a seasonal breeder, the main period being late autumn with a second peak of activity in April (Claus and Weiler, 1985). There is some evidence of the influence of photoperiod on reproduction in the domestic sow; for example, anoestrus is more prevalent in summer, and to a lesser extent February and March, whilst the ovulation rate is lower in summer. Claus and Weiler (1985) found that by reducing the day length artificially from May to August they were able to decrease the interval from weaning to oestrus from 23.6 to 5.7 days. For the most part, the recurrent reproductive cycles of 21 days are interrupted by pregnancy and lactation. The average length of oestrus is 53 hours, with spontaneous ovulation occurring between 38 and 42 hours from the beginning (Signoret, 1971). During lactation, the physical stimuli of suckling suppresses cyclical activity, but many sows show an anovulatory oestrus 2 days after farrowing. When weaning occurs at 5 or 6 weeks, oestrus can be expected in 4–6 days. Earlier weaning results in a slightly longer time interval.

Signs of oestrus

Beginning 3 days before oestrus, the vulva becomes progressively swollen and congested; these features persist throughout oestrus and gradually subside during the 3 days afterwards. Restlessness is an unfailing sign of the approach of heat, and a peculiar repetitive grunt is emitted. With other sows the pro-oestrous animal sniffs their vulvae and may try to ride them, or will be the recipient of such attentions. The boar is sought, and in his presence the pro-oestrous female noses his testicles and flanks and may mount him but will refuse to be mounted. At the height of oestrus the sow assumes a stationary, rigid attitude with her ears cocked, and she appears to be quite oblivious to her environment. She generally remains still during coitus, which lasts an average of 5–7 minutes, but when mating with heavy boars gilts may become fidgety. Burger (1952) demonstrated that oestrus could readily be determined by firmly pressing the loin of the sow with the palms of both hands; the oestrous sow will stand motionless with cocked ears whereas sows not in heat will object to this approach. The same immobilisation response can be elicited if the attendant sits astride the sow, and it can also be obtained in the absence of a boar by reproducing the voice or odour of the boar. The substance responsible for boar odour has been identified as 5α-andost-16-ene-3-one and it is secreted by the salivary glands. In the form of an aerosol it can be sprayed in the vicinity of sows to promote the standing reaction of oestrus. 'Silent heats' occur in about 2% of porcine cycles.

Cyclic changes in the ovaries

The ovaries of the mature cyclical sow are relatively large and mulberry-like, the surface lobulation being due to the elevations of the large follicles and CLs, which, when mature, attain diameters of 0.8–1 cm and 1–1.3 cm, respectively. It is much more difficult to study follicular dynamics in the sow compared with the cow and mare; firstly, there are the problems of identifying an individual follicle amongst a large number, and secondly, because until the recent development of small transrectal B-mode ultrasound transducer probes, it has been almost impossible to study changes sequentially in the same animal. It has been shown that, except during the follicular phase of the cycle, there is continuous proliferation and atresia of follicles so that there is generally a pool of about 50 between 2 and 5 mm in diameter. Between about day 14 and day 16 of the cycle, there is recruitment of follicles, probably under the influence of gonadotrophin stimulation induced because of the decline in progesterone and the withdrawal of the negative feedback effect. A substantial number of these follicles are destined for ovulation, although it is not possible to identify the pre-ovulatory population until day 21–22 of the oestrous cycle. The growth of selected pre-ovulatory follicles during the follicular phase is associated with rapid atresia of small follicles, and a block to their replacement in the proliferating pool, thus indicating some intra-ovarian control mechanism (Foxcroft and Hunter, 1985). The precise nature of the substances is not fully understood, although various substances have been proposed: steroids, growth factors (epidermal/transforming, fibroblast and insulin-like growth factors) and their related binding proteins, and other regulatory substances such as 'follicle regulatory protein' (Hunter and Picton, 1999). The ripe follicle is sea-shell pink colour with a fine network of surface blood vessels and a very transparent focus which indicates the site of imminent ovulation. Haemorrhagic follicles are common. After ovulation there remain a considerable number of follicles of about 0.4 cm, some of which gradually enlarge to 0.9 cm by the succeeding day 18.

Immediately after ovulation, the ruptured follicle is represented by a congested depression, but the accumulation of blood clot soon gives it a conical shape. By day 3, its cavity is filled with dark red blood clot, which by day 6 is replaced by a connective tissue plug or by a slightly yellow fluid; clots may persist up to day 12 and fluid up to day 18. The CLs attain their maximum size at 12–15 days, after which they gradually regress to the next oestrus. They are dark red up to day 3 but then change to, and remain, wine red until day 15. As the CLs regress between days 15 and 18 the colour changes rapidly from wine red to yellow, creamy yellow or buff. The mechanism of luteolysis is not fully understood in the sow, for although $PGF_{2\alpha}$ is secreted by the uterus as early as 12–16 days of the cycle, which is earlier than in other species (Bazer et al., 1982); the CLs are unresponsive to exogenous $PGF_{2\alpha}$ until about 12–23 days after ovulation. During the process of luteolysis, the CLs are invaded by macrophages which produce tumour necrosis factor (TNF); this substance in association with $PGF_{2\alpha}$ probably causes luteolysis (Wuttke et al., 1995). In addition, there is also a suggestion that TNF inhibits oestradiol production, thereby removing a luteotrophic source.

There is further rapid regression of the CLs at the next oestrus, but throughout the succeeding dioestrus the CLs remain as distinct entities; after this they regress sharply to grey pin-head foci. When, therefore, a cyclic sow is slaughtered in the first half of dioestrus the ovaries may show the wine-coloured CLs of the current cycle, also the smaller pale CLs of the previous cycle and the grey specks of the third-generation CLs. During the luteal phases of the cycle, oestrogens are very luteotrophic; as a result it is possible to prolong the life span of the CLs for several weeks, resulting in a pseudopregnancy (Dzuik, 1977).

Endocrine changes during the oestrous cycle

The endocrine changes are shown in Figure 1.30. Oestrogens in the peripheral circulation start to rise at the time that the CLs begin to regress, reaching a peak about 48 hours before the onset of oestrus. The ovulatory LH peak occurs at the beginning of oestrus and 8–15 hours after the oestrogen peak; values remain low and fluctuate throughout the rest of the cycle. FSH concentrations vary considerably; however, there appears to

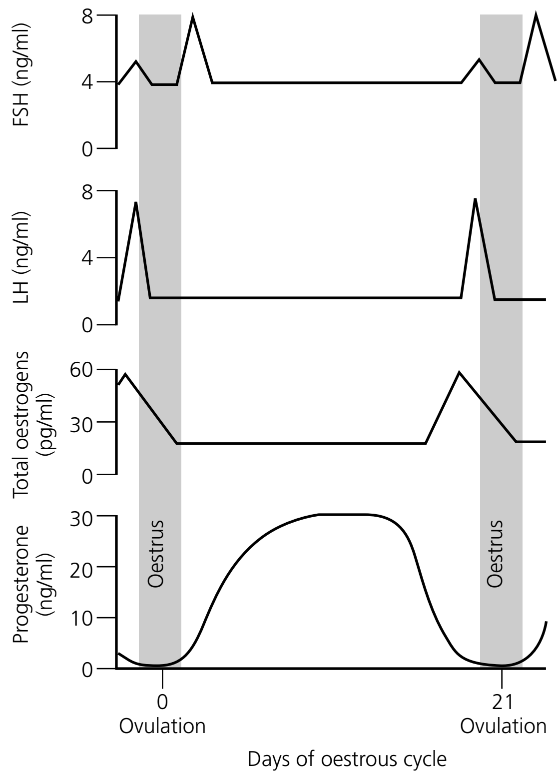


Fig. 1.30 Trends in hormone concentrations in the peripheral circulation of the sow during the oestrous cycle.

be some pattern of secretion. Brinkley et al. (1973) demonstrated two surges, one concurrent with the LH peak and a larger one on day 2 or 3 of the cycle; Van de Wiel et al. (1981) found a similar pattern, the peak coinciding with the minimum value of oestradiol. As with other species, the progesterone concentrations closely follow the physical changes of the CLs. For the first 8 days after ovulation there is a good correlation between progesterone levels and the number of CLs; however, by 12 days it is less obvious (Dzuik, 1977).

Two prolactin surges have been identified (Brinkley et al., 1973; Van de Wiel et al., 1981), the first one concomitant with the pre-ovulatory LH and oestrogen surges and a second during oestrus.

THE BITCH

Reproductive activity in the bitch differs from the polycyclic pattern of other species in that there are no frequent, recurring periods of heat. All bitches have a prolonged period of anoestrus or sexual quiescence between successive heats irrespective of whether they have been pregnant or not; this pattern has been described as monocyclic (Jöchle and Andersen, 1977).

The average interval between successive oestrous periods is 7 months, but it is variable, and there is some evidence that the breed of the bitch can have an effect. For example, for rough collies it is 37 weeks and for the German shepherd 26 weeks (Christie and Bell, 1971); other breeds that were studied had mean intervals between these two figures. Mating does not appear to influence the interval, although pregnancy caused a slight increase (Christie and Bell, 1971). There does not appear to be any seasonal effect on reproductive function since there is a fairly even distribution of the occurrence of oestrus throughout the year (Sokolowski et al., 1977).

The oestrous cycle is traditionally divided into four phases (Heape, 1900).

Pro-oestrus. The bitch has a true pro-oestrus characterised by the presence of vulval oedema, swelling and a sanguinous discharge. Some fastidious bitches show no evidence of discharge as they are continually cleaning the perineum. The bitch is attractive to males but will not accept the male.

Oestrus. The bitch will accept the male and adopts the breeding stance. The vulva becomes less oedematous and the vulval discharge becomes clearer, less sanguinous and less copious.

The duration of pro-oestrus and oestrus combined is about 18 days, i.e. 9 days each. However, this can be very variable, some bitches showing very little sign of pro-oestrus before they will accept and stand for the dog and others producing a copious sanguinous discharge during true oestrus. Some bitches also show evidence of sire preference, which can affect the timing. Ovulation usually occurs 1 or 2 days after the onset of oestrus, although, using laparoscopy, it has been observed that some follicles continue to ovulate up to 14 days later (Wildt et al., 1977).

Metoestrus. This stage starts when the bitch ceases to accept the dog; however, there is dispute about its duration. Some consider that it ends when the corpora lutea have regressed at 70–80 days whilst others measure it in relation to the time taken for repair of the endometrium, 130–140 days.

Anoestrus. At the end of metoestrus the bitch passes into a period of anoestrus without any external signs. The same is also true after parturition following a normal pregnancy. This phase lasts about 3 months before the bitch returns to pro-oestrus.

Signs of oestrus

The first indication that pro-oestrus is approaching is the onset of slight swelling of the vulval lips. This generally precedes the commencement of bleeding by several days. Labial swelling is progressive during the pro-oestrus period. Bleeding attains its maximum early in pro-oestrus and continues at this level into the early part of true oestrus. During the greater part of pro-oestrus the bitch, although attractive to the dog, takes no interest in his attentions. She will not stand for him and generally attacks him if he attempts to mount her. A day or so before the end of pro-oestrus, her attitude changes, and she shows signs of courtship towards the male. These comprise sudden darting movements which end in a crouching attitude with her limbs tense and her face alert. She barks invitingly, but as the dog approaches she moves suddenly again. She will not yet allow him to mount. With the onset of oestrus the invitation to coitus is obvious. She stands in the mating position with her tail slightly raised or held to one side. She remains still while the male mounts and copulates. In the later stages of the copulatory tie, which occupies from 15 to 25 minutes, she becomes restless and irritable and her attempt to free herself may cause the male considerable physical embarrassment. After the first 2 days of oestrus, sexual desire gradually recedes, but with the continued persuasion of the male she will accept coitus until the end of the period. Bleeding, although reduced in amount, generally continues well into oestrus. More often, however, the discharge becomes yellow as oestrus proceeds. Vulval swelling and tumefaction are greatest at the onset of the stage of acceptance. During the course of oestrus the enlarged labia become softer in consistency. Some labial swelling continues into the first part of the metoestrous phase.

Changes in the vagina and uterus

Vaginal smear

The cyclical changes which occur in vaginal cytology have been described in detail (Griffiths and Amoroso, 1939; Hancock and Rowlands, 1949; Schutte, 1967; Rozel, 1975). Vaginal smears stained with either a simple stain such as Leishman's or a trichrome stain such as Shorr's can, with practice, be used to determine the stage of the oestrous cycle. The advent of the 'Diff-Quik' staining method has greatly simplified the procedure. At the onset of pro-oestrus, large numbers of erythrocytes are present; however, when true oestrus occurs the number of erythrocytes is reduced, and the smear consists of superficial cell types from the stratified squamous epithelium, such as anuclear cells, cells with pyknotic nuclei and large intermediate cells. Towards the end of oestrus, numbers of polymorphonuclear neutrophil leucocytes appear in the smear, and these become the dominant cell type during metoestrus. In anoestrus nucleated basal and intermediate cells of the stratified squamous epithelium, together with a few neutrophils, form the characteristic smear.

Figure 1.31 shows the cyclical changes of the cell types.

Vaginal epithelium

The vaginal epithelium during anoestrus is of the low, columnar, cuboidal type and comprises two or three layers only. During pro-oestrus the epithelial cells change to the high, squamous, stratified type and persist in this form until the later stages of oestrus. The stratum corneum and the layers immediately beneath it are lost by desquamation during the pro-oestrous and oestrous periods, leaving a low, squamous structure which becomes converted to columnar epithelium in 1–3 weeks after the end of heat. During metoestrus (and pregnancy) the epithelium is of a higher columnar type than during anoestrus.

During pro-oestrus, oestrus and early metoestrus the epithelium and lamina propria are infiltrated with large numbers of neutrophils which eventually escape into the vaginal lumen.

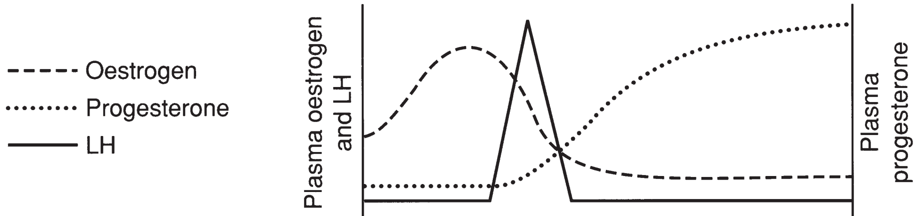
Endometrium

The endometrium shows considerable change during the oestrous cycle. The endometrial glands in pro-oestrus and oestrus are loosely coiled with very obvious lumina and deep epithelial lining. During metoestrus the glands become larger, the lumina smaller and the coiled parts in the basal layer of the endometrium more tortuous. As the bitch passes into anoestrus, there is a reduction in the amount and degree of coiling of the glands.

At about 98 days after the onset of oestrus, i.e. in metoestrus, there is evidence of desquamation of the endometrial epithelium; however, by about 120–130 days the epithelium has been restored by proliferation of cells from the crypts of the endometrial glands.

Changes in the ovaries

During anoestrus the ovaries are oval and slightly flattened. In a bitch of medium size they measure



Cell type		Pro-oestrus Early	Pro-oestrus Late	Oestrus Early	Oestrus Late	Early metoestrus	Anoestrus	Vaginitis pyometra
Para basal		+ + +	+	–	–	–	+ +	±
Small intermediate		+ + +	+ +	–	–	+	+	±
Large intermediate		±	+ +	+ + +	+ + +	–	–	±
Anuclear keratinised		–	+ +	+ + + +	+ + +	±	–	–
Red blood		+	+ + +	+ +	–	–	±	±
Neutrophilis		+	–	–	+	+ + +	+	+ + + +

Fig. 1.31 Changes in the types of cell, and their relative numbers, in vaginal smears from the bitch during the stages of the oestrous cycle, and changes in oestrogen, progesterone and luteinising hormone levels in the peripheral plasma.

approximately 1.4 cm from pole to pole and 0.8 cm from the attached to the free border. No appreciable follicles can be seen, although on section the minute remnants of the CLs of the previous cycle may be seen as yellow or brown spots. In the young bitch, the surface of the ovaries is smooth and regular, but in the aged animal it is irregular and scarred. At the commencement of pro-oestrus, developing follicles have already attained a diameter of 0.5 cm. They progressively enlarge, until at the time of ovulation their size varies from 0.6 to 1 cm. By this time the ovary is considerably enlarged, its size depending on the number of ripe follicles present, and its shape is irregular due to the projection of the follicles from its surface. Owing to the thickness of the follicle wall it may be difficult to distinguish between follicles and CLs. Prior to ovulation, the surface of the follicle shows a slightly raised papule, pin-head-sized, and the epithelium covering it is brown, which contrasts with the flesh colour of the remainder of the follicle. A remarkable feature of the ripening follicle of the bitch is the thickness of its wall, due to hypertrophy and folding of the granulosa cells, which can be seen on section with the naked eye as evidence of pre-ovulatory luteinisation. Ovulation is spontaneous and normally occurs 1 or 2 days after the onset of the period of acceptance. Most of the follicles rupture over a period of 48 hours (Wildt et al., 1977). The oocyte is capable of being fertilised for up to 108 hours after ovulation (Tsutsui and Shimizu, 1975). The CL at first contains a central cavity, but becomes filled by compact luteinised cells by the 10th day after ovulation, by which time the body has attained its full size (0.6–1 cm). CLs now comprise by far the greater mass of the ovary. As a rule an approximately equal number of CLs are found in each ovary, although occasionally there are wide differences. (In this connection it is interesting to note that the numbers of fetuses in the respective cornua in pregnancy frequently differ from those of the CLs in the ovaries on the respective sides.) Embryonic migration into the cornua on the opposite side would appear to be common. On section, the CL is yellowish pink; it remains unchanged in the non-pregnant bitch until about the 30th day after ovulation, after which it slowly atrophies. Visible vestiges may be present throughout anoestrus. During pregnancy the CLs persist at their maximum size throughout, but regress fairly rapidly after parturition. The changes in the ovaries of the bitch are shown diagramatically in Figures 1.32–1.36.

Ultrasound appearance of the ovaries

Using a transabdominal approach via the flank, with the bitch standing and a 7.5 MHz realtime linear array transducer, England and Allen (1989) were able to identify ovarian structures at the onset of pro-oestrus; these were circular and anechoic and were obviously developing antral follicles. When the bitch was in oestrus, they had increased in size, reaching a maximum of 4–13 mm in diameter on day 13 (day 0 being onset of pro-oestrus). The walls of the follicles became thickened from day 10 onwards, presumably due to preovulatory luteinisation; there was no evidence of follicular collapse associated with ovulation. At 25–30 days after the onset of pro-oestrus the ovaries were difficult to identify.

Endocrine changes during the oestrous cycle

The trends in endocrine changes are shown in Figure 1.37. The main feature which distinguishes them from other species previously described is the prolonged luteal phase, illustrated by the persistence of high progesterone levels in the peripheral blood. It is noticeable that progesterone levels start to rise before ovulation has occurred, which confirms the morphological evidence of preovulatory luteinisation of the mature follicles 60–70 hours before ovulation (Concannon et al., 1977). This preovulatory rise in progesterone may provide the stimulus for the bitch to accept the

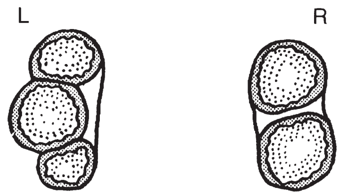


Fig. 1.32 Ovaries of an 8-month-old bitch weighing 6.8 kg after 2–3 days of pro-oestrus. Marked hypertrophy of the granulosa cells lining the follicles.

Fig. 1.33 Ovaries of an 8-month-old bitch weighing 6.8 kg just after ovulation. The follicles of the right ovary are collapsed. The left ovary shows remnants of corpora lutea from a previous heat.

Fig. 1.34 Ovaries of an 8-month-old bitch weighing 6.8 kg late in heat. Corpora lutea are forming; pleats are loose and flesh-coloured. Central cavities.

Fig. 1.35 Ovaries of a bitch weighing 11 kg. Three fetuses in each cornu. Corpora lutea are flesh-coloured. Old remnants are still visible.

Fig. 1.36 Ovaries of a bitch weighing 11 kg 4 weeks after parturition (five fetuses). Corpora lutea are shrunken and cream in colour.

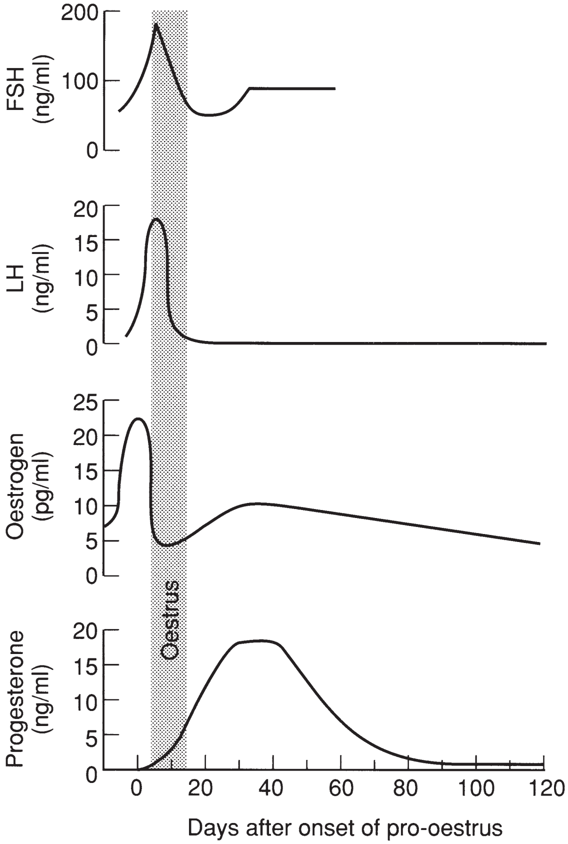


Fig. 1.37 Trends in hormone concentration in the peripheral circulation of the bitch during the oestrous cycle.

male (Concannon et al., 1975). In addition, it can also be used as a method to determine the timing of artificial insemination in that it should not be delayed long after concentrations > 2–3 ng/ml are observed in peripheral plasma (Jeffcoate and Lindsay, 1989; see Chapter 28).

Oestrogens rise rapidly just before the onset of standing oestrus, and are rapidly followed by the LH peak, which lasts much longer than that of other species; ovulation occurs 24–96 hours after this (Phemister et al., 1973; Wildt et al., 1977). FSH concentrations at oestrus reach a peak, coincident with that of LH. Prolactin appears to have a negative correlation with progesterone; thus, as progesterone levels fall towards the end of metoestrus or pregnancy, prolactin increases; it is the major luteotrophic hormone in this species.

Pseudopregnancy

Most, if not all, bitches show some evidence of pseudopregnancy during metoestrus, the intensity and signs being very variable; for this reason it is preferable to refer to *covert* pseudopregnancy, where the bitch will be in metoestrus but will show little or no signs, and *overt* pseudopregnancy. In the latter, the clinical signs will range from slight mammary development and lactogenesis whilst at the opposite extreme the bitch will show all the external signs of pregnancy and will ultimately undergo a mock parturition, with nesting, loss of appetite, straining, emotional attachment to inanimate objects and heavy lactation.

Pseudopregnancy was originally believed to be due to an intensification and prolongation of metoestrus; however, a number of workers have demonstrated that there is no difference in the progesterone concentrations in the peripheral blood of bitches with or without signs of pseudopregnancy. It is likely that the prolactin may well be responsible for initiating the changes. The negative correlation between progesterone and prolactin levels is shown in Figure 1.37, the decline in progesterone appearing to coincide with the rise in prolactin. If bitches undergo ovarohysterectomy when they are pseudopregnant the condition can be intensified and prolonged. Furthermore, antiprolactin drugs such as bromocriptine and cabergoline have been successfully used to cause remission of the signs of pseudopregnancy (Arbeiter and Winding, 1977).

THE CAT

Body weight is the most important factor in determining puberty in non-pedigree cats (Scott, 1970). Females will usually show their first oestrus once a weight of 2.3–2.5 kg has been attained at an approximate age of 7 months. Puberty occurs a month or two later in males at a weight of approximately 3.5 kg. Puberty is also influenced by the season of birth; females born very early in the year may mature in the autumn of the same year, whilst those born later will not normally show oestrus until the following spring (Gruffydd-Jones, 1982). Puberty is much more variable in pedigree cats (Jemmett and Evans, 1977). Oriental queens (such as Siamese and Burmese) may show their first oestrus before 5 months of age, whilst pedigree long-hair cats may not mature until over a year of age.

Free-living non-pedigree and feral cats are seasonally polyoestrus, with a period of anoestrus beginning in the late autumn. Increasing daylight length is the most important factor in inducing the resumption of reproductive activity and the first oestrus will usually be seen soon after the shortest day of the year. If a constant 14 hours of lighting is provided daily, sexual activity will continue throughout the year, and this manipulation of photoperiod will alter the circadian rhythm of melatonin production (Leyva et al., 1985). If the lighting regimen is changed from 14 to 8 hours then cyclic activity will cease immediately (Leyva et al., 1989). There may be a period of apparent lack of oestrous activity in the early summer, but this corresponds with the pregnancy or lactation following mating earlier in the year, rather than true anoestrus.

Some non-pedigree cats have regular oestrous cycles lasting approximately 3 weeks, but others may show no regular pattern (Shille et al., 1979). The duration of oestrus is 7–10 days, and is not significantly shortened by mating (Shille et al., 1979; Gruffydd-Jones, 1982). Oestrous cycle patterns show considerably more variation in pedigree cats. Long-hairs may have only one or two oestrous cycles each year, whilst the period of oestrus may be longer in Oriental queens with a reduced interoestrual interval. Oestrogen concentrations increase dramatically at the time of oestrus from the baseline of 60 pmol/l, and may double within 24 hours, reaching a peak of up to 300 pmol/l (Shille et al., 1979). The principal oestrogen produced by the ovary is oestradiol-17β. The rapid rise in oestrogen concentrations corresponds to an abrupt appearance of behavioural changes indicative of oestrus, and queens do not usually show a distinctive pro-oestrous phase. The oestrual display is characterised by increased vocalisation, rubbing and rolling. The queen becomes generally more active, and she will solicit the attention of a tom. The queen may adopt the mating posture either in response to the tom or occasionally spontaneously. She lowers her front quarters with the hind legs extended, resulting in lordosis. The tail is held erect and slightly to one side. There may occasionally be a slight serous vaginal discharge, but there are usually no changes in the appearance of the external genitalia. The extent of the oestrual display varies considerably between queens but is generally more prominent in the Oriental breeds.

Mating and ovulation

During mating, the tom mounts the queen and grasps her neck with his teeth. The queen's hind legs paddle as he adjusts his position, and this becomes more rapid during coitus, which lasts up to 10 seconds. The queen cries out during copulation, and as the tom dismounts, she may strike out at him, displaying the typical 'rage reaction'. This is followed by a period of frantic rolling and licking at the vulva. As soon as this postcoital reaction has ceased, the tom will usually attempt to mate the queen again, and there may be several matings within the first 30–60 minutes.

The cat is an induced ovulator, and thus mating is important in triggering ovulation. Receptors are present within the queen's vulva which are stimulated during copulation, ultimately resulting in release of LH from the anterior pituitary. Only about 50% of queens will ovulate after a single mating, and multiple matings may be required to ensure adequate release of LH to induce ovulation (Concannon et al., 1980). The ovulatory surge of LH begins within minutes of coitus, peaks within 2 hours and returns to basal values within about 8 hours; peak LH concentrations of over 90 ng/ml have been reported (Tsutsui and Stabenfeldt, 1993). Further matings before the peak of LH concentrations has been reached will lead to additional increments. However, after multiple matings over a period of 4 hours or more, further matings may fail to induce any additional increase in LH concentrations, and this is thought to result from depletion of the pituitary pool of the hormone or development of refractoriness to GnRH (Johnson and Gay, 1981).

Ovulation is an 'all or nothing' phenomenon, and once significant concentrations of LH have been achieved all ripe follicles will rupture (Wildt et al., 1980). The mean ovulatory rate for non-pedigree cats is approximately four, but is more variable in pedigree animals.

Occasionally, ovulation will occur in the absence of any contact with entire toms. Receptors similar to those found in the vulva are also located in the lumbar area (Rose, 1978), and these may be stimulated if the queen is mounted by other females or castrated male cats, or by stroking over this area. Neutered toms may mate queens in oestrus even if they have been castrated prepubertally. However, in a study involving a colony of American short-haired cats in which the queens were housed individually, and where tactile stimulation of the hindquarters and perineal regions by handlers was avoided, ovulation was detected; this was defined if progesterone concentrations were ≥ 4.8 nmol/l in the peripheral circulation. The queens had sight and sound of other cats, including males. It is noteworthy that six of the seven cats that ovulated were older than 7 years of age (Lawler et al., 1993).

Pseudopregnancy

Sterile matings which successfully induce ovulation lead to pseudopregnancy. Concentrations of progesterone are very similar to those of pregnancy for the first 3 weeks, after which levels gradually fall, reaching baseline by approximately 7 weeks (Paape et al., 1975; Shille and Stabenfeldt, 1979) (Figure 1.38). Oestrus will usually occur shortly afterwards.

Nesting behaviour and milk production are rarely seen in pseudopregnant queens, but hyperaemia of the nipples will usually be evident as in pregnancy. The queen's appetite may increase, with some redistribution of fat leading to an increase in abdominal size.

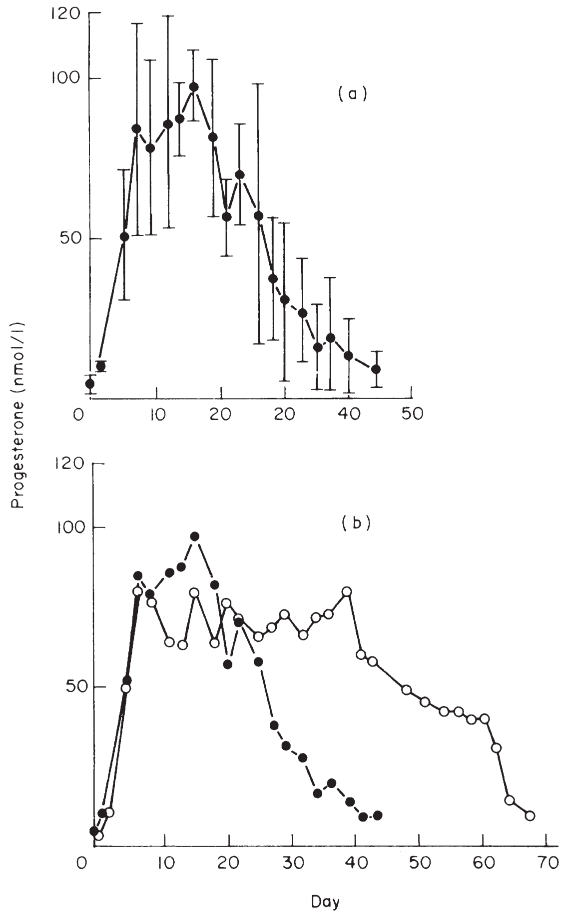


Fig. 1.38 (a) Mean (±SD) plasma concentrations of progesterone throughout pseudopregnancy (four cats). (b) Mean plasma concentrations of progesterone throughout pregnancy (○——○) and pseudopregnancy (●——●). Day 0 represents the day of mating. (Figure reproduced courtesy of Dr T. J. Gruffydd-Jones.)

ARTIFICIAL CONTROL OF CYCLICAL REPRODUCTIVE ACTIVITY

In the management of livestock, or in dealing with companion animals, there are many times when the manipulation of normal cyclic activity ensures optimum production or is convenient for the owner. In the case of seasonal breeders the ability to produce offspring out of season or to advance the time of onset of cyclic activity has advantages. In these and in other species, the ability to ensure that an individual or group of animals does not come into oestrus, or is in oestrus at the same time, has attractions. The methods that are available can be divided into two main groups: firstly, those which do not involve the use of hormones and, secondly, those that do.

Non-hormonal methods

Light

The onset of cyclical activity in the mare, ewe, goat and cat is dependent upon changes in the hours of daylight. The mare and queen are stimulated to activity by a lengthening photoperiod, whilst in the ewe and goat it is the effect of a decreasing photoperiod which is the stimulus.

In ewes, the provision of housing with controlled lighting enables the breeding season to change from the autumn and winter, to spring and summer. Furthermore, by subjecting the ewes to a lighting regimen which does not have any change in duration it is possible to ensure breeding throughout the year, as is the case in equatorial climates.

If mares are stabled at the end of December in the northern hemisphere, and are subjected to artificial light, preferably of increasing duration, then it is possible to advance the onset of normal cyclical activity so that there is oestrus and ovulation.

Both tungsten and fluorescent lights have been used, although the former would appear to be better. The provision of a 200 watt incandescent bulb in each loose-box is adequate, and if it is controlled by an automatic timing device, so that the duration of lighting is increased by 25–30 minutes each week, reproductive activity will be initiated when the mare is receiving 15–16 hours of light each day (Kenney et al., 1975).

Nutrition

The effect of nutrition in initiating reproductive activity in seasonally breeding species is not clear. There is some evidence that the stabling of mares and the provision of good feeding assist in stimulating the onset of cyclic activity in early spring. There is also evidence for the converse, since Allen (1978) has reported that when yarded mares are turned out to fresh spring grass about 80% of them have come into oestrus and ovulated within 14 days. Furthermore, he has found that barren and maiden mares maintained in yards on adequate but mainly dried feedstuffs during the winter and spring remain in anoestrus longer than those which are kept out at grass. An explanation for this is difficult to find, although it may be related to the β-carotene content of the diet, fresh spring grass containing large amounts of this substance.

Improved nutrition can exert a profound effect on ovarian function by increasing the number of follicles which mature and ovulate. This effect is described as ‘flushing’, a practice which has been used in lowland flocks of sheep for many years. By increasing the dietary intake, particularly that of energy, before ewes are tupped it is possible to increase the number of lambs that are born. A similar technique can also be used in the sow to increase litter size. There is no evidence, however, that, provided the ewes are adequately fed, it is possible to advance the onset of the breeding season by this means.

The opinions on the effect of nutrition on reproduction in the sow are conflicting. It is generally assumed that flushing gilts and sows 4–6 days before oestrus increased prolificacy by increasing ovulation rate (Dailey et al., 1972). Whether this effect occurs in adequately fed individuals is difficult to determine (Aherne and Kirkwood, 1985).

Other non-hormonal methods

The presence of a male animal can exert its effect upon the cyclical activity of the female. This is well demonstrated in sheep, where the introduction of a vasectomised tup at the start of the breeding season will stimulate the onset of oestrous cycles in the majority of ewes and can also bring about some degree of synchronisation of cyclical patterns (see Chapter 24).

In sows and gilts the weaning of piglets hastens the return of cyclical ovarian activity postpartum. If litters from a number of sows are weaned at the same time this will result in some degree of synchrony of oestrus.

It has also been shown in gilts and sows that stress associated with a change in environment or transportation can stimulate the onset of oestrus postpartum.

Hormonal methods

A large number of different hormones have been used to manipulate cyclical activity in domestic species. Some of the methods have been based upon attempts to mimic closely the normal endocrine changes that occur; however, some have been rather more empirical.

They can be considered in various groups: (1) preparations which stimulate release of anterior pituitary hormones; (2) preparations which replace or supplement anterior pituitary gonadotrophins; (3) oestrogens; (4) progestogens; (5) prostaglandins; and (6) melatonin.

Preparations which stimulate the release of anterior pituitary hormones

Ovarian steroid hormones, particularly oestrogens, have been shown to exert a positive-feedback effect upon the anterior pituitary and hypothalamus (Figure 1.1). A large number of oestrogens, both naturally occurring and synthetic, have been used to stimulate oestrus. It is likely that their effect is purely a direct one in stimulating oestrous behaviour and changes in the genital tract, but it is possible that they may also stimulate the release of pituitary gonadotrophins. However, synthetic GnRH can be used to stimulate the release of endogenous gonadotrophins; for example, GnRH has been used to induce premature puberty in gilts following equine chorionic gonadotrophin (eCG) stimulation (Webel, 1978). It can be used to stimulate the onset of oestrus in the postpartum cow (Lamming et al., 1979). It has not proved to be effective in inducing oestrus in mares during the seasonal anoestrus (Allen and Alexiev, 1979).

Preparations which replace or supplement pituitary gonadotrophins

It is possible to extract purified FSH and LH from pituitary glands obtained at abattoirs. However, it is expensive and time-consuming to obtain sufficient quantities for routine commercial use other than for superovulation and embryo transfer. Furthermore, there is a danger of transmitting diseases such as bovine spongiform encephalitis (BSE). Fortunately, two readily available substitutes are available: (1) eCG, obtained from the serum of pregnant mares, which has mainly an 'FSH-like' effect but with some 'LH-like' activity, and (2) human chorionic gonadotrophin (hCG), obtained from the urine of pregnant women, which has mainly an 'LH-like' effect but with some 'FSH-like' activity.

Pseudopregnancy can be achieved by mating queen cats with a vasectomised tom or through simulating coitus by swabbing the vagina. Administration of hCG can also be used to induce ovulation. Pseudopregnant queens may not show a return to oestrus for 4–8 weeks.

As has been previously described, premature puberty has been initiated in most domestic species by the administration of eCG. However, both gonadotrophins have also been successfully used to manipulate cyclic activity. In anoestrous gilts and sows, eCG alone or in combination with hCG will promote follicular growth and oestrus (400 IU eCG and 200 IU hCG), but a second injection of hCG 72 hours later will ensure that ovulation occurs. The same technique can be used to synchronise cyclic activity, particularly if used in combination with a progestogen or other pituitary-blocking substance (see below).

The use of eCG alone to induce oestrus in seasonally anoestrous ewes is not very successful, but if progesterone is administered to the ewes before the injection of eCG then there is synchronised oestrus and ovulation in such ewes. However, attempts to stimulate an early return to cyclical activity in lactating ewes have proved to be difficult, particularly in those that are lactating heavily.

In the anoestrous cow, it is possible to stimulate follicular growth and ovulation with eCG treatment. However, the dose–response is variable, and it can frequently result in multiple ovulations. Thus, it is necessary to withhold insemination at this induced oestrus. Unfortunately, in many cases, the cow will then return to the anoestrous state.

Combinations of eCG/hCG have been used to induce oestrus in the anoestrous bitch, sometimes in combination with oestrogens. The induction of a behavioural response has usually been good but the numbers of bitches which ovulated and subsequently conceived has usually been poor.

Surprisingly, eCG does not appear to stimulate ovarian activity when given to mares in winter anoestrus. The reasons for this are probably twofold; firstly, it may be that the dose required to stimulate follicular development is large and, secondly, it is likely that eCG alone is not responsible for stimulating the wave of accessory follicles during early pregnancy. Human menopausal gonadotrophin (hMG) is extracted from the urine of menopausal women; this has a high 'FSH-like' action. It is used to superovulate cows for embryo transfer, but the author is not aware of it being used elsewhere.

Oestrogens

The administration of oestrogens, either synthetic or naturally occurring, has been used to induce oestrus in animals that are anoestrus, especially in the bitch. In most cases they have a direct effect on the tubular genital tract and on behaviour; however, it is doubtful that they initiate ovarian activity and ovulation; in fact, in large doses they could result in pituitary inhibition.

Progestogens

Progesterone and progestational compounds have been used extensively in most domestic species as a method of controlling the oestrous cycle, particularly synchronisation within groups of females. In general, the principle behind their use is that the exogenous progestogens act in the same way as a CL, resulting in a negative feedback effect upon the anterior pituitary and a suppression of cyclic activity initiated by the release of gonadotrophins. When the source of progestogen is withdrawn, or its effect declines, there is a return to cyclic activity.

Uses in the mare. In some racehorses and show-jumpers it is desirable to prevent the mare from coming into oestrus at an inopportune time; in some cases it may be desirable to synchronise a group of animals. A daily injection of progesterone at a dose rate of 0.3 mg/kg is effective in preventing oestrus, with a return to a normal fertile heat 3–7 days after treatment ceases (Van Niekerk, 1973). Potent oral progestogens are now also available for suppressing oestrus and synchronising groups of mares when withdrawn (Webel, 1975). One of these, allyltrenbolone or altrenogest, has been used successfully in a number of ways:

- To stimulate the onset of cyclical activity. The hormone, which is incorporated into a clear, yellow vegetable oil, is administered at a dose rate of 0.044 mg/kg body weight mixed in the feed for 10 days and then stopped. A good response will be obtained when given in the late transitional phase from anoestrus to cyclical activity when follicles are present. The results are better if increased lighting is used.
- To suppress oestrus – for example, for shows or other functions. It should be fed for 15 days at a dose rate of 0.044 mg/kg.
- To suppress oestrus in mares with prolonged oestrus or other aberrant sexual behaviour.
- To control the time of oestrus so that effective use can be made of a stallion or artificial insemination. The hormone should be fed for 15 days and then stopped, so that the mare should come into oestrus 2–3 days later.

Uses in the cow. Progestogens can be used to suppress oestrus as described in the mare but there are few practical indications for this purpose. However, there is ample indication for their use to synchronise groups of cows and heifers for artificial insemination and to overcome the problems of oestrus detection.

Progesterone was first used by daily injection to synchronise oestrous cycles in groups of cows in 1948 (Christian and Casida, 1948). A large number of synthetic substances have since been used and it is generally accepted that following treatment of randomly cycling animals with these compounds for 18–21 days, there is fairly good synchronisation of oestrus 4–6 days after the cessation of treatment. Unfortunately, as with other species, fertility at the first oestrus may be lower than normal, the most likely reason for this being impaired sperm transport as a result of the atypical hormone balance after withdrawal of the progestogen.

Good synchronisation and fertility, following double fixed-time artificial insemination, was reported by Wishart and Young (1974) using a synthetic progestogen (Norgestamet). The hormone was given as a subcutaneous implant at the same time as an injection of oestradiol valerate. The implant was removed after 9 days, and following two inseminations at 48 and 60 hours afterwards, conception rates were 65%.

The main disadvantage of such a scheme is the need to handle the cattle a second time to remove the implant; it should not be used in lactating dairy cows where their milk is used for human consumption.

Another method of administering progestogens is in the form of a progesterone-releasing intra-

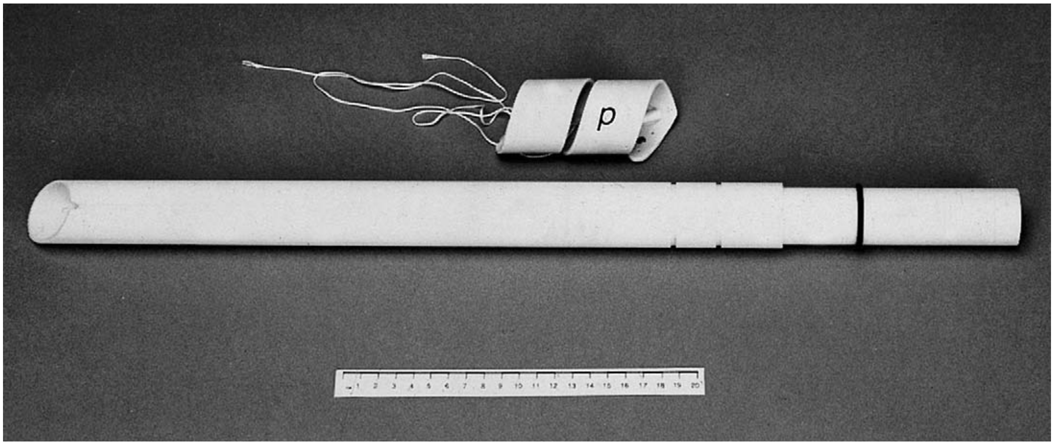


Fig. 1.39 Progesterone-releasing intravaginal device (PRID) (p) with speculum/applicator.

vaginal device (PRID) (Figure 1.39) or controlled internal drug release device (CIDR-type B) containing 1.9 g of progesterone (Figure 1.40). The PRID, which is a stainless steel coil covered with an inert elastomer incorporating 1.55 g of progesterone, is placed in the vagina, using a special speculum, and whilst it is in position progesterone is absorbed, producing concentrations in the peripheral blood comparable with the maximum levels of dioestrus. When the coil is removed after 12 days, the cow will come into oestrus in 2–3 days. Good conception rates have been obtained following two fixed-time inseminations at 57 and 74 hours after the removal of the device. Some of the coils also contain a small capsule of oestradiol benzoate. The CIDR, which is a 'T'-shaped device with flexible arms impregnated with progesterone, functions in a similar manner.

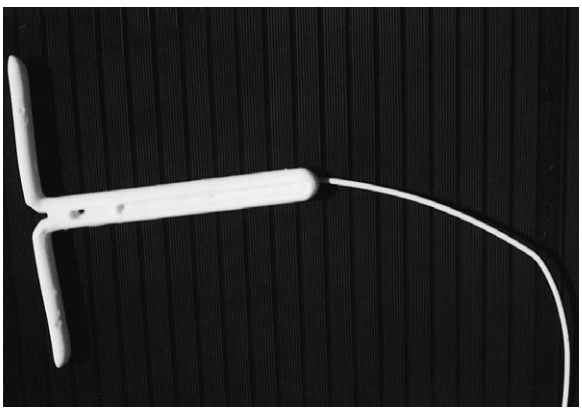


Fig. 1.40 Controlled internal drug release (CIDR) device.

In the case of PRID and Norgestamet (see above), the oestradiol benzoate or oestradiol valerate is used as an antiluteotrophic and luteolytic agent. However, there is good evidence that oestrogens are only weakly luteolytic, especially when used in the early luteal phase (Smith and Vincent, 1973). This is probably the reason why in an earlier study on the use of Norgestamet to synchronise heifers only 75% were observed in oestrus within 4.5 days of implantation (Drew et al., 1979). In a study using Norgestamet, Peters (1984) found that, of eight cows treated with oestradiol valerate during the first 6 days of the oestrous cycle, in only one was there prevention of luteal function. Thus in those cows where this occurred the CL would have persisted beyond the time that the implant was in situ, and hence they would have returned to oestrus only when the CL had regressed spontaneously. By using $PGF_{2\alpha}$ at the time of removal of the implant, synchronisation approaching 100% should be achieved. Better results are obtained if the prostaglandin is injected 24 hours before removal. Norgestamet is available commercially as a 3 mg implant; on insertion, 3 mg of Norgestamet and 5 mg of oestradiol valerate are injected as a 2 ml dose intramuscularly.

In a study comparing the effect of the stage of the oestrous cycle when PRIDs are inserted, and the degree of synchronisation, it was found that it was much better when they were inserted on day 13 or 14 compared with days 2–4 (Cumming et al., 1982). When PRIDs were inserted for 12 days and $PGF_{2\alpha}$ injected 24 hours before removal, very good synchronisation was achieved (Roche and Ireland, 1981; Folman et al., 1983), with a pregnancy rate of 67% following fixed-time artificial insemination at 56 hours.

The same PRID has also been used successfully to induce oestrus in dairy and beef suckler cows that are anoestrus (Lamming and Bulman, 1976), as has Norgestamet and CIDR. This will be discussed further in Chapter 22.

Uses in the ewe. Progestogens have been widely used in controlling reproduction in the ewe, either on their own or in conjunction with other hormones. They have been used to induce oestrus in the anoestrous ewe during the non-breeding season, and also for synchronisation of groups of ewes that are already showing cyclical activity. Most of the progestational substances are administered via the intravaginal route in the form of impregnated sponges or tampons (Figure 1.41). Provided that the progestogen is correctly incorporated into the sponge, it is readily absorbed at a sufficient rate to ensure a full negative feedback effect on pituitary function. Although progesterone was used initially in the sponges, the potent short-acting analogues, notably fluorogestone acetate (FGA) and medroxyprogesterone acetate (MAP), have superseded it.

When intravaginal sponges are used outside the normal breeding season, it is necessary to use eCG as a source of gonadotrophin at the end of the progesterone priming period. The onset of normal cyclic activity can be determined by running vasectomised rams with a harness several weeks before. However, it is possible to use a simple rule-of-thumb calculation to determine if eCG is necessary: from the lambing records of the non-synchronised flock, calculate the date when 50% of the ewes had lambed, then, if the sponges are not to be inserted earlier than 150 days before the same date for the current year, eCG will not be required (Henderson, 1985).

The dose of eCG required is such that it should stimulate oestrus and ovulation without causing superovulation. Some approximate dose rates are listed in Table 1.2.

Opinions vary on the time of injection of eCG. Whilst it is claimed that better results are obtained if it is injected 48 hours before sponge removal, the advantage is so small that the additional handling of the ewes does not make it cost-effective.

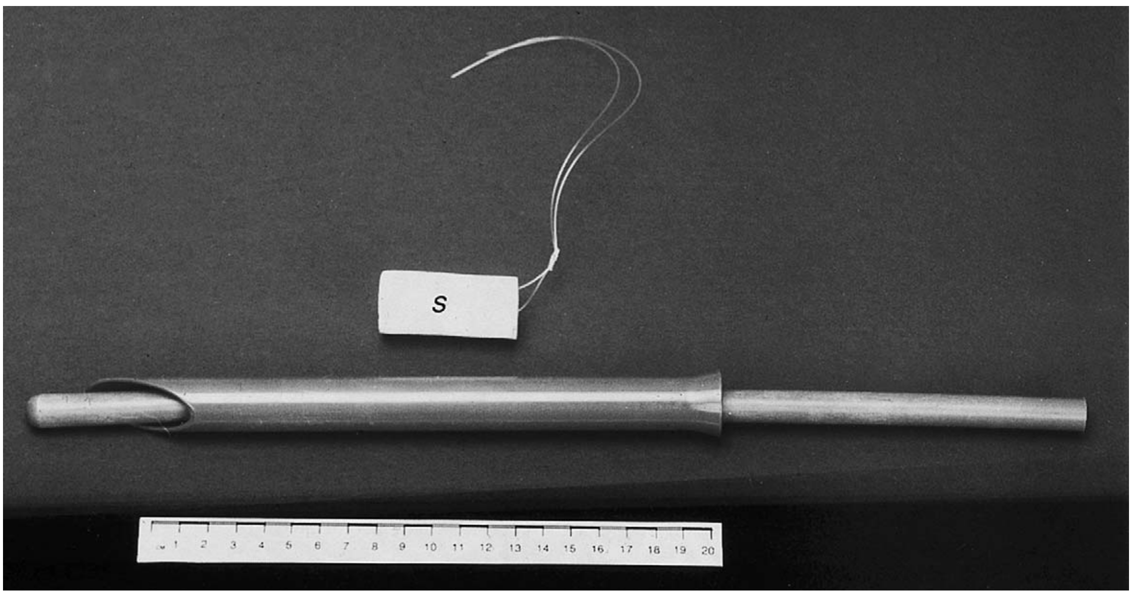


Fig. 1.41 Progestogen-impregnated intravaginal sponge or tampon (s) with speculum and introducer.

Table 1.2 Dose of eCG (IU) required to induce 'out-of-season' oestrus in ewes following withdrawal of progestogen sponges (from Henderson, 1985)

Month	*Dorset Horn, Finn/Dorset*	*Suffolk, Suffolk cross Greyfaces*	*Scottish half-breeds, mules,*
July	600–500	750–600	Poor results
August	400–300	500–400	750–600
September	0	300–0	500–300
October	0	0	0

Fertility may be reduced when ewes are mated at the first synchronised oestrus; this may be due to poor absorption of the progestogen from the sponge or to an effect of the abnormal steroid balance on sperm transport and survival. However, if ewes fail to conceive at the first oestrus there is usually good synchrony at the second, when better conception rates are likely.

Attempts to induce oestrus in the early postpartum and lactating ewe have been unsuccessful.

Uses in the goat. The same type of sponge used in sheep is quite successful for synchronisation of oestrus and, in conjunction with eCG, for the initiation of oestrus during the normal period of anoestrus. Some manufacturers produce a specific sponge for use in goats with a higher dose rate of progestogen. Goats object to the insertion of the sponges, particularly the applicator, much more than ewes, and in maiden goats it is preferable to insert a lubricated sponge with the use of a finger only (Henderson, 1985). Goats show intense oestrus 36–48 hours after sponge removal. A specially designed CIDR has been successfully used in Australia (Rita et al., 1989).

When eCG is used to stimulate oestrus, dose rates of 600–500 IU should be used in July, 500–400 IU in August, and 300–0 IU in September; from October onwards eCG is not required (Henderson, 1985).

Uses in the sow and gilt. A number of different progestogens have been tried unsuccessfully to synchronise oestrus in sows and gilts. Altrenogest/allyltrenbolone is now sold in many countries for this purpose. This progestogen effectively suppresses follicular maturation when fed daily at a dose rate of 15–20 mg, with no apparent effect upon the life span of the CLs. When fed at lower dose rates, i.e. 2.5–5 mg daily, follicular growth was not inhibited and cystic follicles developed by 10 days after the start of treatment (Martinat-Botte et al., 1985). Similar problems have been encountered with other progestogens.

Allyltrenbolone or altrenogest is sold as an oily solution within a pressurised container and calibrated so that a single 5 ml volume (0.4 w/v) contains the required dose. In an extensive trial involving 1223 gilts of various breeds, Martinat-Botte et al. (1985) found that after feeding the compound for 18 days there was good synchronisation of oestrus 5–7 days after withdrawal. There were breed differences in the degree of synchronisation. In cross-bred gilts, good farrowing rates (average 64–73%) were obtained following fixed-time artificial insemination on days 6 and 7 after withdrawal of the progestogen; litter sizes ranged on average from 9.5 to 9.8. When pure-bred and cross-bred gilts were inseminated at observed oestrus the overall farrowing rates were improved.

A similar study, involving 2215 gilts and sows, was undertaken to see if synchronisation after weaning could be improved following feeding of altrenogest/allyltrenbolone. It was found that there was some improvement following short-term feeding at a dose of 20 mg from 3 days before the weaning date. It was improved by boar proximity. This same progestogen has been administered with some success as a subcutaneous implant at the base of the ear (Selgrath and Ebert, 1993).

Much of the interest in developing suitable progestational compounds was inhibited when a non-progestational, pituitary-inhibiting substance was developed. This substance, Methallibure, was effective in regulating cyclic activity, and sows and gilts which had been on treatment had good fertility. Unfortunately, it was withdrawn for safety reasons because if fed to pregnant animals it had a severe teratogenic effect on the developing fetuses.

Uses in the bitch and queen cat. A number of different progestogens, such as megoestrol acetate, proligestone and medroxyprogesterone acetate, have been used to suppress oestrus in the bitch; these are available for oral administration as tablets, or by injection. They can be used to postpone the onset of oestrus when administered during anoestrus, or to prevent oestrus from occurring at the first signs of pro-oestrus. The latter is not too difficult to achieve in the bitch. Postponement can be maintained for over a year by injections of progestogens at intervals of 3–5 months or, after a 40-day course of daily oral administration, by tablets twice per week. Prevention of oestrus can be achieved by a single injection of the progestogen or by the administration of oral progestogen at a higher dose rate than for postponement, but for a shorter duration.

Unfortunately, following the administration of progestogens, the time interval before the onset of the next oestrus is rather unpredictable if treatment is not continued. Furthermore, there is good evidence that continued and frequent use of such preparations can predispose to reproductive disorders, particularly cystic glandular hyperplasia of the endometrium. Because of this problem, owners should be warned of the possible dangers, particularly if they wish to use the bitch subsequently for breeding.

In the cat, suppression of oestrus may be desirable for a number of reasons, but particularly for planning of litters throughout the year and to allow the queen a period of rest from sexual activity after a litter and to enable her to regain condition before being bred again. If a queen is allowed to call repeatedly without mating, this may lead to considerable loss of condition due to relative inappetance during oestrus, particularly in Oriental breeds, which have short interoestrual intervals and long periods of oestrus. In addition, breeders report that difficulties are sometimes encountered in breeding from queens that have been allowed to call incessantly and have been unmated for long periods.

The most widely used method of oestrus suppression is the administration of progestogens. Injectable depot forms are available of proligestone and medroxyprogesterone acetate which will suppress oestrus for up to 7 months or more following a single injection, and can be repeated every 5 months to achieve permanent oestrus suppression. Loss of pigmentation in the area overlying injection sites is occasionally encountered. Oral progestogens have the advantage of greater flexibility, and the most commonly used of these is megoestrol acetate. This can be used to prevent an individual oestrous period by administering 5 mg as soon as signs of oestrus are observed. However, this approach is less suitable in cats than in dogs in view of the very rapid and sudden onset of oestrous behaviour. Postponement is achieved by administering 2.5 mg daily or weekly dependent on whether treatment is begun during the breeding season or anoestrus, although, in some queens, lower dosages may prove effective.

Many queens treated with progestogens will show behavioural changes, most commonly lethargy and weight gain (Øen, 1977). A proportion of treated queens may also develop endometritis. A more serious side-effect which has occasionally been reported, and may not be reversible in some cats, is the development of diabetes mellitus (Moise and Reimers, 1983).

Prostaglandins

Since the length of the interoestrus interval in most domesticated species is controlled by the duration of the life span of the CL, premature lysis, induced by the administration of $PGF_{2\alpha}$ or its analogues, can be used to manipulate the normal pattern of cyclic activity.

The CLs of the cow, mare, sow, ewe and goat normally respond to the administration of exogenous prostaglandins, but in the bitch and the queen the CLs are generally unresponsive unless subjected to repeated doses. It is important to examine, in the first five species mentioned, when the CLs are responsive or are refractory; this is summarised for four species in Figure 1.42. In the cow, mare, ewe and goat the pattern is fairly similar, the new developing CL being refractory for 3–5 days after ovulation. At the other end of the oestrous cycle the CL is unaffected by exogenous prostaglandin, since it is already regressing

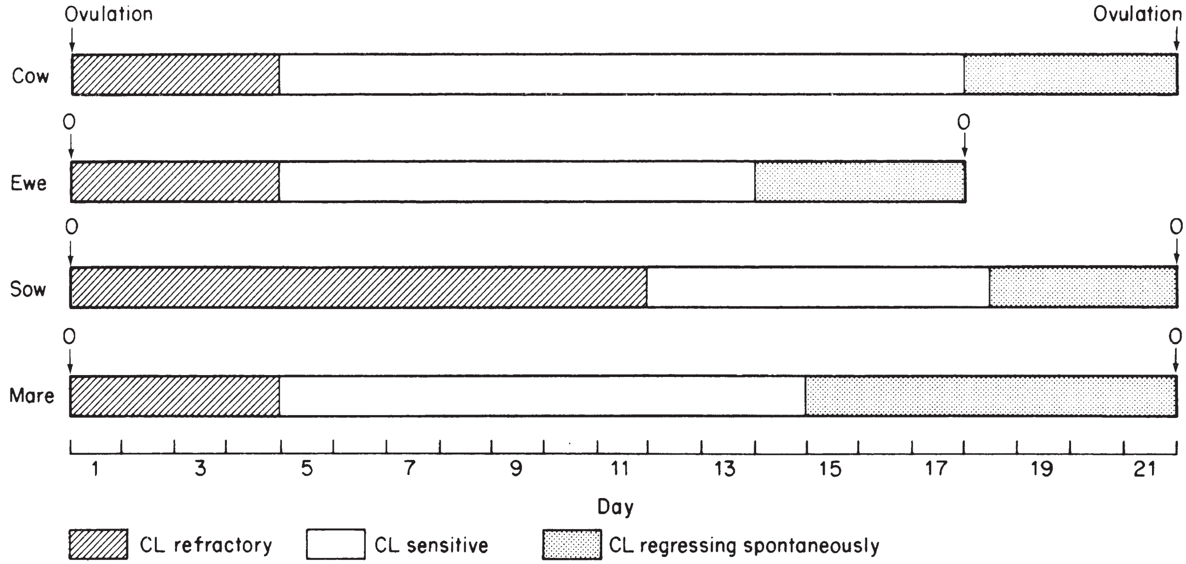


Fig. 1.42 Sensitivity of the corpus luteum (CL) of different species to $PGF_{2\alpha}$ during the oestrous cycle.

under the influence of its own endogenous luteolysin; there is no evidence that this can be accelerated. Therefore in the cycles of the cow, goat, mare and ewe the CL is responsive for 13, 13, 10 and 9 days, respectively. The sow, however, is different since the CL is refractory for up to 11 days after ovulation, and thus is responsive for a much shorter period of only 7 or 8 days.

Since prostaglandins are abortifacients, they must not be used in animals that might be pregnant. If there is any doubt, pregnancy diagnosis must be performed.

Uses in the cow. Prostaglandins have been used successfully to synchronise oestrus in groups of cows and heifers. This technique has applications in beef cows and heifers, and in dairy heifers where oestrous detection is frequently difficult, thus enabling the routine use of artificial insemination at a predetermined time. The availability of artificial insemination in such situations allows the use of semen from genetically superior sires and thus can result in the improved genetic potential of the offspring.

It has been found that if two injections of $PGF_{2\alpha}$ or one of its synthetic analogues, e.g. cloprostenol, are given at an interval of 11 days to a group of cows or heifers at randomly different stages of the oestrous cycle, then 3–5 days after the second injection all the animals treated will come into oestrus and ovulate at about the same time. It has also been shown that if they are inseminated twice at a fixed time of 72 and 96 hours or 72 and 90 hours after the second injection, conception rates are comparable with those following artificial insemination or natural service at a spontaneous oestrus. A single fixed-time injection results in a reduction in conception rates. Figure 1.43 shows how three cows at different stages of the oestrous cycle can have their heats synchronised with the double-injection technique. Those animals which have a sensitive CL at the time of the first injection, as in cow B, will have an induced oestrus 3–5 days later, and if this is observed it is possible to save an additional injection of $PGF_{2\alpha}$ by inseminating them at this stage. The time interval from the last injection of $PGF_{2\alpha}$ to oestrus and ovulation depends upon the stage of follicular development when it is administered. It has been shown by sequential transrectal ultrasonography, that the dominant follicle in the first wave loses its ability to ovulate when it has reached the late plateau or regression phase of development if luteolysis is induced (Fortune et al., 1991). As a result, it will be the dominant follicle of the next wave that will grow and ovulate, resulting in a longer interval than if premature luteolysis occurs when the dominant follicle is in the mid-static phase of development. Thus oestrus and ovulation

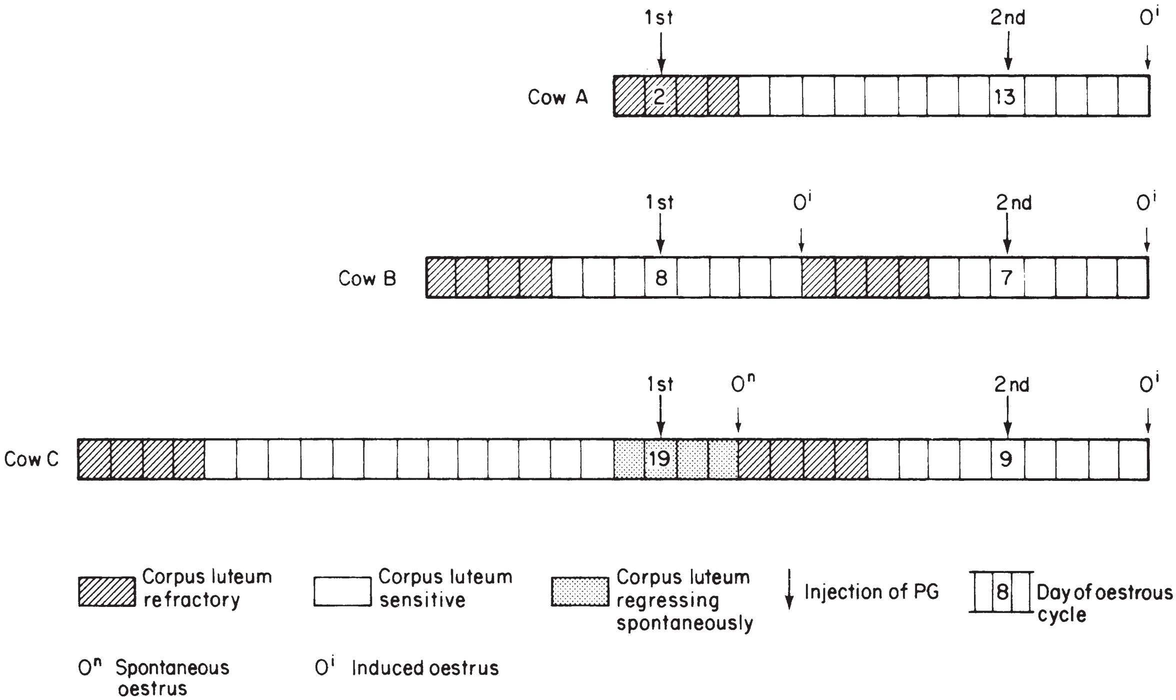


Fig. 1.43 Synchronisation of oestrus in cows by the administration of two injections of $PGF_{2\alpha}$ or an analogue at an 11-day interval.

synchronisation regimens would be much improved if the second injection of $PGF_{2\alpha}$ could always be given near the end of the growth phase of the dominant follicle, and probably accounts for the better results obtained when combinations of progestogens and $PGF_{2\alpha}$ are used (Garcia et al., 1999).

The efficiency of synchronisation, following the double-injection regimen, is usually much better in heifers than in cows. It is not known precisely why this should be so, but one possible explanation is that frequently in cows (as opposed to heifers) the progesterone concentrations remain low for a much longer period of time after ovulation than normal (see Figure 1.28). This phenomenon, referred to as 'long-low progesterone', has been recorded as occurring in up to 15% of cows in some herds. Presumably the delay in the CL reaching a sensitive stage interferes with the synchronisation scheme outlined in Figure 1.42.

Largely to reduce cost and to improve the pregnancy rates, a compromise regimen has been used. All animals are injected with $PGF_{2\alpha}$ on the same day and observed for oestrus during the following 5 days. Any identified in oestrus are inseminated as normal, and those not identified receive a second injection of prostaglandin followed by fixed-time artificial insemination. Any individuals exhibiting oestrus a few days after fixed-time artificial insemination should be reinseminated.

There is some suggestion that pregnancy rates are improved after a prostaglandin-induced oestrus where cows are inseminated in relation to an observed oestrus. In a review of 17 trials using cloprostenol, McIntosh et al. (1984) found that there was evidence of improved pregnancy rates in 13 of the trials. There is no physiological explanation for this response, but the authors suggest that it might be a management phenomenon; oestrus can be anticipated over a restricted period of time and there is the opportunity for interaction between several animals in oestrus which may improve the timing of artificial insemination. The use of more complex hormone treatment regimens to improve fertility are discussed at the end of this chapter.

Several points are worthy of further consideration. Firstly, it is important to liaise with the local artificial insemination (AI) organisation before starting the regimen, since it will be able to ensure the availability of inseminations when they are required. Secondly, if DIY AI is used, then the person responsible may quickly suffer from inseminator fatigue if they are not very experienced. Thirdly, particularly in heifers, it is important to ensure that they are receiving adequate nutrition since disappointing conception rates have been obtained when feeding has been inadequate (see Chapter 22). The use of prostaglandins to overcome oestrous detection problems and to treat pathological conditions will be described later (see Chapter 22).

Uses in the mare. There are fewer practical indications for synchronising groups of mares or fillies, although with the increase in the use of AI and embryo transfer in this species it is likely to be utilised more in the future (see Chapter 31 and 35). When it is used, the onset of oestrus is generally well synchronised 3 days after treatment, although the subsequent ovulation has a time spread of 7–12 days (Allen, 1978). Some improvement has been achieved by the injection of hCG or GnRH on the second or third day of the induced oestrus (Allen and Rowson, 1973).

Prostaglandins, both $PGF_{2\alpha}$ and the synthetic analogue cloprostenol, are useful in the breeding management of mares. By enabling mares to be mated on predetermined days it is particularly useful where either the mare or the stallion has to travel a distance for service, and eliminates the need for the frequent teasing of mares. It is also useful if a heat is missed, especially the foal heat, since it enables oestrus to be induced prematurely and obviates the need to wait for the next spontaneous heat.

Uses in the ewe. When $PGF_{2\alpha}$ or an analogue is given to ewes with sensitive CLs, oestrus occurs 36–46 hours after injection (Haresign and Acritopolou, 1978). In order to synchronise a group of ewes at randomly different stages of the oestrous cycle it is necessary to give two injections 8 or 9 days apart. Conception rates and prolificacy following natural matings have been comparable with unsynchronised ewes (Haresign and Acritopolou, 1978). There are obvious advantages of using such a technique in conjunction with AI since it could enable the use of genetically superior sires in many flocks.

Uses in the goat. Two doses of $PGF_{2\alpha}$ each of 2.5 mg, or 100 μg cloprostenol at 10 to 11 days, are effective in synchronisation of oestrus. When a single fixed-time AI was used at 72 hours after the second injection, pregnancy rates of 44.7% were obtained (Simplicio and Machado, 1991).

Uses in the sow. Reliable synchronisation of oestrus in groups of sows and gilts would have many applications, particularly in conjunction with the use of AI and enable batch farrowing to occur. Unfortunately, prostaglandins and their analogues are not luteolytic until the 11th or 12th day of the oestrous cycle; thus, it is not possible to devise a regimen of injections which will synchronise groups of animals with randomly distributed cyclic activity.

However, it is possible to prolong the life span of CLs in the pig with injection of oestrogens on days 10–14 of the oestrous cycle; once this has been done, prostaglandins can be injected after 5–20 days to induce oestrus 4–6 days later (Guthrie and Polge, 1976). Another approach that has been used is to induce luteolysis, by prostaglandin administration, of accessory CLs produced by the injection of eCG and hCG at any stage of the oestrous cycle (Caldwell et al., 1969).

Uses in the bitch and queen cat. Prostaglandins do not readily cause luteolysis in these species.

Hormone combinations

Uses in the cow. In cattle, it has been known that ovulation cannot be synchronised very closely using a simple synchronisation regimen such as prostaglandins or progestogens alone (Coulson et al., 1979), resulting in poor pregnancy rates particularly in parous cows rather than nulliparous heifers when fixed-time AI is used (Peters and Ball, 1994). This is for two reasons: firstly, because of the variability of the luteolytic refractory period of the CL after ovulation and, secondly, the complexities of the follicular waves that occur in cattle. Thus a variety of different regimens have been used in an attempt to manipulate folliculogenesis, and can be summarised as follows:

- A combination which has been utilised for some time involves intravaginal progestogens, such as these applied with a PRID or CIDR, for 8–10 days, followed by the administration of $PGF_{2\alpha}$ or analogue on the day before withdrawal. Such regimens will result in 95% of cows being in oestrus within 5 days (see the review by Odde, 1990).

- Another approach involves treatment with GnRH on day 0, followed by $PGF_{2\alpha}$ on day 7, followed by GnRH on day 9 or 10. There are two rationales behind the use of the first GnRH treatment. Firstly, it ensures that a new dominant follicle has emerged at the time of prostaglandin treatment and can grow and ovulate. Secondly, it can extend the life span of the CL in late dioestrus so that it is still responsive to $PGF_{2\alpha}$ 7 days later (Peters et al., 1999). The second injection of GnRH should ensure better synchronisation of ovulation by stimulating the preovulatory LH surge (Coulson et al., 1980). Cows are inseminated once, in the case of the regimen described by Mawhinney et al. (1999) 68–72 hours after prostaglandin treatment. Pregnancy rates obtained in this study, which involved cows as opposed to heifers, were 44% compared to 52% in the control animals which were inseminated at observed oestrus.
- Exogenous steroid hormones can modify folliculogenesis. Oestradiol has been used to suppress the inhibitory effect of the dominant follicle, thereby allowing the emergence of a new follicular wave. Much better results have been obtained by using a combination of oestradiol and progestogens, which by inhibiting both FSH and LH secretion, suppressed folliculogenesis. Thus when their influence waned a new follicular wave emerged at a predictable time irrespective of the stage of the oestrous cycle at which they were given (Garcia et al., 1999).
- GnRH is given at the same time as a CIDR is inserted and, 24 hours before the latter is removed at 10 days, $PGF_{2\alpha}$ is injected. The GnRH stimulates folliculogenesis, so that at the time of $PGF_{2\alpha}$ treatment a dominant follicle is present which is capable of maturation and ovulation once the negative-feedback effect of progesterone has been removed with the withdrawal of the CIDR.

Such combinations of hormones can reduce the need for repeat inseminations, whilst ensuring acceptable pregnancy rates; however; they can be costly and also require additional handling of animals. It is important to stress these facts to farmers before embarking upon such regimens. Mawhinney et al. (1999), using the $PGF_{2\alpha}$ and double GnRH regimens, showed that such an approach can increase the number of cows in calf by specific time intervals after calving in seasonally calving herds, and reduce the mean calving–conception interval by 15 days. However, they stress that improvements may be obtained in herds with average or below-average fertility, rather than in herds with good fertility.

Prolactin inhibitors

In bitches, the use of prolactin inhibitors such as cabergoline and bromocriptine in anoestrus can induce a fertile oestrus with normal pregnancy rates if they are mated; the mode of action is unknown.

Melatonin

The pineal gland controls reproductive activity in seasonal breeding species such as sheep, goats, horses and cats by the secretion of melatonin. Perhaps not surprisingly, it cannot been used to modify seasonal activity in the mare because it would be necessary to inhibit the secretion of melatonin or neutralise its effect to advance the time of onset. However, in the ewe and doe, which are short-day breeders, it has been used commercially to advance the timing of the onset of the breeding season. The hormone is administered as an implant containing 18 mg of melatonin which is inserted subcutaneously at the base of the ear.

It is critical that rams (and bucks) should be separated from the ewes so that they are out of sight, sound and smell at least 7 days before the insertion of the implant. They must remain separated for at least 30 days and not more than 40 days, when rams (or bucks) should then be reintroduced. Peak mating activity occurs 25–35 days later. Melatonin should not be used in ewe lambs.

The breeding season can be successfully advanced by 2–3 months with good fertility.

Immunisation procedures

Increased lambing rates have been obtained by the use of an immunogen, produced by conjugating a derivative of the natural ovarian hormone androstenedione with human serum albumen. When injected into ewes the conjugate stimulates the production of antibodies to androstenedione, which binds free, naturally occurring androstenedione in the blood. This results in an increase in the ovulation rate and the number of lambs born; the precise mode of action is not fully understood (Scaramuzzi et al., 1983; Harding et al., 1984).

The conjugant is injected twice, 8 and 4 weeks before tupping, although if ewes have been treated in the previous season one injection only is required (4 weeks before tupping). The effect of immunisation is to increase the lambing percentage by about 25%.

It is important that only those ewes which are likely to be fed adequately during pregnancy should be treated because of the dangers of pregnancy toxaemia; for this reason, mountain and hill breeds should not be treated.

Immunisation against inhibin, which has been used experimentally to increase the ovulation rate in cattle and sheep, may well become available for commercial use.

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2 Development of the conceptus

As the ripe follicle is about to rupture, the fimbriated end of the uterine tube is applied to it and, at ovulation, the follicular fluid and egg are discharged. If the female has been mated at the current oestrus, spermatozoa will be waiting in the ampulla of the uterine tube for the arrival of the oocyte. Although the nucleus of only one sperm is required to fuse with that of the ovum, it has been estimated that approximately a million sperms are necessary to create a suitable environment for fertilisation. Following fertilisation, cleavage of the zygote begins and, as a result of peristaltic contractions and ciliary currents in the uterine tube, it is propelled towards the uterus. When it reaches the uterus, at 3–4 days in cattle and at 5–8 days in the dog and cat, the zygote consists of 16–32 cells in the form of a morula. With further cell division and cell orientation the morula becomes hollowed out to form a blastocyst. Up to the time of shedding of the zona pellucida at the ninth day there is little absolute growth of the mammalian egg from its original dimension of 0.14 mm. The fertilised egg of the ewe reaches the uterus at the eight-cell stage on day 3, while in the sow it passes down the uterine tube within 2 days of ovulation and arrives in the uterus at the four to eight-cell stage. Tubal transport of the fertilised eggs of the mare probably takes 5–6 days, by which time they are at the blastocyst stage, but van Niekerk and Gernaeke (1966) have shown that unfertilised equine eggs remain in the tubes for several months, where they slowly degenerate.

The variable duration of travel by the fertilised egg in the uterine tube of the domestic species appears to be determined positively by the degrees of activity of the tubal muscle and cilia and negatively by the muscular constriction either at the tubal isthmus or at the uterotubal junction; both positive and negative factors are probably influenced by variable concentration of ovarian steroids and possibly by locally produced prostaglandins.

From the time of its arrival in the uterus until attachment, the zygote is propelled or aspirated in the uterine lumen, where it is nourished by the uterine milk. In the polytocous species, the blastocysts are arranged throughout the uterus so as to utilise the uterine space effectively; thus there occurs free migration of embryos between the cornua, regardless of the side of ovulation. In the monotonous cow such migration hardly ever occurs, but in the ewe it is not uncommon.

After the ninth day, the blastocyst elongates rapidly. For example, the sheep embryo is 1 cm long at 12 days, 3 cm at 13 days and 10 cm at 14 days, while by day 13 in the sow the apparent length of each blastocyst is about 33 cm, but its real length when disconnected from the much corrugated endometrium is 55–191 cm; by day 21 in the mare the blastocyst measures 7 × 6.5 cm, but in the cow it extends almost throughout the pregnant horn. Embryonic attachment to the uterus occurs at the following times: 12 days, cow; 14 days, sow; 15 days, ewe; 13–17 days, dog and cat; and 25–30 days, mare.

Interesting facts about the intrauterine mobility and location of the equine embryo have been obtained by means of ultrasonic echography by Leith and Ginther (1984). The location of the vesicle was observed in seven mares over a 2-hour period daily from days 9–17. There was increasing mobility from day 9 to day 12, and a plateau of high mobility persisted until day 14, after which there were few location changes on day 15 and none on days 16 and 17, indicating that fixation had occurred. On days 9 and 10 the vesicle was at least twice as likely to be in the uterine body as in a uterine horn; on days 11–14 this proportion was reversed, and beyond day 15 the embryo was always cornually disposed.

The bovine embryo itself, although differentiating fast, elongates slowly as compared with the chorion, and at a month after mating it is only just over 1 cm long. The chorionic vesicle, which is at first string-like with a central distended sphere of amnion containing the embryo, is progressively filled by allantoic fluid to form an extensive allantochorionic sac; this first begins to distend the gravid cornu at about 35 days (Figure 2.1). At this time, the chorion already extends into the non-gravid horn; its length is about 40 cm and at its widest part in the dependent portion of the gravid horn it is 4–5 cm in diameter. The early development of the sheep is very similar to that of the cow, but the equine conceptus does not show the initial rapid elongation of the blastocyst-chorion. For example, at 35 days the equine chorion is oval rather than cylindrical and is more distended by the allantoic fluid. This causes an earlier, more discrete uterine enlargement than in the cow, and this is helpful in early clinical pregnancy diagnosis (Figure 2.2).

The allantois, which is an outgrowth of the embryonic hindgut, spreads out into the chorionic vesicle; as a protruding sac, it makes contact outwardly with the chorion to form the vascular allantochorion, and inwardly fuses with the amnion, to give rise to the allanto-amino. The allantochorion, which eventually surrounds the allanto-amnion, is separated from it by allantoic fluid. When the vas-

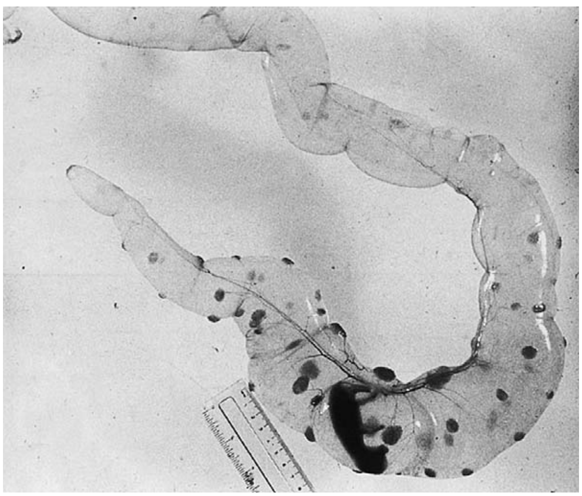

Fig. 2.1 Bovine conceptus at 60 days. Note the fetus contained within the discrete – almost spherical – amniotic sac, with an elongated allantochorion and evidence of the formation of cotyledons.

Fig. 2.2 Equine conceptus at 7 weeks. The allantochorion has been opened to show the yolk sac and early fetus within the amnion.

cularisation of the chorion by the allantois is complete (at 40–60 days in the cow) the allantochorion is ready to participate in placental function (Figures 2.1 and 2.2). Prior to this time, the embryo has been nourished through its chorion and amnion by diffusion from the uterine milk. In the ruminant uterus, where the allantochorion contacts the uterine caruncles, finger-like processes or villi containing capillary tufts grow out from the allantochorion into the crypts of the maternal caruncles, which are also surrounded by capillary plexuses. Thus is formed the characteristic ruminant cotyledon, or placentome, through which nutrient and gaseous exchange between the mother and fetus takes place. On average, there are some 120 functioning cotyledons in the cow (Figure 2.3) and about 80 in the sheep, arranged in four rows along each of the uterine horns. It will be recalled that the chorion, and following it the allantois, extend into the non-gravid horn, and thus it is normal in the ruminant for there to be numerous functioning cotyledons in the non-pregnant horn. The pregnant uterus of the mare, sow, bitch and cat shows no cotyledons, for in these species the villi are dispersed over the placental area.

During early development of the ruminant embryo, there occurs an extensive fusion between the allanto-amnion and the allantochorion, thus largely obliterating the cavity of the allantois. As a result, where it lies over the amnion, the allantois is reduced to a narrow channel. Here its shape

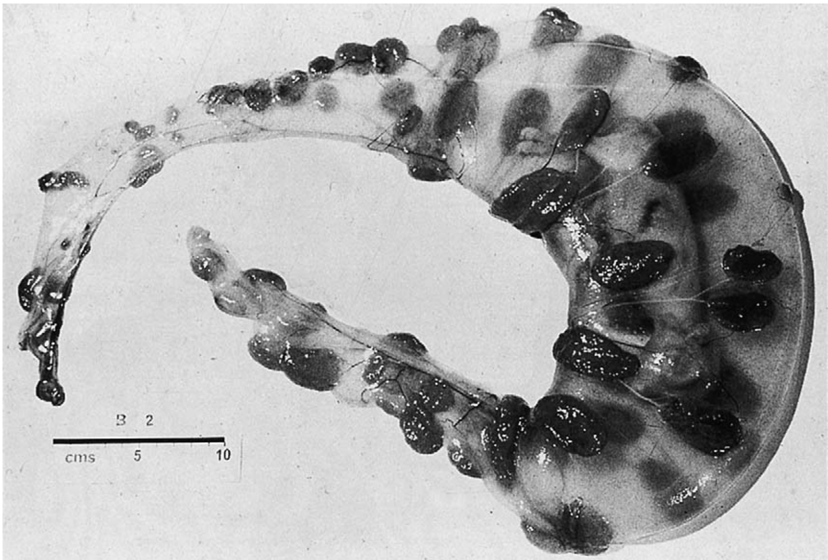

Fig. 2.3 Bovine conceptus at 90 days. Note the relatively larger amniotic sac compared with Figure 2.1, and obvious cotyledons which are larger towards the centre of the allantochorion.

resembles the letter 'T' with the stem coming out of the urachus, along the umbilical cord and then diverging as the two cross-pieces over the lateral face of the amnion. Consequently there is little allantoic fluid over the amniotic area; most of it lies in the extremities of the allantois, one of which lies in the non-gravid horn (Figures 2.3–2.5). A similar fusion takes place between the amnion and allantois of the pig. However, studies of bovine

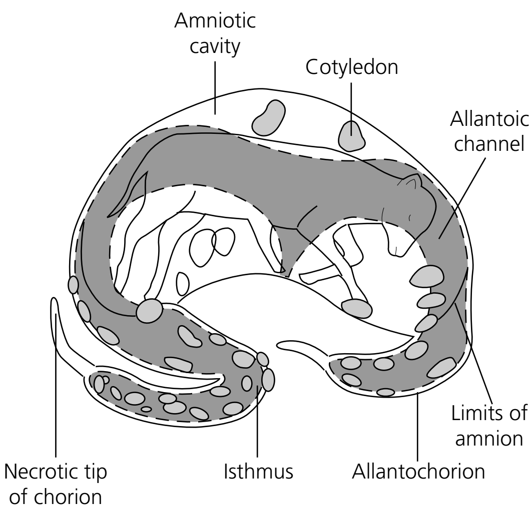

Fig. 2.4 Bovine conceptus showing the allantochorion of early pregnancy. (After Zeitzschmann, 1924.)

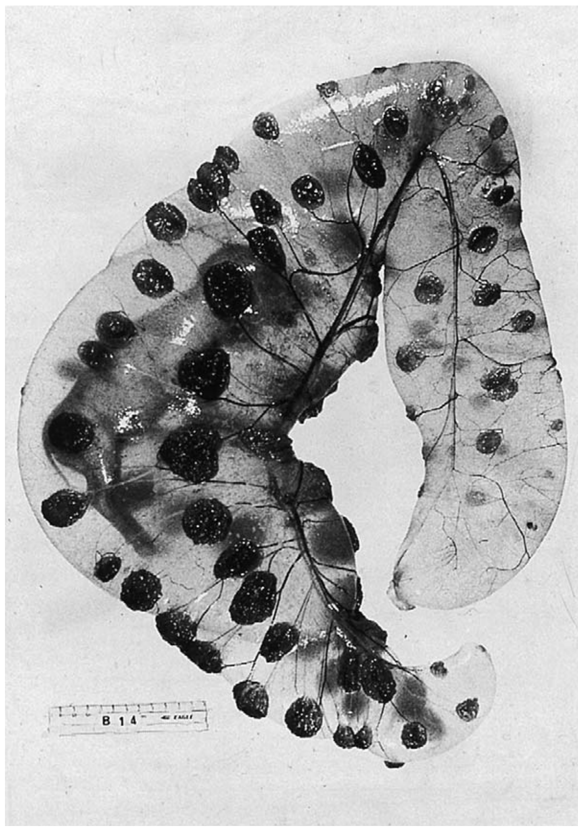

Fig. 2.5 Bovine conceptus at 115 days. Note the blood supply to and from the developing cotyledons.

uteri in late gestation (Arthur, 1959) have shown that, with the increasing pressure of accumulating allantoic fluid, the allantochorion tends to become separated again from the allanto-amnion so that at term the allantois may almost surround the amnion. Thus the final arrangement of the two fetal sacs may closely resemble that of the horse, in which species the amnion, except for its attachment at the umbilicus, floats freely in the allantoic fluid throughout gestation (Figures 2.6 and 2.7). The relationships of the fetal sacs at birth will be referred to again in Chapter 6 on parturition.

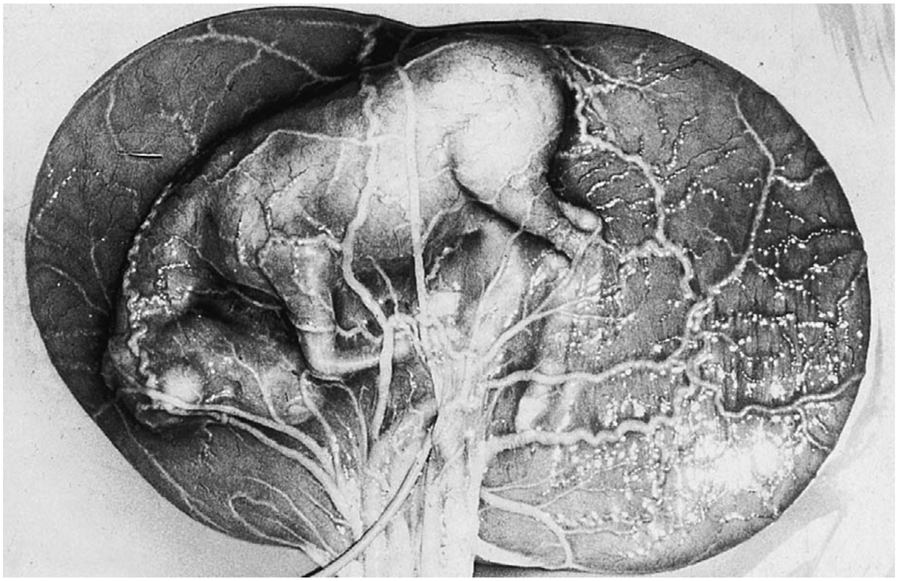

Fig. 2.6 Equine fetus of approximately 3 months of age. The allantochorion has been incised to expose the fetus enclosed within the almost transparent amnion. Note the umbilical cord at the bottom of the picture and the tortuous blood vessels on the surface of the amnion.

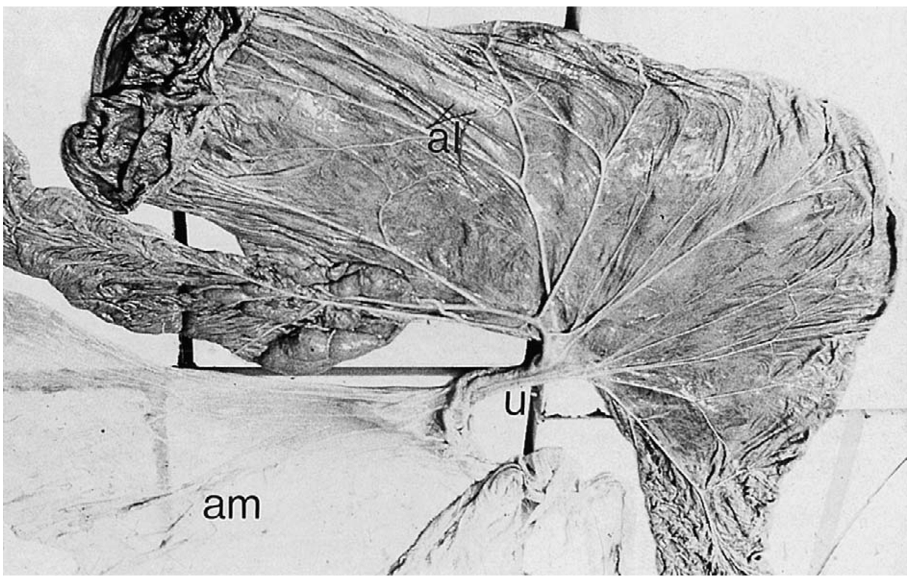

Fig. 2.7 Equine fetal membranes at term. Note the denser allantochorion (al), which is red in colour with its smooth inner surface exposed, the amnion (am), which at term is almost transparent, and the umbilicus (u).

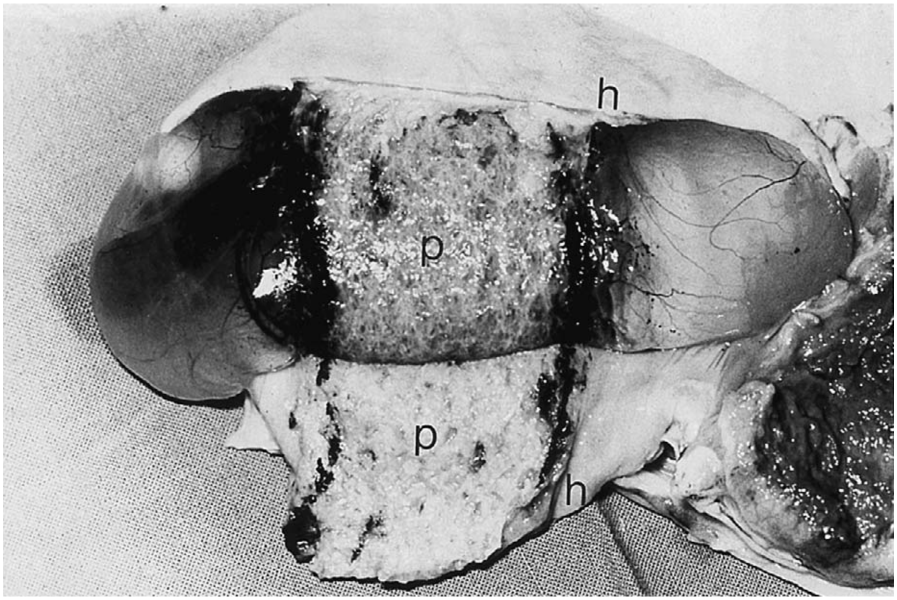

Fig. 2.8 Canine conceptus at approximately 8 weeks of gestation, partially contained within part of the uterine horn, showing marginal haematomata on the allantochorionic sac (h), and the areas of placentation (p).

In carnivora, as in the domestic herbivora, the allantois also grows out into the cylindrical chorion, but only the central part of it becomes vascularised and serves as a placenta. The amnion is surrounded by allantoic fluid, as in the horse (Figures 2.8 and 2.9). A 35-day beagle embryo is 35 mm in crown–rump length and increases by 6 mm per day between the 35th and 40th days (Evans, 1983).

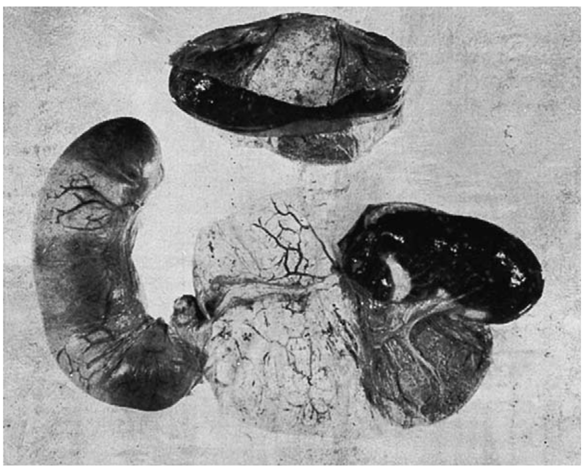

Fig. 2.9 Uterus from a pregnant cat near term, dissected to show the placental areas and zonal placenta.

TYPES OF PLACENTA

Placentae may be classified according to the way the villi are distributed on the fetal chorion. Thus, where they are uniformly dispersed, as in the mare and sow, the placenta is said to be *diffuse*. Where they are grouped into multiple circumscribed areas, as in the ruminant, the placental arrangement is called *cotyledonary*, while in the bitch and cat the villi are disposed in the form of a broad encircling belt forming a *zonary* placenta.

Formerly, the placentae were differentiated according to whether or not maternal tissue separated off with the fetal tissue at birth. Thus, of the domestic mammals, the placentae of the bitch and cat were said to be deciduate and those of the remainder non-deciduate.

More recently, embryologists have favoured Grossers (1909) division of placental types in which the degree of proximity of the maternal and fetal blood circulations is the criterion of classification. Such a concept recognises the phagocytic property of the trophoblast, or chorionic epithelium, that may be exerted on tissues with which it comes in contact. In the simplest, or *epitheliochorial* type of placenta, seen in the horse and pig, the

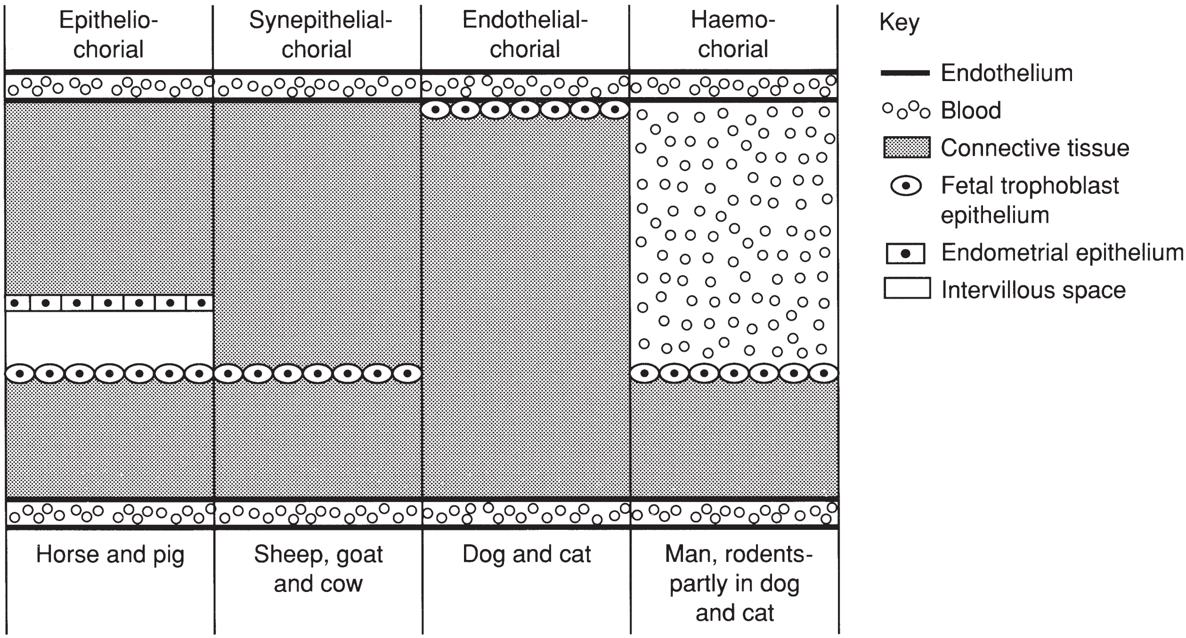

Fig. 2.10 Diagrammatic representation of types of placenta found in domestic species based on Grosser's original classification.

chorion is everywhere in contact with the endometrium, and there is no loss of maternal tissue. In the cow, the placenta is described as being *synepithelialchorial* (Wooding, 1992). Soon after embryonic attachment a syncytium is formed on the maternal side of the placentome by the fusion of binucleate cells derived from the trophectoderm and the endometrium. Unlike the sheep and goat the syncytium is only temporary, as fairly soon the syncytial plaques are overgrown by the rapid division of the remaining maternal epithelium (King et al., 1979). In the third, *endotheliochorial* type, there is further invasion of the endometrium by the trophoblast, which is now apposed to the maternal capillaries. Such a type is typical of the carnivora. In the *haemochorial* placenta of primates only the tissues of the chorionic villi separate the fetal and maternal blood. The placenta of the dog and cat is partly haemochorial in that the main zonary placenta of endotheliochorial type is flanked by marginal haematomata – 'the green border' in the dog and 'brown border' in the cat – in which an accumulation of maternal blood between the uterine epithelium and the chorion directly bathes the chorionic villi that project into it. When separation of the canine placenta begins at parturition, it is the escape of this altered blood from the marginal haematoma which gives the characteristic green colour to the normal parturient discharges. A simple diagrammatic representation of the types of placenta based on Grosser's (1909) original classification is shown in Figure 2.10.

The degree of intimacy of the maternal and fetal placental blood vessels is the basis for the variable function of the 'placental barrier' of different species. This is of interest in certain diseases of the fetus and newborn. For example, in the haemolytic disease of foals antigens from the fetus pass across the placenta to the mother but the resultant antibodies can return to the foal only via the colostrum, whereas in women similar antibodies may traverse the placenta and cause an antigen –antibody reaction in the unborn fetus.

FETAL FLUIDS

Sheep

The studies of Malan and Curson (1937) and Cloete (1939) have revealed that the total volume

Table 2.1 Volume of fetal fluids in the sheep (ml) (data from Malan and Curson, 1937 and Cloete, 1939)

Month of gestation	*Amniotic*	*Allantoic*	*Total*
1	3	38	41
2	169	89	258
3	604	131	735
4	686	485	1171
5	369	834	1203

of fetal fluid increases with advancing age of the conceptus, but that the separate fetal fluid volumes show different tendencies. Thus, during the first 3 months, apart from an initial preponderance, the allantoic fluid accumulates slowly, e.g. 131 ml at 3 months, whereas the increase in amniotic fluid occurs largely during this time and at 3 months reaches 604 ml. In the fourth month the increase in allantoic fluid is greatly accelerated to 485 ml, while the amniotic fluid increases only slightly. During the last (fifth) month of gestation the allantoic fluid almost doubles its volume to 834 ml but the volume of amniotic fluid diminishes to 369 ml (Table 2.1). When twins are present the totals of fluid are approximately doubled (Arthur, 1956).

Cattle

The total quantity of fetal fluid of cattle increases progressively throughout pregnancy; it averages about 5 litres at 5 months and 20 litres at term. Sharp rises in the total quantity occur between 40 and 65 days, between 3 and 4 months and again between $6\frac{1}{2}$ and $7\frac{1}{2}$ months. The first and last of these are due to allantoic and the second to amniotic increases. For nearly the whole of the first third of pregnancy, when the conceptus consists of an elongated allantochorion with a central spheroidal amnion – closely investing the relatively small embryo – there is more allantoic fluid; during most of the second third of pregnancy amniotic fluid predominates but for the greater part of the final third allantoic fluid is again clearly in excess (Figure 2.11).

Throughout gestation the allantoic fluid is watery or urine-like. In the first two-thirds of pregnancy the amniotic fluid is similar, but for the

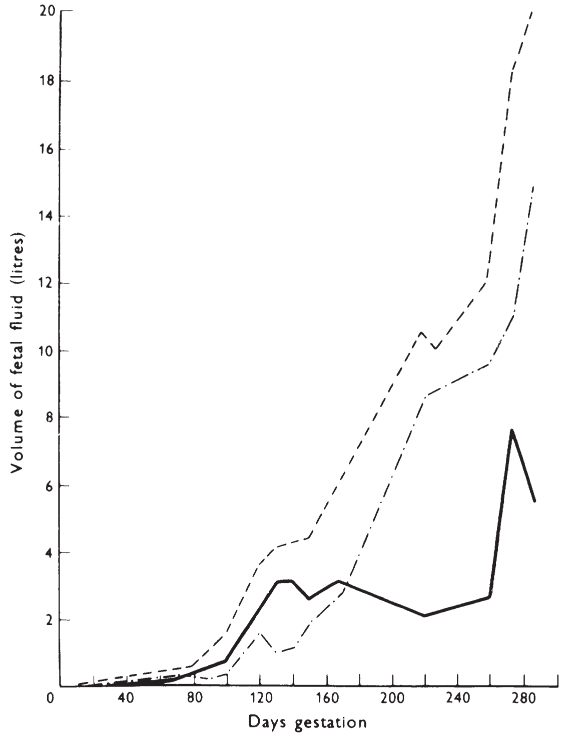

Fig. 2.11 Volume of fetal fluids at successive stages of pregnancy in the cow. –––––, total; –·–·–·–; allantoic; ————, amniotic (from Arthur, 1969).

remainder of gestation it is a mucoid fluid. The latter change gives it the lubricant property which is so helpful at parturition. At birth the allantoic sac forms the first and the amnion the second 'water-bag'. The allantochorion is thicker and tougher than the transparent amnion.

Horses

Ranges of weights of the total fetal fluid for each month of pregnancy in the mare as given by Richter and Götze (1960) are shown in Table 2.2.

Serial values for the separate amounts of amniotic and allantoic fluids are not available, but the observations of Arthur (1956) (Table 2.3), together with data provided by Amoroso (1952) and Zietzschmann (1924), suggest that the trends are similar to those of cattle. Thus, allantoic fluid predominates in early as well as late pregnancy and measures 8–15 litres at term. The amniotic fluid volume differs from that of cattle. It is low during the first 3 months: for example, only 27 ml

Table 2.2 Weight of fetal fluids in the horse

Month of gestation	*Weight of total fluid (kg)*
1	0.03–0.04
2	0.3–0.5
3	1.2–3.0
4	3.0–4.0
5	5.0–8.0
6	6.0–10.0
7	6.0–10.0
8	6.0–12.0
9	8.0–12.0
10	10.0–20.0
11	10.0–20.0

Table 2.3 Volume of fetal fluids in the horse

Estimated gestation (months)	*Fetal body length (cm)*	*Amniotic (ml)*	*Allantoic (ml)*
3	10	30[a]	2300
$4\frac{1}{2}$	20	100	4090
6	44	6200	4090
	77	3700	4800[b]
9	80	9200	—
	81.5	1670	5210

[a] Estimated volume in a preserved specimen
[b] Some allantoic fluid lost in transit

at 74 days. Thereafter it increases more rapidly and at mid-pregnancy equals the volume of allantoic fluid, while at term it is 3–5 litres.

Pigs

The volume of allantoic fluid shows an early rise to about 130 ml at 1 month. Thereafter it increases gradually to nearly 200 ml, although a terminal decrease to 100 ml is sometimes seen. During the first 2 months the amount of amniotic fluid does not exceed 20 ml; in the next months it rises to a maximum which may vary from 75 to 200 ml; thereafter there is wide variation with a tendency to decline.

Carnivora

In kittens of fetal body length up to 9 cm the amniotic fluid rises gradually to 10–15 ml, after which there is some decrease followed by a slight rise just before term. The allantoic fluid starts with a more rapid rise, and at mid-term is higher than the amniotic (20 ml), but towards the end of gestation declines to about 6 ml.

FORM AND FEATURES OF THE FETAL SACS

Ruminants

Throughout gestation the amnion enclosing the fetus, together with the larger portion of the allantochorion, remains in the uterine horn corresponding to the ovary with the corpus luteum; a smaller ‘limb’ of allantochorion projects across the uterine body into the other horn. Most of the allantoic fluid gravitates to the poles of the allantochorion, which lie in the dependent parts of the uterine horns, and the uterine distension thus caused in cattle is the chief clinical sign of early pregnancy. By the third month, considerable fluid (up to 0.75 litres) has accumulated in the spherical amnion, and it now gives rise to the main palpable mass in the pregnant horn (see Figure 2.3).

On the inner face of the amnion of ruminants, particularly near the umbilicus, are numerous raised, rough, discrete, round foci called amniotic plaques. They are rich in glycogen but of unknown function and disappear after 6 months of gestation. Towards the end of pregnancy smooth, discoid, rubber-like masses float in the amniotic and occasionally in the allantoic fluids. They probably comprise aggregations of fetal hair and meconium around which salts are deposited from the fetal fluids. They are called ‘hippomanes’ and are of no functional significance.

Mare

Much of the allantochorion and the greater part of the amnion are contained within the gravid horn with a direct continuation of similar width into the uterine body. The part of the allantochorion which projects into the non-gravid horn is much narrower and is about two-thirds the length of the gravid horn segment, but in the rare bicornual pregnancy the allantochorion occupies both cornua to a similar extent.

Projecting into the allantoic fluid are peculiar invaginations of the allantochorion. They are first found at a fetal body length (FBL) of about 11 cm and occur in juxtaposition with the endometrial cups whose secretion accumulates in them. Their size corresponds with the secretory activity of the endometrial cups, being largest at FBL 15–20 cm, and regressing after FBL 30 cm. When distended with secretion they are appropriately called allantochorionic pouches. They are few in number, not more than six, and are sometimes absent. The endometrial cups are crateriform structures which are disposed in a concentric manner at the base of the pregnant horn. They are present from the sixth to the 20th week of gestation, and in them the equine chorionic gonadotrophin (eCG) is produced. The endometrial cups are formed from cells which invade the endometrium from the trophoblastic girdle of the embryo (Allen and Moor, 1972); this invasion provokes a reaction by the maternal tissue and leads to the dehiscence of the endometrial cups at about day 140.

The immunological importance of the endometrial cups in protecting the 'foreign' conceptus has been demonstrated by Allen (1982). In interspecies transfers of fertilised eggs between horses and donkeys no endometrial cups were formed, and the donkey fetuses died at 80–90 days.

The surface of the allantochorion adherent to the endometrium is red in colour and has a 'velvety' appearance and texture. The area adjacent to the internal opening of the cervix is devoid of placental villi, giving rise to the so-called called 'star' (Figure 2.12). The inner surface of the allantochorion which is outermost when the placenta is shed has a smooth surface (see Figure 2.7).

Sow

The uterine surface of the sow's allantochorion is studded with small, round, grey foci called 'areolae' in which villi are absent. They occur opposite focal aggregations of uterine glands.

RELATIONSHIP BETWEEN FETAL MEMBRANES OF TWINS AND MULTIPLE FETUSES

The relationship of the membranes of contiguous fetuses is simplest in carnivora, in which, although the extremities of the allantochorionic sacs impinge on each other, they remain separate. The next gradation is in the mare pregnant with twins; here, apparently owing to the lack of uterine length, the distal pole of one allantochorion invaginates the

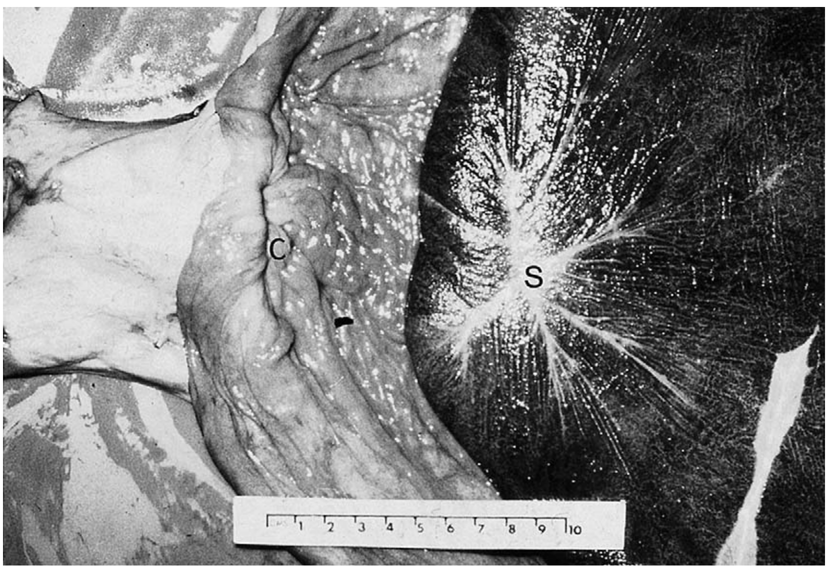

Fig. 2.12 Outer surface of the allantochorion showing the 'star' (s) which is adjacent to the internal opening of the cervix (c) and is devoid of placental villi.

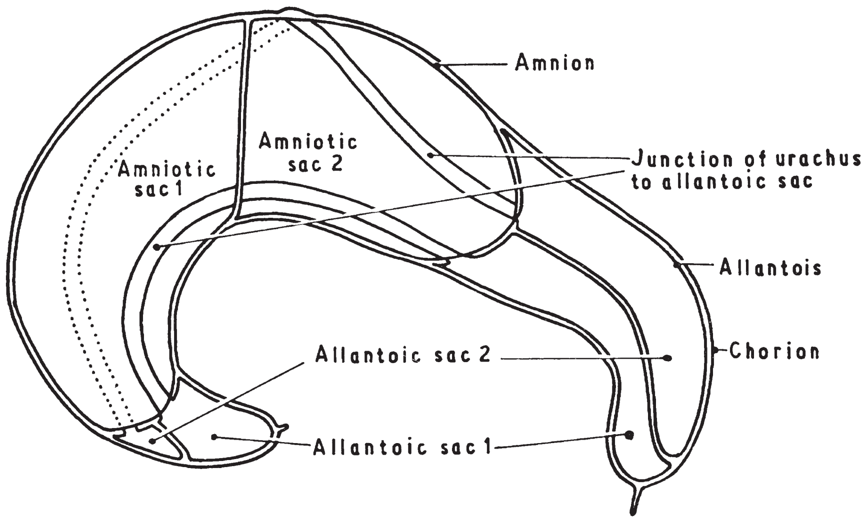

Fig. 2.13 Fetal membranes of bovine twins in unicornual gestation (courtesy of M. J. Edwards).

proximal extremity of the other. According to Williams (1939) this results in an unequal sharing of the uterine space and is the reason for the commonly observed disparity of twin foals. Adjacent membranes of porcine fetuses tend to become 'glued' together by a gelatinous substance but the junction is not a strong one. In 66% of cases the adjacent chorions grow together and the intervening tissue breaks down with the formation of a common chorion, and occasionally with a common allantoic cavity. In the rare case of anastomosis of allantoic blood vessels of porcine fetuses of unlike sex, a basis for the 'freemartin' condition exists, as in cattle (see Chapter 4).

In the majority of twin or triplet pregnancies of sheep and cattle, contiguous chorionic sacs coalesce (Figures 2.13 and 2.14) and in many cases the allantoic cavities are confluent, while in cattle – but only in about 0.8% of sheep – allantoic vascular intercommunication is the rule. Such anastomosis, according to Lillie (1917), is present between bovine fetuses from the 40th day, and it forms the main premise of his theory of origin of the bovine freemartin (see Chapter 4).

Of 25 sets of equine twins, 11 were found by Vandeplassche et al. (1970) to have blood chimerism, thus indicating vascular anastomosis between the twin placentae. However, on dissection, the genital organs of the female members of

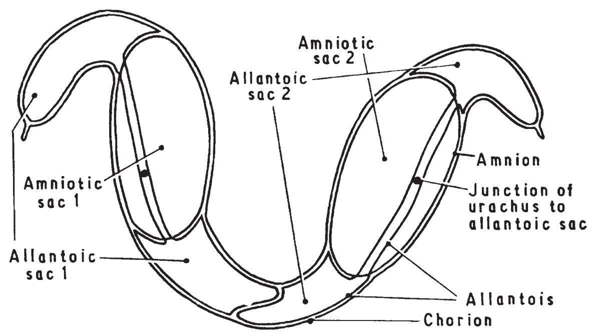

Fig. 2.14 Fetal membranes of bovine twins in bicornual gestation (courtesy of M. J. Edwards).

four heterosexual chimerical pairs were found to be normal while five other mares of heterosexual chimerical pairs were found clinically to have normal genitalia, and they had normal oestrous cycles and became pregnant. The fact that blood chimerism has been found in adjacent fetuses of horse, sheep and pig – as well as cattle – gestations indicates that there is a basis for the freemartin condition in these species also. The observed incidence of freemartinism is very low, however, in pigs and sheep (see Chapter 25) and is nil in horses, and this may be due, according to Vandeplassche et al. (1970), to late allantoic vascular anastomosis in these three species as compared with the early (30-day) fusion in cattle.

FETAL MOBILITY DURING PREGNANCY

While discussing placental relationships it is instructive to consider the subject of fetal mobility within the uterus. Obviously in all species, within the amnion, fetal movement around both longitudinal and transverse axes is possible. Rotation about the first is limited by the length of amniotic umbilical cord and about the second when the length of the fetus exceeds the width of the amnion. In cattle, not more than a three-fourths revolution around the long axis is possible and, although several turns around the transverse axis may occur, a complete revolution of the bovine umbilical cord is not normal and has been seen only in mummified fetuses. In equine and porcine fetuses, however, complete revolutions of the amniotic portion of the umbilical cord are common.

Another possibility of intrauterine fetal movement is the potential mobility of the amniotic sac (with contained fetus) within the allantochorion. Owing to the extensive fusion of the allantochorion to the allanto-amnion in the cow, ewe and sow, such mobility is impossible (except perhaps near term) whereas in the mare, bitch and cat such movement does take place and leads to twisting of the allantoic portion of the umbilical cord.

Data collected from pregnant bovine uteri by the present author and from equine specimens by Vandeplassche (1957) (Table 2.4) indicate that initially anterior and posterior presentations occur in equal numbers. At between 5 and 6 months of pregnancy the body length of the fetal calf exceeds the width of the amnion and thus at this stage the final polarity of 95% of fetuses in anterior presentation is adopted. In the mare, however, at 6½ months, 40% of fetuses are still in posterior presentation; the final gestational presentation of 99% of foals anteriorly disposed is not taken up until the ninth month. Messervy (1958), from observations made during laparotomy of pregnant mares, has also concluded that the presentation of a foal may alter after the eighth month. It would seem likely that these changes of longitudinal presentation that occur during late gestation in the mare are due to movements of the amnion within the allantochorion. The reason for the final overwhelming proportion of anterior presentations in the mare and cow is not known.

The size of the bovine fetus at the various stages of pregnancy is given on p. 84 and the fetal growth curve is shown in Figure 2.15.

Richardson et al. (1976) have shown that long bone length (conveniently radius and tibia) is a reliable indicator of fetal age from 50 days of

Table 2.4 Pregnant equine uteri: disposition of fetus

Months of gestation	*No. of cases*	*Anterior presentation*	*Posterior presentation*	*Right horn pregnant*	*Left horn pregnant*
2–3½	4	2	2	2	2
3½–4½	12	7	5	6	6
4½–5½	9	4	5	6	3
5½–6½	16	9	7	11	5
6½–7½	12	8	4	7	5
7½–8½	11	9	2	8	3
8½–10	4	4	0	3	1
10½–11	4	4	0	3	1
11–12	3	3	0	2	1
Total	75	60	25	48 64%	27 36%
10	1	Transverse presentation		Bicornual pregnancy	
5	1			Twin pregnancy	

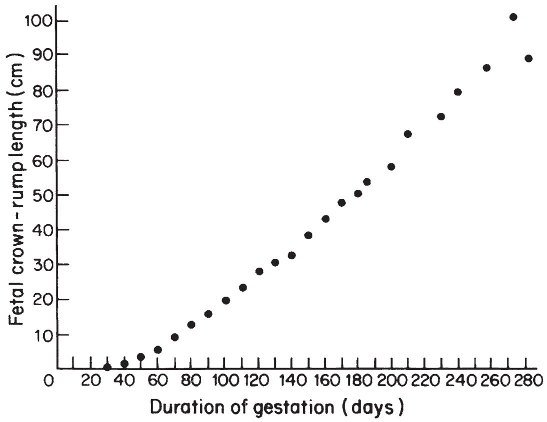

Fig. 2.15 The growth curve of the bovine fetus.

Table 2.5 Length of ovine fetal long bones at various stages of conception

Days after conception	*Length of radius (mm)*	*Length of tibia (mm)*
50	4.8	5.0
60	10	12
70	16	19
85	25	32
100	36	47
110	47	63
120	56	76
130	67	91
140	74	100
150	79	107

gestation to term in the sheep, and may be obtained radiographically in the living fetus or by post-mortem measurement. Mean values for the respective ages are shown in Table 2.5.

Richardson (1980) has provided the following formulae for calculating the age of the fetus from its crown–anus length:

$$\text{Pig } X = 3\ (Y + 21)$$

$$\text{Calf } X = 2.5\ (Y + 21)$$

$$\text{Lamb } X = 2.1\ (Y + 17)$$

where X is the developmental age in days and Y is the crown–anus length in centimetres.

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3 Pregnancy and its diagnosis

MATERNAL RECOGNITION OF PREGNANCY

In most domestic species, the establishment and maintenance of pregnancy require that the luteal phase of the oestrous cycle is prolonged by the persistence of a single corpus luteum (CL) or a number of corpora lutea (CLs). As a result of the persistence of the luteal tissue, progesterone concentrations remain elevated. This results in a negative feedback on the hypothalamus and anterior pituitary with a resultant inhibition of follicular development and ovulation and, in polyoestrous species, a prevention of return to oestrus. In many species, the placenta subsequently replaces or supplements the luteal source of progesterone.

In Chapter 1 the importance of the CL in regulating the periodicity of the oestrous cycle was discussed, and the role of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), produced by the endometrium, in causing regression of the CL and the consequent return to oestrus was described. The presence of a viable, developing embryo(s), however, prevents the CL from regressing and thus, in polyoestrous species, inhibits the return to oestrus. This phenomenon was described by Short (1969) as the 'maternal recognition of pregnancy'. It is particularly interesting because a maternal endocrine response is detectable before the blastocyst is attached to the endometrium by microvilli, which either directly or indirectly prevents regression of the CL. In five of the domestic species the time of maternal recognition of pregnancy has been determined (Table 3.1).

Table 3.1 Time of maternal recognition of pregnancy (after Findlay, 1981)

Species	*Day of maternal recognition of pregnancy*	*Day of definite attachment*
Sow	12	18
Ewe	12–13	16
Cow	16–17	18–22
Mare	14–16	36–38
Goat	17	

The sheep

In sheep, early work by Moor (1968) and Martal et al. (1979) demonstrated that the conceptus produces a protein. In recent years it has been characterised as existing in three or four isoforms of molecular weight about 18 000; originally it was named ovine trophoblast protein or oTP-1. This substance has been shown to be a type 1 interferon, which together with the same substance produced by the bovine conceptus, is classified as a tau interferon (IFN-τ). It is produced by the trophectoderm from about day 10, when the blastocyst starts to elongate.

Details of the mechanism of luteolysis have been described in Chapter 1. The effect of IFN-τ in the maternal recognition of pregnancy is to alter the dynamics of $PGF_{2\alpha}$ secretion at this early stage of pregnancy, compared with the same stage of the oestrous cycle. The number of luteolytic pulses is reduced between days 15 and 17, yet surprisingly the basal secretion of $PGF_{2\alpha}$ is increased at this time. It is likely that IFN-τ prevents the rise in endometrial oestrogen receptors which precedes the rise in endometrial oxytocin receptors, which is necessary for the secretion of $PGF_{2\alpha}$. The consequence of this is that there is a reduction in the synthesis of $PGF_{2\alpha}$ from arachidonic acid. Tamby et al. (1993) found that endometrial tissue from pregnant ewes (days 12, 14 and 16) had lower phospholipase A_2 (PLA_2) activity than tissue from non-pregnant ewes on the same days after ovulation. Endometrial homogenates on day 16 of pregnancy exerted a greater inhibition of pancreatic PLA2 activity than did homogenates from non-pregnant ewes, and in the endometrial tissue from the same pregnant sheep oxytocin induction of phospholipase C activity was absent. However, oxytocin and arachidonic acid could induce $PGF_{2\alpha}$ secretion at these times, and thus arachidonic acid availability in the endometrium of pregnant ewes may be the limiting factor in preventing the synthesis of $PGF_{2\alpha}$ (Thatcher et al., 1995).

The cow

In the cow, the importance of the blastocyst in prolonging the life span of the CL was shown by the studies of Northey and French (1980). They found that, if the blastocyst was removed at day 17 or day 19, the interoestrus intervals were extended to 25 and 26 days, respectively, compared with those in which the embryo was removed at day 13, or which were not mated; in the latter cases the intervals were 20–21 days. The antiluteolytic signal produced by the bovine conceptus is a protein with a molecular weight of 24 000. Originally it was called bovine trophoblast protein (bTP-1), and was found to cross-react immunologically with oTP-1 as well as having a high amino acid sequence homology with both oTP-1 and IFN-α; it also possesses antiviral activity (Bazer et al., 1991). As in sheep, it is now classified as tau interferon (bIFN-τ), with maximum secretion occurring between days 16 and 19 of gestation; it is first secreted at the time of elongation of the blastocyst and, unlike oIFN-τ, continues to be secreted until day 38 of gestation (Bartol et al., 1985; Godkin et al., 1988). When it is infused into the uterine lumen of non-pregnant cyclical cows between days 14 and 17, the life span of the CL is extended. Similar results have been obtained following the administration of recombinant bovine INF-α_1 using the same route of administration, and also intramuscularly (Plante et al., 1989).

As in the ewe, the luteolytic patterns of $PGF_{2\alpha}$ are attenuated in the cow in early pregnancy; however, the luteolytic pulses are abolished without major increases in basal secretion (Thatcher et al., 1995). It is likely that bIFN-τ exerts its antiluteolytic effect by modifying oxytocin receptors, thereby inhibiting the synthesis from arachidonic acid and subsequent release of $PGF_{2\alpha}$, although unlike in the ewe, it has been shown that in the cow it occurs at least initially without a change in oestrogen receptors (Robinson et al., 1998). It has also been shown that bIFN-τ also induces several endometrial proteins, one chemotactic cytokine, and another referred to as ubiquitin cross-reactive protein (UCRP); their functions are as yet unknown (Hansen et al., 1999).

The goat

In the goat, the removal of conceptuses from the uterine lumen between days 13 and 15 does not prolong the life span of the CL, but removal on day 17 increases the interoestrus interval by 7–10 days. The caprine conceptus secretes a protein, originally designated cTP-1, which as in other ruminants is cIFN-τ (Gnatek et al., 1989).

The sow

In the sow, maternal recognition of pregnancy is not controlled by a single event but rather a series of complex biochemical and cellular interactions. The porcine conceptus has been shown to convert progesterone to oestrone and oestradiol-17β as well as another isomer of 16α, 17-oestradiol (Fischer et al., 1985). The production of oestrogens increases with the rapid elongation of the blastocyst so that the conceptus is able to stimulate locally a large surface of endometrium. Oestrogen production by the conceptus plays a vital role in the maternal recognition of pregnancy and the extension of the life span of the CLs. The administration of exogenous oestrogens parenterally from day 12 in non-pregnant gilts has been long known to be luteotrophic, thereby prolonging the life span of the CLs and extending the interoestrous interval (Kidder et al., 1955). The secretion of oestrogens by the blastocyst at the time of elongation also stimulates the release of calcium, specific polypeptides and proteins. These may play a role in the establishment of pregnancy. In addition to the initial secretion of oestrogens at day 11, the second sustained release of oestrogens is necessary between days 14 and 18 for luteal persistence beyond day 25

(Geisert et al., 1990). Several mechanisms, by which the conceptus-secreted oestrogens prevent luteal regression, have been proposed. These include a direct luteotrophic effect, a reduction in the endometrial synthesis, and release of $PGF_{2\alpha}$. However, there is evidence that they probably exert their effect by altering the transport of $PGF_{2\alpha}$ from an endocrine (towards the uterine vasculature) to an exocrine (into the uterine lumen) direction, thereby preventing $PGF_{2\alpha}$ reaching the CLs (Bazer et al., 1984). The fate of the intrauterine $PGF_{2\alpha}$ is not known; however, fetal membranes readily metabolise it to PGFM (15-keto-13, 14-dihydro-$PGF_{2\alpha}$), which is inactive. The oestrogen stimulation of calcium secretion into the uterine lumen also appears to be involved in the process.

As in ruminant species, the pig conceptus produces interferons at the time of elongation (11–17 days of gestation) (Cross and Roberts, 1989); to date two interferons have been identified, namely IFN-γ and a type 1 interferon. Since the infusion of total conceptus-derived secretory proteins into the uterine lumen failed to prolong the life span of the CLs in cyclic sows (Harney and Bazer, 1989), their precise functions are not known. It has been postulated that they may have a specific protective antiviral role, or that they may act in conjunction with conceptus-derived oestrogens in prolonging the life span of the CLs (La Bonnardière, 1993).

The mare

In the mare, the mechanisms responsible for the recognition of pregnancy are less well understood. However, evidence of the importance of the developing conceptus has been shown by its removal at varying stages of gestation; if it was removed at 10, 15 and 20 days, then the return to oestrus was 22.3, 38.0 and 47 days respectively (Hershman and Douglas, 1979). A low molecular weight protein has been identified in the uterine flushings of mares in dioestrus; this persists during pregnancy (McDowell et al., 1982). The equine conceptus also produces oestradiol and oestrone (Berg and Ginther, 1978; Zavy et al., 1979), which enhance the production of a glycoprotein, uteroferrin, by the uterus. PGF secretion from the pregnant uterus is apparently blocked, since lower concentrations of PGF have been identified in the uterine venous blood of pregnant mares compared with non-pregnant mares (Douglas and Ginther, 1976), and similarly lower concentrations of PGFM in the peripheral circulation (Kindahl et al., 1982). There does not appear to be any sequestration of PGF in the uterine lumen during early pregnancy, but when endometrial tissue was incubated in vitro with material of conceptus origin there was a reduction in PGF synthesis or secretion (Berglund et al., 1982). The importance of the migration of the conceptus within the uterine lumen until it becomes 'fixed' at 16–18 days of gestation at the base of the uterine horn has been demonstrated in some elegant experiments by McDowell et al. (1988). By restricting the mobility of the conceptus using ligatures at various parts of the uterus, the maternal recognition was compromised so that the CL regressed spontaneously. It is likely that the stimulus elicited by the migratory conceptus in its contact with the endometrium is comparable with the stimulus associated with the rapid elongation of the blastocyst in ruminants and the pig.

The bitch

The bitch is atypical in that the luteal phase (metoestrus/dioestrus) is prolonged in the absence of pregnancy, there being very little difference in the interoestrous intervals of bitches that have been pregnant or non-pregnant. Until more is known about the mechanisms which control the life span of the CL, maternal recognition of pregnancy in this species will not be fully understood.

The queen cat

Little is known about the maternal recognition of pregnancy in the queen.

Early pregnancy factor

Possible mechanisms involved in the endocrine recognition of pregnancy resulting in failure of luteolysis have already been discussed. However, there is some evidence that a maternal response can be identified within hours of fertilisation and may well be involved in protecting the embryo, and subsequently the fetus, from being rejected by the dam as a semi-allograft. In 1974, Morton and her co-workers discovered that in pregnant mice it was possible to identify a substance in the peripheral blood from 4 to 6 hours after mating and for 2 weeks of gestation. This substance, called early pregnancy factor (EPF), has been identified in sheep, cattle and pigs as well as man and several other species. The method by which it exerts a protective role for the early embryo is not known, but it has been suggested that it binds to the T lymphocytes, thus preventing recognition of the embryonic antigen (Koch, 1985). It can be identified using the rosette inhibition test (Morton et al., 1974). The identification of this substance is now used as a method of detecting pregnancy at a very early stage (see below).

METHODS OF PREGNANCY DETECTION

A variety of methods can be used to detect pregnancy; many are common to all species whereas others are species-specific. There are four broad categories which are: management, clinical, ultrasonographic and laboratory-based.

Ultrasonography

Ultrasonographic methods have become increasingly popular in recent years mainly because they are accurate and can provide an immediate determination of the animal's status, thereby assisting the husbandry and management of livestock. At this point it is appropriate to describe the scientific principles behind the technology.

Three types of ultrasound have been used for pregnancy diagnosis. The ultrasonic fetal pulse detector was the first type that was used. This is based upon the Doppler phenomenon, in which high-frequency (ultrasonic) sound waves emitted from a probe, placed on the exterior of an animal or in the rectum, are reflected at an altered frequency when they strike a moving object or particles, e.g. the fetal heart or blood flowing in arteries. The reflected waves are received by the same probe; the differences in frequencies are converted into audible sounds and amplified.

The ultrasonic amplitude depth analyser (A-mode) relies upon a transducer head that emits high-frequency sound waves and receives the reflected sound, which is shown as a one-dimensional display of echo amplitudes for various depths, usually on an oscilloscope but also on the newer light-emitting diodes. This has been used successfully in many species, notably the sow.

A more recent development is that of the B (brightness) mode, which has become a very versatile tool in studying reproductive events in many species, in particular the mare (see Chapter 26). Readers who wish to extend their knowledge of this technique are recommended to consult Ginther (1986).

It is worthwhile outlining briefly the principles behind the technique. The probe, or transducer, as it should be called, is applied to the skin surface or inserted into the rectum. The transducer contains numbers of piezoelectric crystals which, when subjected to an electric current, expand or contract and produce high-frequency sound waves. When these sound waves are transmitted through tissues a proportion, depending upon the characteristics of the tissue, will be reflected back to the transducer, where the returning echoes will compress the same piezocrystals, resulting in the production of electric impulses which are displayed as a two-dimensional display of dots on a screen. The brightness of the dots will be proportional to the amplitude of the returning echoes and hence will provide an image ranging from black, through various shades of grey, to white.

Liquids do not reflect ultrasound, and thus are depicted as black on the screen, i.e. non-echogenic, whereas solid tissues such as bone or cartilage reflect a high proportion of sound waves, i.e. they are echogenic and appear white on the screen. Since a tissue–gas interface can result in up to 99% of the sound waves being reflected, it is important that air should not be trapped between the transducer face and the tissues to be examined. For this reason, a coupling medium or gel (usually methyl cellulose) is applied to the transducer face before it is placed on the skin or rectal mucosa so that air is eliminated. It is also important to select an area that is relatively hairless, or alternatively it may be necessary to clip the hair.

The technique is frequently referred to as real-time ultrasound or imaging. This just implies that there are live or moving displays in which the echoes are recorded continuously. The transducers may have the piezocrystals or elements arranged side by side in lines (hence they are referred to as linear array transducers); the field under examination and the two-dimensional image are in the shape of a rectangle. Sector transducers contain a single crystal which oscillates or rotates to produce a fan-shaped beam. They allow ready access to most of the thoracic and abdominal viscera, although very superficial structures may not be readily identified because of the shape of the beam. Sector scanners require less skin surface contact, which can reduce the time required to examine each animal; hence they are used for the transabdominal approach, especially in sheep. Linear transducers are usually cheaper to buy and more robust, and they produce a rectangular image which is easier to interpret. The transducer should be small enough to be cupped in the hand, smooth in contour, waterproof and easy to clean (Boyd and Omran, 1991).

Each transducer produces ultrasonic waves at frequencies of between 1 and 10 MHz. The most commonly used frequencies are 3.5, 5 and, more recently, 7.5 MHz. The lower-frequency transducers give better tissue penetration but poorer resolution. Since using the transrectal approach the structures requiring imaging are within a few centimetres of the transducer head, high-frequency equipment is the most effective. Thus, in the case of the mare, using a linear array transducer transrectally to diagnose pregnancy, it is possible to identify a 3–4 mm conceptual vesicle with a 5.0 MHz transducer, whereas a 3.5 MHz transducer will only identify a vesicle of 6–7 mm diameter.

PREGNANCY AND ITS DETECTION IN THE MARE

Endocrinology

The endocrine changes in the mare during pregnancy are particularly unusual, when compared with other domestic species, because of the development of temporary hormone-producing structures, the endometrial cups.

After ovulation and the formation of the corpus haemorrhagicum and the CL, plasma progesterone concentrations in the peripheral plasma rise to 7–8 ng/ml by 6 days. They persist at about these levels for the first 4 weeks of gestation, but there is frequently a transient fall at about 28 days after ovulation to 5 ng/ml (Holtan et al., 1975), followed by a later rise. Published values for progesterone in the blood and plasma vary considerably between laboratories. This is because there are other progesterone-like substances which cross-react with the antisera during the assay; for this reason several authors refer to 'total progestogen' levels.

In the early part of the second month of pregnancy, the endometrial cups are formed. These are discrete outgrowths of densely packed tissue within the gravid horn, derived as a result of the invasion of fetal trophoblast cells into endometrium, where they subsequently give rise to the endometrial cup cells (Moor et al., 1975). Usually, there are about 12 cups present at the junction of the gravid horn and body as a circumferential band. The endometrial cups produce pregnant mare serum gonadotrophin (PMSG), which is now referred to as equine chorionic gonadotrophin or eCG. It is first demonstrable in the blood 38–42 days after ovulation, reaches a maximum at 60–65 days, declines thereafter and disappears by 150 days of gestation (Figure 3.1). eCG has both 'follicle-stimulating hormone (FSH)-like' and 'luteinising hormone (LH)-like' activity, and it is generally assumed that, in association with pituitary gonadotrophins, it provides the stimulus for the formation of accessory CLs (Allen, W. R., 1975) and regulates luteal steroidogenesis (Daels et al., 1998). These structures start to form between 40 and 60 days of gestation, either as a result of ovulation, in the same way that the CL of dioestrus is formed (32%), or as a result of luteinisation of anovulatory follicles (68%) (Squire et al., 1974). Because of the presence of the accessory CLs, the progestogen concentrations in the peripheral circulation increase, to reach and maintain a plateau from about 50 to 140 days and then decline (Figure 3.1). By 180–200 days the concentrations are below 1 ng/ml, and they remain so until about 300 days of gestation, when they increase rapidly to reach a peak just before foaling and subsequently decline rapidly to very low levels immediately after parturition.

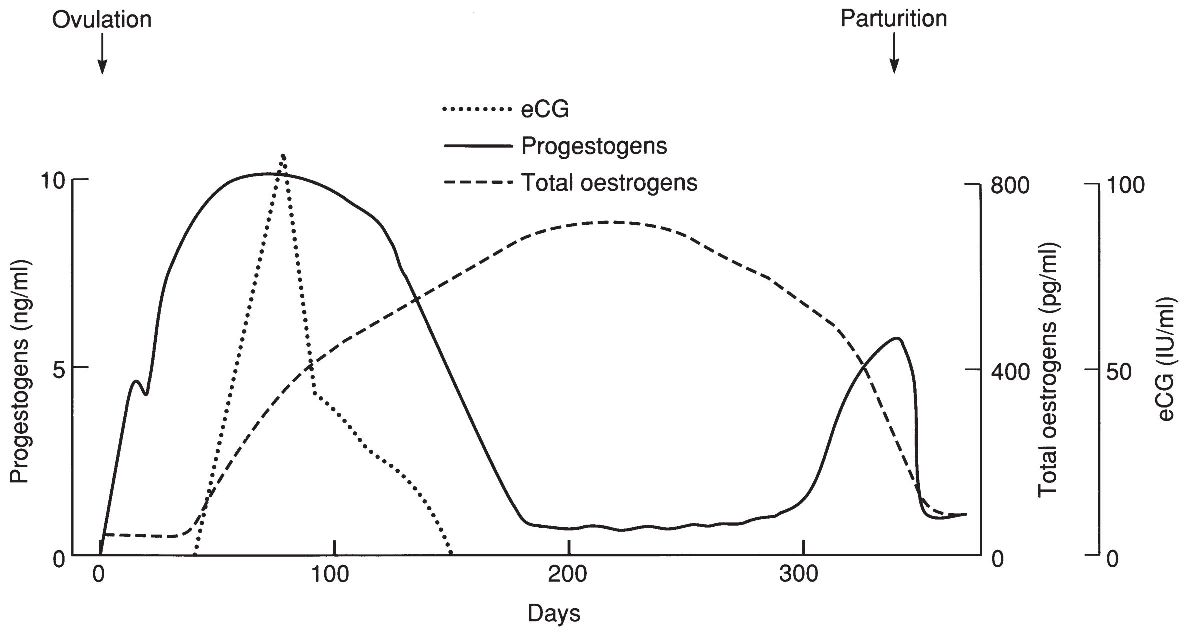


Fig. 3.1 Schematic representation of the trends in hormone concentrations in the peripheral circulation of the mare during pregnancy and at parturition; eCG, equine chorionic gonadotrophin.

Concentrations of total oestrogens in the peripheral circulation during the first 35 days of pregnancy are similar to those of dioestrus, although there is a temporary production of oestrogen by the embryo at 12–20 days (Mayer et al., 1977). After this time they increase to reach a plateau between 40 and 60 days, at values slightly above those that occur before ovulation, about 3 ng/ml; the rise is probably due to the increased follicular development associated with eCG production. After day 60 it is likely that the increase is due to the activity of the fetus or placenta (Terqui and Palmer, 1979). Maximum values are observed at about 210 days, the main source being the fetal gonads (Cox, 1975), with a gradual decline towards the time of foaling and a precipitous fall post-partum. The main oestrogens in the mare are oestrone and a ketonic steroid equilin; oestradiol-17β, oestradiol-17α and equilenine are also present.

Prolactin levels show no distinct pattern, there being considerable variation within and between mares, but there is some evidence of a slight increase towards the end of gestation (Nett et al., 1975).

The main source of progesterone in early pregnancy is the true corpus luteum and the accessory corpora lutea. The true corpus luteum is active for the first 3 months of gestation, and regresses at the same time as the accesssory corpora lutea (Squire and Ginther, 1975).

The placenta must take over the production of progesterone after the regression of the accessory corpora lutea, and although concentrations fall in the peripheral circulation they remain high in the placental tissue and must maintain pregnancy by virtue of a localised effect (Short, 1957). When ovariectomy is performed at 25–45 days of gestation, mares will abort or resorb the fetus; when it is performed after 50 days the response is variable; between 140 and 210 days the pregnancy is continued uninterrupted to term. Thus after 50 days there is evidence of a non-ovarian source of progesterone, and by 140 days the ovaries are no longer necessary for the maintenance of pregnancy (Holtan et al., 1979).

Changes in the genital organs

Conception to 40 days

Ovaries. The corpus luteum verum can only be palpated per rectum for 2 to 3 days after its formation. Thereafter, although it persists for 5 or 6 months, it cannot be identified. In pony mares there is some palpable follicular development at about 15 days, whilst during the next 14 days there is quite a marked increase in folliculogenesis with a large number of follicles less than 3 cm diameter giving the ovaries a 'bunch of grapes' appearance. Ovulations during this period are rare (Allen, W. E., 1975).

Uterus. During late dioestrus and oestrus, the uterus is soft and the endometrium is oedematous. After ovulation, tone increases and the uterus becomes more tubular; these textural changes are not marked in the non-pregnant animal, in which they subside after the CL begins to regress at 10–14 days, but in the pregnant mare the CL persists, and the tone of the uterus increases to a maximum at 19–21 days, when the conceptus causes a soft, thin-walled ventral cornual swelling close to the uterine body. The horn involved is not necessarily on the same side as the ovary which produced the ovum, because there is extensive mobility of the conceptus within the horns and uterine body before fixation occurs between days 16 and 18. Most clinicians have reported more equine pregnancies in the right horn – for example, Vandeplassche (1957) noted 64% – although in ponies there is only a small majority of right-horn pregnancies. The excess of right-side pregnancies, coupled with the slightly greater incidence of left-side ovulations, indicates a major embryonic migration from left to right in horses generally. There is, however, good evidence that other factors can influence the horn in which pregnancy occurs. In a survey of 937 thoroughbred mares, Butterfield and Mathews (1979) found that there was no significant difference between the numbers of right- or left-horn pregnancies: 469 left and 468 right. However, when they examined the results for mares that conceived when they were not lactating, there was a significantly greater number of right-horn pregnancies. There is also good evidence that implantation usually occurs on the opposite side to that of the previous pregnancy. Feo (1980) found that 19 of 22 mares which conceived at the foal heat were pregnant in the opposite horn to that of the previous gestation. Allen and Newcombe (1981) found that in 82.5% of cases the conceptus was present in the opposite horn.

The conceptual swelling of the uterine horn protrudes ventrally and cranio-caudally, but not dorsally, and grows slowly during the phase of organogenesis, i.e. until about 30 days. Thereafter, growth is faster and the swelling progressively extends to the tip of the pregnant horn. Twins are usually disposed at the base of each horn, and in this situation there will be two groups of endometrial cups. If both twins are present in the same horn, only one set of cups will be present.

Vagina and cervix. During early pregnancy, the vagina becomes progressively paler and dryer and is covered by thin, tacky mucus. The cervix is small and tightly closed; the external os is gradually filled by a plug of mucus and points eccentrically.

40–120 days

Ovaries. This period is characterised by marked ovarian activity, with multiple follicular development causing one or both ovaries to become temporarily larger than during oestrus, in some cases very much larger. Ovulations, forming accessory CLs, and luteinisation of anovular follicles occur. Follicular activity has usually subsided by 100 days, and the CLs begin to regress. In pony mares, Allen (1971) found ovulations in pregnancy between 21 and 112 days, with the highest incidences between days 40–42, 54–56 and 63–66.

Uterus. The conceptus completely occupies the pregnant horn by about 60 days, after which the body and then the non-pregnant horn are invaded by the allantochorionic membrane. The pregnant horn now changes from a transverse to a longitudinal disposition in the mare's abdomen. By 100 days the fluid-filled uterus is a somewhat tense swelling on the pelvic brim. At this time the small fetus, closely enveloped in the amnion, is floating in a relatively large volume of allantoic fluid.

120 days to term

Ovaries. With the gradual regression of all luteal elements and follicles the ovaries become progressively smaller and harder and are drawn forwards and downwards by the gravid uterus. Except in very large mares they can usually be palpated throughout pregnancy.

Uterus. Gradual distension of the uterus by the fetus and fluids causes increased tension on the utero-ovarian ligament, and the anterior border of the uterus sinks downwards and forwards. After the eighth month the fetus normally assumes an anterior longitudinal presentation and ventral position (see Chapter 8 for definitions of these terms). Except in very large mares the fetus can be palpated throughout this period. Fremitus can be detected in the uterine arteries although it is less obvious than in the cow.

Methods of pregnancy diagnosis

Management methods

Failure to return to oestrus is a good sign that a mare is pregnant. The demonstration of the signs of oestrus (see Chapter 1) usually requires the presence of a teaser stallion, although some mares will respond to geldings and to androgenised geldings. It is preferable that mares should be accustomed to the teasing routine, which should commence 16 days after service and continue for a further 6 days.

False positives will occur:

- if the mare has a silent heat, a common problem when the foal is at foot
- if the mare becomes anoestrous as a result of lactation or environmental factors
- if the mare has a prolonged dioestrus yet has not conceived (see Chapter 26)
- if the mare has a prolonged luteal phase associated with embryonic death; this is referred to as 'pseudopregnancy' (see Chapter 26).

False negatives will occur in a few mares which will show oestrus at this time although they are pregnant.

Clinical methods

Vaginal examination. This is best done using a speculum; however, manual exploration can be used. The vaginal mucosa is pale pink, the mucus is scant and sticky, and the cervix small and tightly closed; the external os is gradually filled with thick, tacky mucus, although it is not really apparent as a plug, and points eccentrically.

False positives can occur in early pregnancy because the vagina is indistinguishable from that seen in dioestrus. Errors can also be made as a result of prolonged luteal phase and pseudopregnancy.

Rectal palpation. The presence of *follicles* during the third week after service does not necessarily indicate that the mare is returning to heat. Follicles are normally present during the first 3 months of gestation, and give considerable size to the ovaries.

Uterine tone is marked at 17–21 days of pregnancy, when the uterine cornua can be palpated as resilient tubular organs. If no conceptual swelling is palpable, then this tone should only be interpreted as suggestive of pregnancy. The uterine body and non-pregnant horn remain tonic until at least day 50 of gestation. Marked uterine tone may also be found in: the puerperal mare covered at the foal heat; acute endometritis; and pseudopregnancy, that is when early embryonic death is followed by autolysis or expulsion of the conceptus but the uterus retains the texture of pregnancy because of the persistent CL.

Palpation of the conceptus is first possible at 17–21 days, when it is a small soft swelling of 2.4–2.8 cm in diameter or is an apparent 'gap' in the otherwise tonic horn. It is more easily felt between 21 and 30 days, but still only the cranioventral portion of the distension can be appreciated. At 25 days, the conceptual swelling is 3–3.4 cm. At 30 days, its dorsoventral diameter is 3–4 cm. At 35 days, it is 4.5–6 cm and at 40 days 6–7 cm in diameter – about the size of a tennis ball. Thereafter, it is not possible for the conceptual swelling to be completely cupped within the palm of the hand. By 60 days, it is becoming oval in shape and measures approximately 13 × 9 cm, whilst by 90 days it has increased to approximately 23 × 14 cm.

There is a natural variation in the size of the conceptual swelling in mares of similar size and ovulation dates owing to the variation in the volumes of fetal fluids present. However, swellings that appear small for the given stage of pregnancy should be re-examined later to exclude the possibility of resorption (hence the clinician should keep written records of the findings for later comparisons). Twin conceptuses can be identified up to 60 days. After this, a single conceptus is likely to involve both horns, and the swelling becomes more diffuse. Care must be taken not to confuse a partially filled urinary bladder with the pregnant uterus during the 70–100-day period, or with an inflated large colon during days 90–120; when in doubt a search should be made for the ovaries in order to establish an anatomical link between them and the uterus via the utero-ovarian ligament.

At about 100 days, it is often possible to *ballotte the fetus* as it floats in the fetal fluid of the uterine body. Growth of the fetus and reduction in tension of the fetal sacs enable the examiner to palpate parts of the fetus in the uterine body from the end of the fourth month onwards. It may be difficult to locate between the fifth and seventh months in large pluriparous mares, and very occasionally in mares near term. The palpable absence of a non-pregnant uterus and tension in the mesovarium are reassuring features.

False positive results by rectal palpation can be obtained either when, in rare instances, it is confused with pyometra (see Chapter 26), or when, in the very early stages, the uterine tone due to incomplete involution might be assumed to be due to pregnancy in mares which have been served at the foal heat, and in those that have developed a pseudopregnancy. It should also be remembered that mares may suffer embryonic or fetal death with resorption or abortion after they have been confirmed pregnant.

False negative results can be obtained if there is confusion over the service date, i.e. later than the one recorded, or if the uterus is not palpated completely.

Ultrasonographic methods

The Doppler and A-mode ultrasonographic methods are of little or no value in the mare; however, B-mode has been used extensively in this species (Figure 3.2). With this imaging mode with a 5.0 MHz transducer, the earliest gestational age that pregnancy has been confirmed in the mare is 9 days, when the conceptual vesicle appears as a black sphere of about 3 mm in diameter. By 11 days the vesicle has been identified in 98% of ponies and horses examined (Ginther, 1986). The conceptus at this stage moves freely within the uterus, and has been identified in all parts of the uterine horns and in the uterine body just cranial to the cervix; in fact, it is found more frequently in the body at this stage. Rapid growth of the conceptual vesicle occurs from 9 to 16 days, with evidence of some reduction in growth rate from 16 to 28 days, before it increases. This plateau in growth may be an artefact because, with a reduced turgidity of the vesicle, it is capable of being compressed by the uterine walls. This is also associated with an apparent change in the shape of the vesicle from spherical, to oval, to triangular; it then becomes irregular in outline. Fixation at the base of the horn occurs between 16 and 18 days, when the diameter of the conceptual vesicle is about 19–24 mm (Ginther, 1986).

Twin ovulations are very common in mares, especially in thoroughbred and draught mares where they can occur in up to 25% of ovulations. The birth of live twins is relatively uncommon, ranging between 0.8 and 3% depending upon the breed. The reasons for the discrepancy are:

- fertilisation failure
- death of one or both embryos before or after fixation
- death of one fetus, which is relatively uncommon
- abortion of both fetuses (see Chapter 26). This is the most common sequel and is obviously the most costly.

Whilst the detection of double ovulations by B-mode ultrasound has enabled better management, and hence presentation of the problem, it is still possible for double ovulations to go undetected. Thus, early identification of twin embryos, preferably between days 12 and 14 before fixation occurs, can enable more effective management of the problem (see Chapter 26). For this reason it is important to scan the whole of the uterine body and uterine horns.

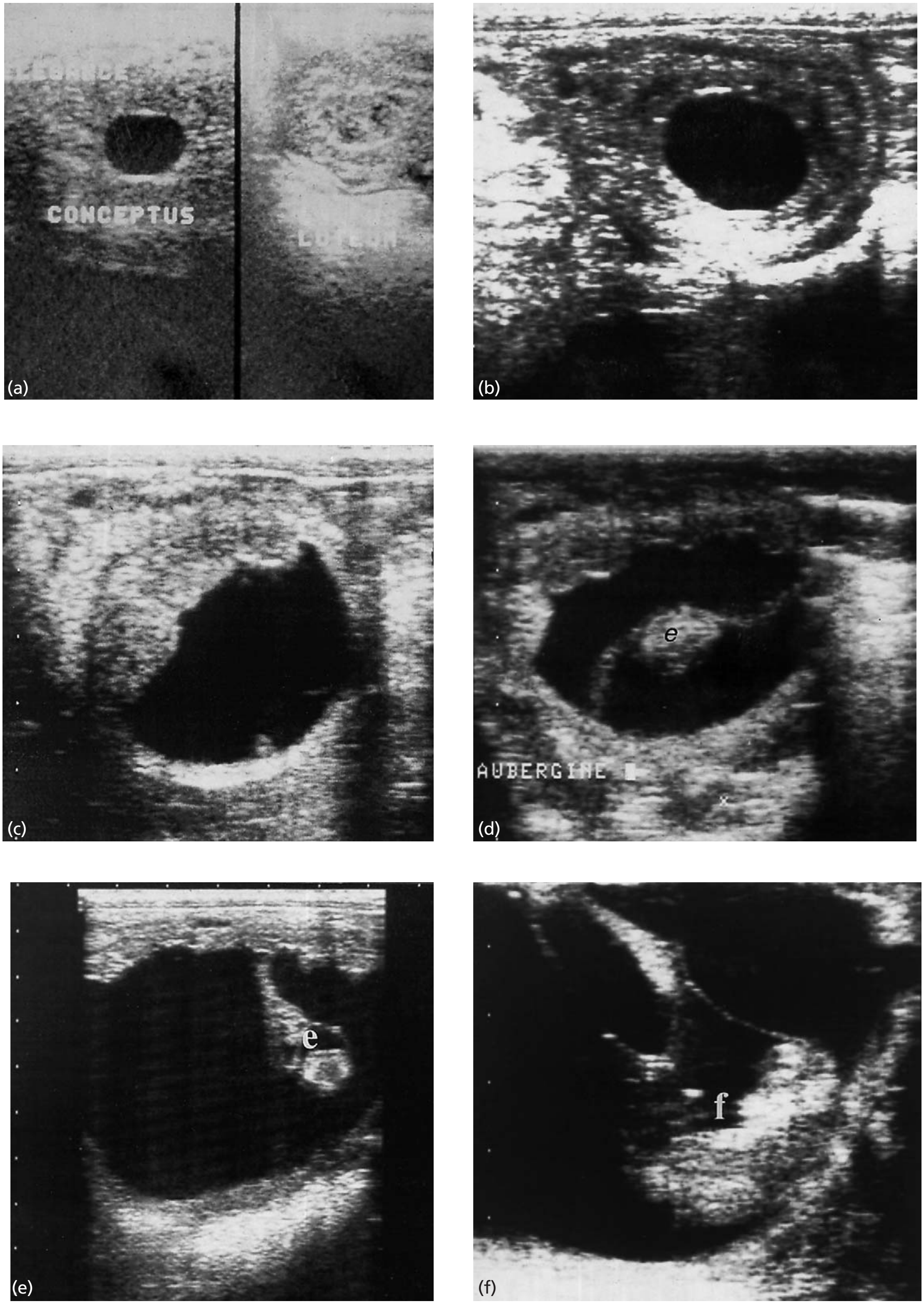

CONCEPTUS
(a)
(b)
(c)
AUBERGINE
e
(d)
e
(e)
f
(f)

Fig. 3.2 Transrectal B-mode ultrasound images of the pregnant uterus of the mare, and its content, using a 7.5 MHz linear transducer. Scale in cm. (a) Conceptus at 13 days (left), which is 9 mm in diameter with a dorsal and ventral specular echo. Adjacent ovary (right) showing corpus luteum verum. (b) Conceptus at 16 days; note that it is starting to lose its spherical shape. (c) Conceptus at 21 days; note embryo at 5 o'clock and thickening of uterine wall extending from 8 o'clock to 10 o'clock. (d) Conceptus at 30 days; note embryo (e) which is starting to move towards the dorsal pole of the vesicle. (e) Conceptus at 35 days; note embryo (e) and that the yolk sac has now regressed leaving only the anechoic allontoic sac. (f) Conceptus at 50 days; note fetus (f).

In addition to the detection of pregnancy, it is also possible to determine the sex of the foal; however, this requires that the person has good-quality equipment, as well as considerable experience of transrectal ultrasonography and interpretation of ultrasonic images. The method is based on determining the relationship of the genital tubercle to surrounding structures. The genital tubercle is the embryonic precursor of the penis in the male and clitoris in the female. As the fetus develops, the tubercle moves from a position between the hind legs to near the tail in the female, and near the umbilicus in the male. The optimum gestational age is between 59 and 68 days (Curran and Ginther, 1989), although accurate results were obtained up to 99 days (Curran, 1992); it becomes more difficult after this stage.

Laboratory methods

Milk or blood progesterone. As can be seen in Figure 3.1 in the pregnant mare, plasma progesterone concentrations remain elevated just before, or during, the time when the mare would have returned to oestrus. Blood or milk samples collected 16–22 days after service should have elevated progestogen concentrations in pregnant mares, whilst in non-pregnant mares the levels would be low and typical of those obtained at oestrus. Although Hunt et al. (1978) have quoted 100% accuracy in diagnosing pregnancy using this method, false positive results occur with a prolonged luteal phase, and, in general, the method is not very reliable.

Identification of eCG. Blood samples should be collected, preferably between 50 and 90 days after service, although it is possible to identify the hormone between 40 and 120 days. The test is performed on serum.

Originally, biological methods were used to identify the presence of the gonadotrophin. The most frequently used method was the injection of serum into $3\frac{1}{2}$-week-old immature mice, a positive result being the production of ripe follicles in the ovaries and the presence of a swollen enlarged uterus.

Immunological methods are now used: either gel diffusion or the haemagglutination inhibition technique. The latter method is available commercially in a simple kit form; all of the reagents are provided, and it is possible for the procedure to be carried out in any veterinary practice laboratory.

False negative results are obtained if the blood sample is taken either too early or too late, and for this reason it is important to sample at the optimum times stated above. Some mares produce low levels of eCG that are briefly sustained and which cannot be detected using the method.

False positive results are obtained as a result of embryonic or fetal death, either after the blood sample was collected or in some cases before. Once the endometrial cups have formed, they will persist and still secrete eCG, even if the fetus has died. They regress at the time that they would have done if the pregnancy had continued normally. For this reason, owners should always be warned of the problems, to save subsequent disappointment, and certificates should indicate that a positive result has been obtained with an explanation of the significance of such a result.

Blood oestrogens. A method of detecting pregnancy by determining the concentration of total oestrogens in the peripheral blood has been suggested by Terqui and Palmer (1979). By 85 days of gestation the concentration should exceed the maximum values obtained in non-pregnant mares.

Urinary oestrogens. Oestrogens (oestrone and oestradiol-17β) are present in the urine of pregnant mares in sufficient amounts for accurate detection by a chemical method (Cuboni, 1937) between 150 and 300 days of gestation. A modification of this test (Cox and Galina, 1970) can be carried out easily by the practitioner. Very little equipment is required and the results are easy to interpret.

Because the presence of blood oestrogens depends on a functional placenta, false positives, as seen with the eCG test, do not arise. This chemical test is not sensitive enough to identify the urinary oestrogens of oestrus. It is nearly 100% accurate between 150 and 300 days.

Serum early pregnancy factor. Early pregnancy factor (EPF) is an immunosupressive glycoprotein associated with early pregnancy, first identified in the mouse (Morton et al., 1974) and subsequently identified in a number of domestic species, including the horse. Using the rosette inhibition test it has been possible to detect the presence of EPF in the serum from peripheral blood from as early as 7–10 days after ovulation (Branco et al., 1989; Ohnuma et al., 1996). At present, it is still only in the developmental stages but eventually it is likely to become available for routine use.

Optimum time for diagnosing pregnancy

The optimum time, as with all species, is as early and as accurately as possible. In thoroughbred mares in particular, where twin ovulations are common, the early identification of the resultant twin conceptuses, should they be present, is important (see Chapter 26). The use of transrectal B-mode ultrasonic imaging has enabled early identification, which is important since termination of pregnancy in the case of twins must be carried out before the endometrial cups develop. It is important to stress that good results depend upon good equipment and knowledgeable interpretation of the images. Similarly, many of the earlier changes in uterine tone can be identified only by an expert using sequential palpation.

If the mare is not pregnant and has not been observed in oestrus, or if she is in a prolonged luteal phase or anoestrus, the necessary action can be taken (see Chapter 26).

Hazards of pregnancy diagnosis

There is no evidence that careful palpation of the genital tract will cause failure of the pregnancy. In those mares where fetal death occurs after a normal rectal examination has been performed, the pregnancy would have failed in the absence of the examination.

PREGNANCY AND ITS DETECTION IN THE COW

Endocrinology

The main source of progesterone for the maintenance of pregnancy in the cow is the CL, the placenta producing only small amounts. The results of ovariectomy and removal of the CL are controversial. Up to about 200 days of gestation, removal of the ovary containing the CL, or ablation of the CL either surgically or with the use of $PGF_{2\alpha}$, usually results in abortion. However, after this stage until just before term, pregnancy usually continues. Some difference has been noted between the effects of ovariectomy and CL removal, which would suggest that the ovarian stroma may produce some progesterone, whilst the localised effect of placental hormones upon uterine function must also be considered.

The hormonal changes during pregnancy are illustrated in Figure 3.3. Progesterone concentrations in the peripheral circulation during the first 14 days of gestation are similar to those of dioestrus; thereafter, those of the non-pregnant cow decline sharply from about the 18th day after ovulation (see Figure 1.28). In the pregnant cow, there is normally only a slight decline at this stage with a rapid recovery. Thereafter the concentration increases slightly during pregnancy until it starts to decline at about 20–30 days prepartum. Oestrogen concentrations during early and mid-gestation are low, less than 100 pg/ml; however, towards the end of gestation, in particular after day 250, oestrogen concentrations increase to reach peak values 2–5 days prepartum of 7 ng/ml oestrone sulphate and 1.2 ng/ml oestrone (Thatcher et al., 1980). These rapidly decline from about 8 hours prepartum to low levels immediately postpartum.

Both FSH and LH concentrations remain low during gestation and show no significant fluctuations. Prolactin is low during pregnancy until just before calving, when it increases from basal concentrations of 50–60 ng/ml to peak values of 320 ng/ml 20 hours prepartum, until a subsequent decline to basal concentrations by 30 hours postpartum. Bovine placental lactogen is present in the peripheral circulation of the dam at about

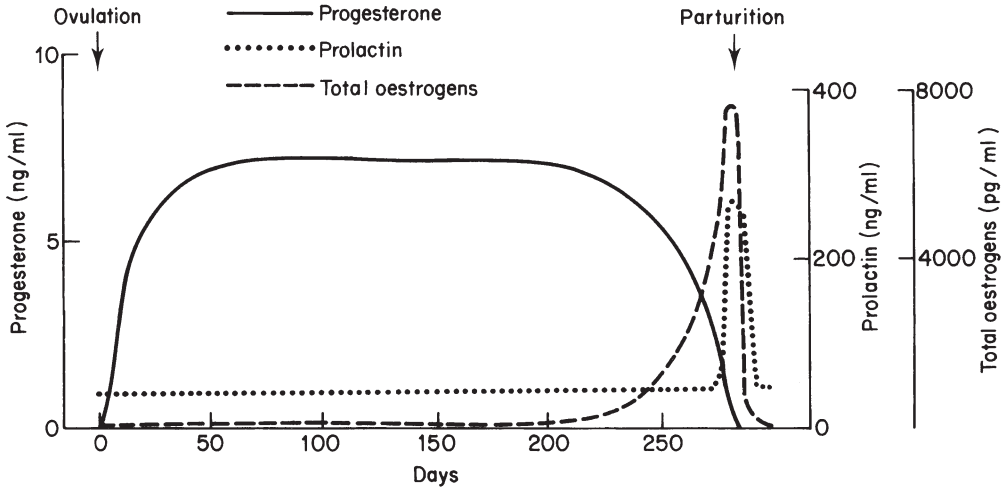


Fig. 3.3 Schematic representation of the trends in hormone concentrations in the peripheral circulation of a cow during pregnancy and at parturition.

160 days of gestation, increasing dramatically to maximum concentrations of 1000 ng/ml between 200 days and term (Bolander et al., 1976). The role of this hormone is still unclear but it appears to have prolactin and growth hormone-like activities.

Changes in the genital organs

Ovaries. In the cow, the CL of pregnancy remains at its maximum size throughout the whole of the period of gestation. Fundamentally it is indistinguishable from the fully developed CL of dioestrus, but there are certain features by which its persistence can be recognised when examined post-mortem. The chief one is that the protrusion of the structure from the surface of the ovary is less marked and the epithelium over it is white and scarred. The CL of dioestrus often contains a central lacuna, whereas during pregnancy this becomes filled. It is considered by some that the CL of pregnancy is larger than that of dioestrus. The author's series indicates that, if this is the case, it is too slight to be of significance, and moreover that there are considerable variations in the weights of pregnancy CLs in individuals (3.9–7.5 g) which bear no relationship to the duration of pregnancy. The colour of the CL of pregnancy, however, differs somewhat from that of dioestrus. There is a wider range from yellow through orange to light brown, and the appearance of the luteal tissue is duller. Figures 3.4–3.9 show examples of bovine ovaries (natural size) obtained from gravid genital tracts recovered after slaughter. Note the variations in the shape of the ovaries and the position of the CL of pregnancy. In addition, these sagittal sections also show that folliculogenesis and regression continue throughout pregnancy.

As pregnancy advances, the position of the ovaries changes. Their location, however, in non-gravid animals is not constant. In heifers and young cows they are generally situated on each side of, and slightly below, the conjoined cornua at the level of the pelvic brim. They may lie in the pelvic cavity. In multiparous animals they are often situated in the abdominal cavity 5–8 cm in front of the pelvis, where their detection is more difficult. Consequent on the increase in the weight of the uterus and hypertrophy of the ovarian and uterine ligaments, the ovaries pass deeper and deeper in the abdominal cavity as pregnancy advances. From the fifth month onwards the weight and size of the uterus is such that it sinks down into the abdomen to rest on the abdominal floor. Hammond (1927) found the weight of the uterus and its contents in primigravidae at 5 months to be 48.4 kg. Provided that the animal is

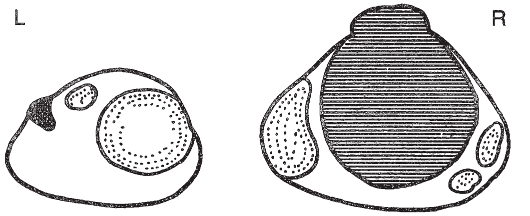


Fig. 3.4 Ovaries of the cow at 35 days of pregnancy. Fetal body length (FBL) 1.6 cm; corpus luteum verum (CLV) yellow.

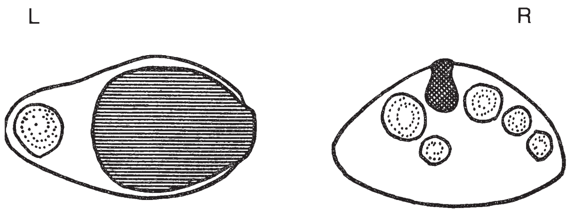


Fig. 3.5 Ovaries of the cow at 48 days of pregnancy. FBL 3.4 cm; CLV orange.

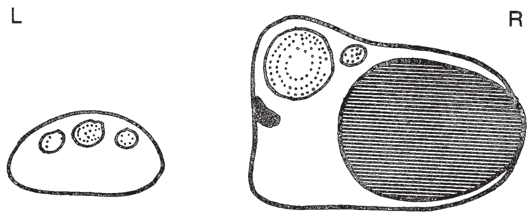


Fig. 3.6 Ovaries of the cow at 70 days of pregnancy. FBL 6.3 cm; CLV yellow–orange.

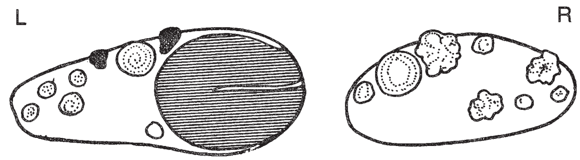


Fig. 3.7 Ovaries of the cow at 100 days of pregnancy. FBL 16 cm; CLV yellow–brown.

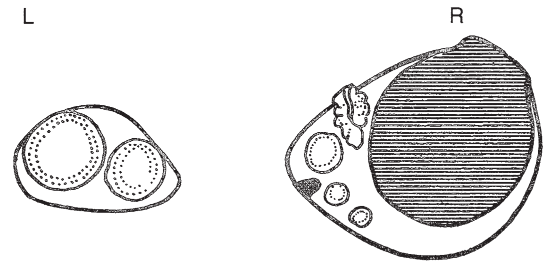


Fig. 3.8 Ovaries of the cow at 120 days of pregnancy. FBL 25 cm; CLV orange.

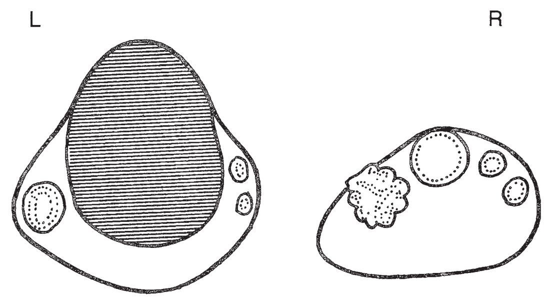


Fig. 3.9 Ovaries of the cow at 190 days of pregnancy. CLV orange.

comparatively easy to examine, it is generally possible to palpate the ovaries with reasonable certainty up to day 100, by which time in heifers the one on the gravid side is about 8–10 cm in front of, and slightly below, the pelvic brim, and that on the non-gravid side a little nearer the pelvis. In occasional cases both ovaries may be detected as late as day 150, although by this time there is a risk that they will be confused with cotyledons. In the later stages of pregnancy it is not so much that they are beyond the reach of the hand as that one is unable to depress the rectum sufficiently deeply into the abdomen to locate them.

Uterus. During the early stages, detection of an increase in size of the uterus affords strong evidence of pregnancy, but the recognition of these changes necessitates an appreciation of the size of the quiescent uterus in subjects of varying ages and parity (Figure 3.10 and Table 3.2), the quantities of fluid present in the respective fetal sacs and the disposition of those sacs in the uterus (see Figure 2.11).

At 28 days of pregnancy the amniotic sac is spherical in outline and about 2 cm in diameter. It occupies the free portion of the gravid horn. The allantoic sac is about 18 cm long, but the amount of contained fluid is insufficient to distend it, and its width is negligible. It occupies almost the whole of the gravid cornu. At this stage the embryo is 0.8 cm long, a quite inappreciable size.

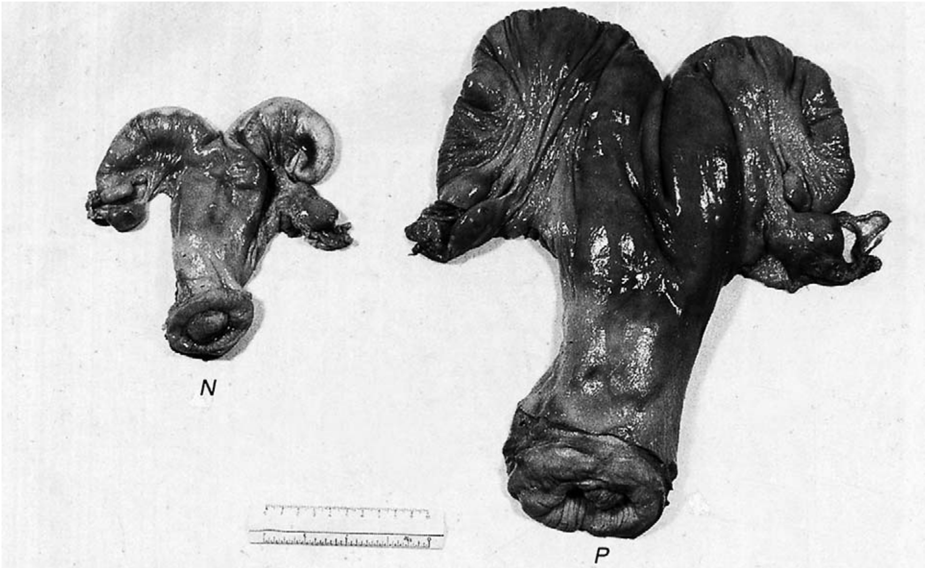


Fig. 3.10 Genital tracts of anulliparous heifer (N) and pluriparous (seven calves) cow (P).

Table 3.2 Uterine dimensions (non-gravid organ) (cm)

	Unbred nulliparous heifer	*Pluriparous cow*
Width of conjoined cornua immediately anterior to cervix	2.5	4.0
Width of each cornu at external bifurcation	2.0	3.5
Length of externally connected parts of cornua	9.0	14.0
Length of free portions of cornua	15.0	20.0
Thickness of wall of cornua	0.5	1.2
Length of cervix	5.0	10.0
Width of cervix	3.0	5.0

At 35 days, fetal body length is 1.8 cm and the diameter of the spherical amniotic sac 3 cm. They still occupy the free part of the cornua. The conjoined portions of the cornua and the free portion of the non-gravid cornua are not appreciably changed. It is possible, particularly in a heifer easy of examination, that the distension in the free part of the gravid cornua will be detected.

At 60 days the fetal crown–rump length is approximately 6 cm. The amniotic sac is oval and tense, having a transverse measurement of about 5 cm. This causes the free part of the gravid horn to be distended to a width of about 6.5 cm, compared with 2–3 cm in the quiescent stage in the heifer and young cow. In such subjects this distension may be recognised.

At 80 days the fetus measures 12 cm and the total quantity of fluid is about 1 litre. Distension of the free part of the gravid horn varies from 7 to 10 cm, while that of the conjoined part is but little greater than normal. The greater length of the gravid horn can often be detected.

By 90 days, uterine distension is such that it can be detected with accuracy in the great majority of cases. The conjoined cornua are tense, the gravid one having a width of about 9 cm and the non-gravid one about 4.5 cm. In most individuals the organ is still high up at the pelvic brim and it is generally possible to pass the hand well over the curvature of the distended horn, but in some multigravid cows the uterus lies in the abdomen, and to palpate it effectively it is necessary to retract the organ. Sometimes it is possible to detect the fetus at this stage. Tapping of the distended cornua with the fingers may reveal the fetus rather like a piece of wood floating in the fluid beneath. By gently squeezing the uterus one may be able to pick up the fetus. Its body length is about 15 cm.

By the fourth month the uterus sinks below the pelvic brim, and distension is less easy to recognise as the fluid gravitates towards the extremities of the cornua. The cervix lies on the pelvic brim.

Changes in the size and shape of the gravid uterus during the first 4 months of gestation are shown in Figure 3.11.

Fetus. Several workers (Hammond, 1927; Winters et al., 1942) have recorded fetal body lengths (crown–rump) during the various stages of pregnancy. Data have been collected chiefly from pregnant heifers, and thus allowance must be made for the greater size of the fetus in cows, particularly in the later periods of pregnancy (see Table 3.3). The fetal bulk in relation to these body lengths will be appreciated.

During the period 120–160 days, it will be possible to palpate the fetus in more than 50% of cases. The presented extremity will lie within reach in front of, and below, the pelvic brim. In some cases, the fetus may be touched transiently at the commencement of examination and then sinks into the depths of the uterus beyond reach. Similarly, if a series of examinations of an individual is made during this period, the fetus may be detected on some occasions and not others.

Table 3.3 Fetal body length in cows at various stages of pregnancy

Pregnancy (months)	*Fetal body length (cm)*
1	0.8
2	6
3	15
4	28
5	40
6	52
7	70
8	80
9	90

Between $5\frac{1}{2}$ and $7\frac{1}{2}$ months the fetus is detected less often than during the previous period. The author would put it at 40–50%. In favourable cases the fetal head and/or flexed limbs are palpated just anterior to the pelvic brim. Touching the fetus often provokes reflex movement.

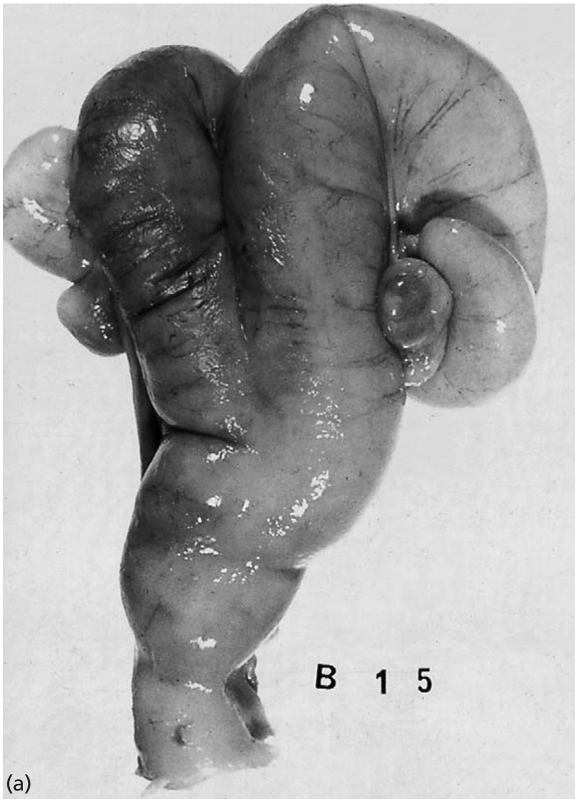
From $7\frac{1}{2}$ months to the end of gestation the fetus will, in the majority of cases, be detected readily. Again, however, cases will be encountered, especially in deep-bellied, multiparous cows in which the fetus cannot be detected, at any rate on a single examination, even to term. Several authors (Rüsse, 1968; Dufty, 1973) have shown variations in myometrial tone during late pregnancy.

The latter author found that in a large number of Hereford heifers, which were examined daily by rectal palpation near term, it was frequently impossible to palpate the fetal calf. The reason for this finding was the considerable relaxation of the myometrium, which allowed the calf to descend into the abdomen.

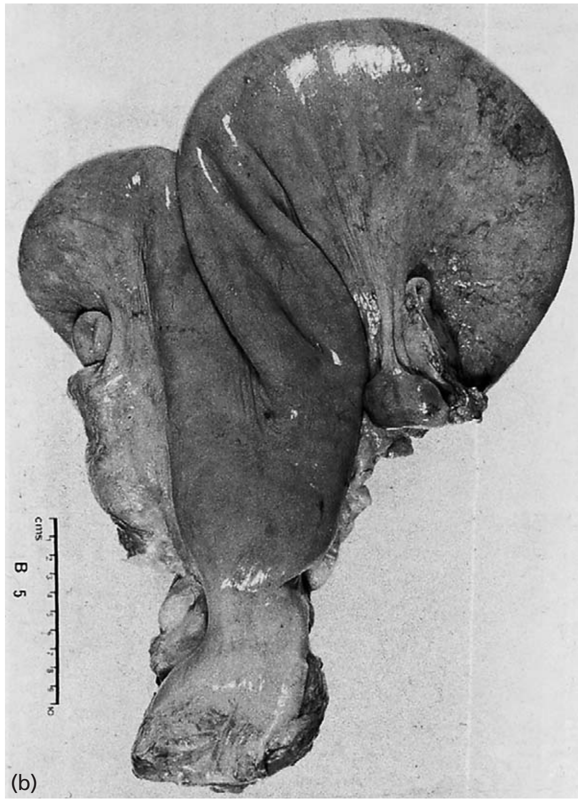
Non-gravid cornu. The extent to which the allantochorionic sac occupies the non-gravid horn varies greatly. In the great majority of bovine pregnancies the sac occupies some part of it, in some extending to the apex. In others, the caudal two-thirds or one-half only is occupied, while in exceptional cases the non-gravid horn is entirely unoccupied by fetal membranes. In the majority of cases, the non-gravid horn also plays its part in placentation and its cotyledons hypertrophy, although the degree of cotyledonary enlargement is not as great as that of the pregnant horn. Occasionally the non-gravid horn, although occupied by the allantochorion, plays no part in placentation and its cotyledons remain undeveloped. In such cases, and also in those in which the non-gravid horn is unoccupied, the cotyledons in the gravid horn, particularly those in the region of the fetal trunk, become grossly hypertrophied and may at the time of parturition be as large as 8 × 12 cm.

Caruncles. Detection of the hypertrophied cotyledons is evidence of pregnancy, but variations occur in their size at the various stages of

Fig. 3.11 Gravid genital tracts from cows at different stages of gestation. (a) Right horn at 6 weeks. (b) Right horn at 12 weeks. (c) Left horn at 16 weeks. (d) Right horn at 19 weeks.



(a)



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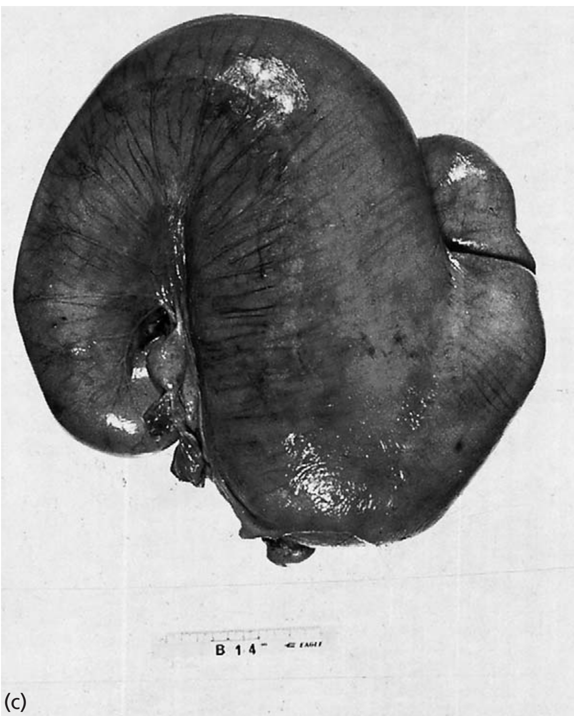

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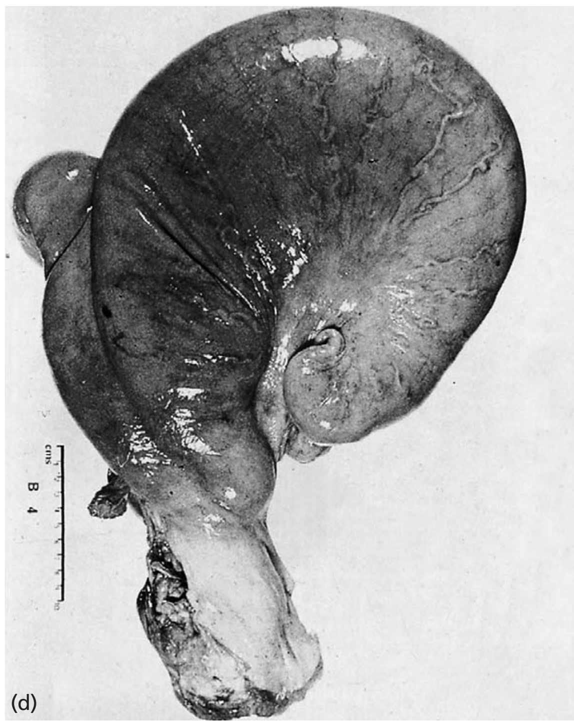


(d)

pregnancy in different individuals. This is probably due to differences in number. Again there is variation throughout the same uterus. Those situated about the middle of the gravid cornu are larger than those of the extremities, while those in the non-gravid horn are smaller than those in the gravid one. (Occasionally there is no placentation in the non-gravid horn.) As pregnancy continues, they become progressively larger, until in the terminal stages they may be 5–6 cm in diameter, but because the pregnant uterus sinks into the abdomen, it may not be possible to palpate cotyledons from the fifth to the seventh month.

Uterine arteries. Evidence of pregnancy is afforded by hypertrophy of the middle uterine arteries and a characteristic change in their pulse wave, which is referred to as fremitus.

Pregnant side. It is generally accepted that dairy cows are more often pregnant in the right horn, and that the CL is in the ovary on the side of the pregnant horn. In a large series of pregnant bovine uteri examined by the author the proportion has been 60% right-side pregnancies to 40% left-side; in only one case was the fetus present in the horn opposite to the ovary containing the CL; another case showed a CL of normal size in each ovary with a single fetus in the right horn. In a series of 1506 uteri of dairy cattle examined in the USA, Erdheim (1942) found the fetus in the right horn in 1015 (67.4%) and in the left in 474 (31.4%). In a series of 2318 uteri of beef cattle, however, the side incidence of pregnancy was approximately equal: right, 1178 (50.8%), and left, 1121 (48.3%). Among all Erdheim's specimens there was one exceptional single pregnancy in which he found the CL in the left ovary, and fetus in the right horn. Of 133 pregnant uteri from Swedish Highland cattle, Settergren and Galloway (1965) found 59.4% pregnant on the right side and 40.6% on the left. This series also included one specimen in which the CL was in the left ovary and the fetus in the right horn.

Twinning. There is an incidence of 1.04% of twins in dairy cattle and 0.5% in beef cattle. Individual breed records show higher figures: 2.7–8.85% for Brown Swiss, 3.08–3.3% for Holsteins, 2.8% for Ayrshires, 1.95% for Guernseys and 13% for Jerseys (Meadows and Lush, 1957; Johansson, 1968). The rate increases with age, figures for Holsteins showing 1.3% for heifers rising to 7% in 10-year-old cows.

In the majority of cases one CL is present in each ovary and a fetus in each horn. Not infrequently, however, two CLs are found in a single ovary with gestation bicornual. In 25 pairs of twins in Erdheim's series five were unilateral. This observed natural preponderance of bilaterally disposed twins conforms with the experimental results of Rowson et al. (1971), which showed that induced twin pregnancies were more stable when an embryo was transplanted into each horn than when two embryos were placed in one horn. Arthur (1956) and Erdheim (1942) each encountered only one case of identical twinning – a single CL with two developing fetuses – and the aggregate recorded statistics for identical twinning is 4–6% of all twins. The incidence of bovine triplets is 1 in about 7500 single births.

Methods of pregnancy diagnosis

A variety of different methods have been, and are still, used to detect the presence or absence of pregnancy in the cow. These range from the identification of substances that are present in body fluids using laboratory assays, and from different ultrasound modes, to simple clinical methods such as transrectal palpation; the latter has been the most ubiquitously used method for the last 70 years (see Figures 3.12–3.15). Table 3.4 gives a list of the methods of pregnancy diagnosis, and the earliest times that they can be used. In terms of efficient livestock production, it is the early identification of the non-pregnant cow that is important, since immediate measures can be taken to attempt to induce pregnancy again as soon as possible.

Management methods

Failure to return to oestrus and persistence of the corpus luteum. Failure of regression of the CL at about 21 days, as determined by transrectal palpation, provides a method of anticipating that the cow is probably pregnant. It is seldom used as a practical procedure and there are reasons for the CL persisting in the absence of

Table 3.4 Methods of pregnancy diagnosis in the cow

Method	*Earliest time*
Early pregnancy factor (EPF)/early conception factor (ECF)	3 days
Realtime ultrasound (direct imaging)	13 days
Failure to return to oestrus and persistence of corpus luteum	21 days
Progesterone concentration in plasma and milk	21–24 days
Assay of pregnancy-specific protein B (PSPB)	24 days
Palpation of the allantochorion (membrane slip)	33 days
Unilateral cornual enlargement and disparity in size, thinning of the uterine wall, fluid-filled fluctuation of enlarged horns	35 days
Palpation of the early fetus when the amnion loses its turgidity	45–60 days
Palpation of the carnucles/cotyledons	80 days
Hypertrophy of the middle uterine artery until presence of fremitus	85 days
Oestrone sulphate in blood or milk	105 days
Palpation of the fetus	120 days

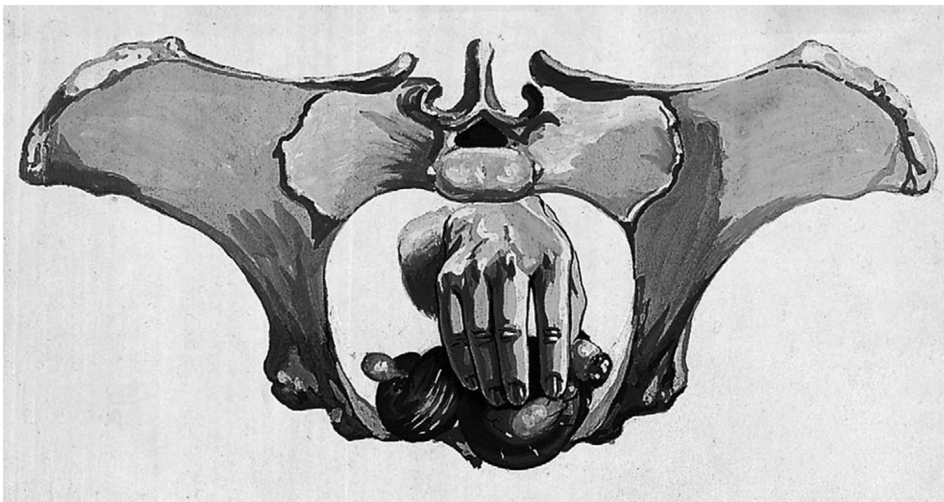


Fig. 3.12 Detection of pregnancy in the cow by rectal examination. Uterus gravid 70 days.

pregnancy (see Chapter 22). Rectal examination at about this time in an individual which was close to, or at, oestrus would demonstrate the presence of a turgid, coiled uterus and a mucoid vaginal discharge.

How reliable is failure to return to oestrus as a method of diagnosing pregnancy? This will be dependent on the efficiency and accuracy of oestrus detection. In a large group of dairy cows it can be expected that about 50% or fewer in many cases will become pregnant after artificial insemination, and thus 50% will not. Those that are pregnant will not return to oestrus, whilst those that are not pregnant will return. However, a substantial number returning to oestrus will not be detected, and thus will incorrectly be assumed to be pregnant, although there will be an opportunity for detection at subsequent oestruses. Assuming an oestrus detection rate of 60% (see Chapter 1), 30% of cows will be incorrectly assumed to be in calf. This constitutes a large error and fully justifies the use of other diagnostic procedures.

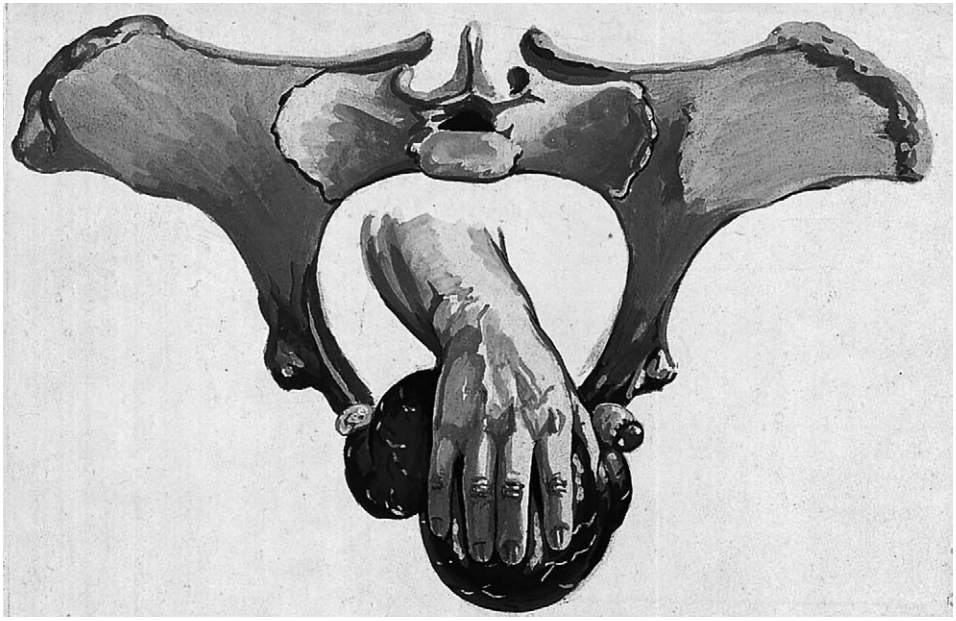


Fig. 3.13 Detection of pregnancy in the cow by rectal examination. Uterus gravid 90 days.

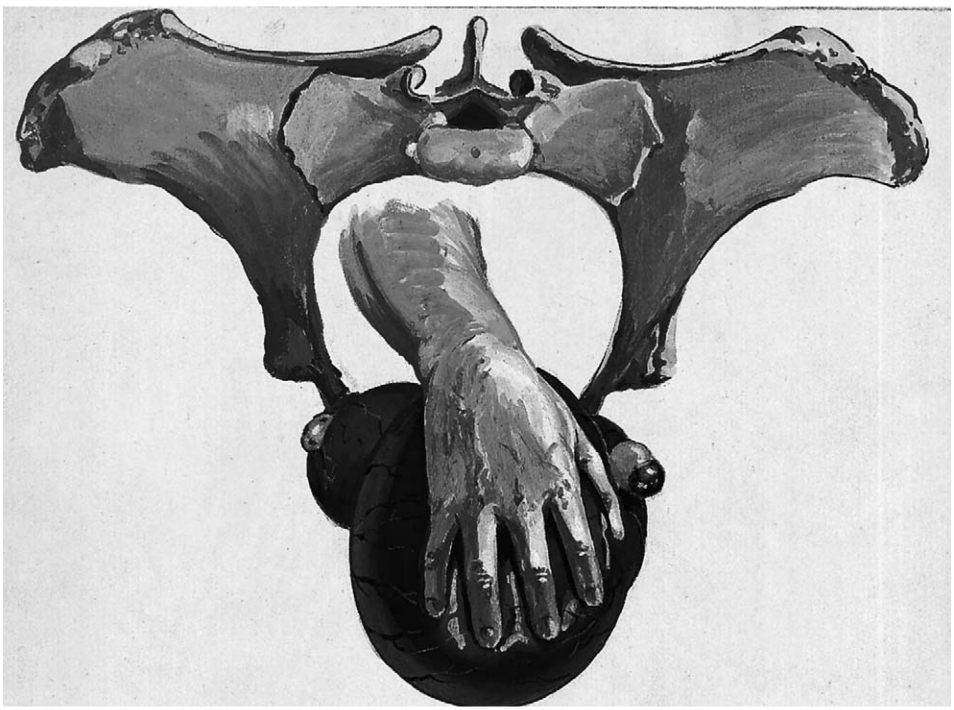


Fig. 3.14 Detection of pregnancy in the cow by rectal examination. Uterus gravid 110 days.

Fig. 3.15 Detection of pregnancy in the cow by rectal examination. Pregnancy approaching term.

Mammary glands. Mammary changes during pregnancy are best observed in primigravida. The teats of the pregnant heifer begin to enlarge about the fourth month, and with a little experience it is an easy matter to distinguish them from those of the non-pregnant or early pregnant animal. From the sixth month the mammary glands become more firm to the touch and their enlargement can be seen. Hypertrophy is progressive and is particularly marked during the terminal month. As parturition approaches, the glands become grossly enlarged and oedematous and the teats take on a waxy, tumefied appearance. The abdominal wall, particularly in the region of the umbilicus, may also become swollen by oedema. In the dry milch cow, mammary enlargement occurs during the last 14 or so days of pregnancy. After the fourth month a honey-like secretion may be withdrawn from the teats of pregnant heifers.

Abdominal ballottement. This is often possible as early as 7 months of gestation in some small breeds such as the Jersey. However, in some fat cows of large breeds it is sometimes impossible even at term.

The method involves fairly vigorous pummelling of the ventral abdomen and flank with clenched fists. The object is to push the fetus, which is floating in the fetal fluids, away from the body wall and then identify it as it swings back against the fist which is kept pressed against the abdominal wall.

Laboratory methods

Identification of early pregnancy factor/early conception factor. Early pregnancy factor (EPF) is an immunosuppressive glycoprotein associated with pregnancy. It was first identified in the mouse (Morton et al., 1974) and subsequently in a large number of domestic species; in the cow it has a molecular weight of 200 000 (Threlfall, 1994). Commercially available test kits are available which use the 'dip-stick' principle and can detect early conception factor (ECF) in serum and milk from as early as 3 days after artificial insemination, although more accurate results are obtained if samples are taken later at 7 to 8 days (Adams and Jardon, 1999; Threlfall and Bilderbeck, 1999). The test, which is still in the developmental stage, obviously has important practical application in the early identification of the non-pregnant cow in the luteal phase after an unsuccessful insemination; thus the cow can be treated with $PGF_{2\alpha}$ to induce a premature oestrus, when it can be rebred, saving time (see Chapter 22).

Assay of pregnancy-specific protein B. This protein has been identified in the maternal serum of cows from 24 days of gestation; the concentration is measured by radio-immunoassay (Sasser and Ruder, 1987). It is secreted by the binucleate cells of the trophoblastic ectoderm (Reimers et al., 1985), and thus its presence can be used to confirm pregnancy. However, since it has a long biological half-life it can also be identified in serum for many weeks postpartum; for the same reason, false positives can occur after embryonic or fetal death.

At present, it can only be measured by radio-immunoassay (RIA) but, with the development of suitable enzyme-linked immunosorbent assay (ELISA) methods, it could well become an 'on-farm' diagnostic test (Sasser and Ruder, 1987). Using RIA to detect the protein, Humblot et al. (1988) reported a 90% accuracy at 30 days of gestation. It has been shown that there is a good correlation between pregnancy-specific protein B (PSPB) in peripheral plasma concentrations and fetal numbers, and thus the method can be used to identify twins (Dobson et al., 1993; Patel et al., 1995).

Progesterone concentration in plasma and milk. In 1971, Robertson and Sarda described a method of diagnosing pregnancy by the determination of the progesterone concentration in the plasma of cows. Since the CL persists as a result of the pregnancy, if a blood sample is taken at about 21 days after the previous oestrus, progesterone levels remain elevated. If the cow is not pregnant and is close to or at oestrus then the progesterone levels will be low; this can be seen if the progesterone curves in Figures 1.28 and 3.3 are studied. Although this is a perfectly valid and reliable laboratory method, it has the one disadvantage that it requires the collection of a blood sample.

In 1969, Heap et al. showed that progesterone crossed the mammary gland and appeared in milk. Laing and Heap (1971) confirmed that the changes in progesterone concentrations in the milk closely followed those in the blood or plasma. Furthermore, since progesterone is very soluble in milk fat there were higher concentrations per unit volume in milk than in the blood or plasma. Heap et al. (1973) described the use of the technique to diagnose pregnancy, and since then a large number of different workers in many different countries of the world have described similar methods. The technique depends upon the herdsperson collecting about 20 ml of milk, usually at the afternoon milking because the fat content is higher, into a glass or plastic bottle. Then a tablet of potassium dichromate and mercuric chloride as a preservative is added; provided that the sample is not exposed to high temperature or excessive ultraviolet light, there is very little loss of progesterone activity.

Initially, progesterone concentrations in the milk were assayed using radio-immunoassay; it is an effective method of measurement but requires the use of radio-isotopes and the equipment to measure radioactive emissions. It can only be performed in a specialist laboratory and hence has the big disadvantage of taking several days before a result is known.

A number of qualitative 'cow-side' tests have been developed which can be used on the farm and hence enable the herdsperson to obtain a result within 1 hour of collecting the milk sample. All the necessary reagents and equipment are provided in kit form. Semiquantitative or fully quantitative tests are also available but these are designed for use in a veterinary practice laboratory, since they require a minimum amount of equipment and some expertise. Both tests are based on ELISA.

The basic principle of the assay is as follows. The plastic wells of the microtitre plates are precoated with a specific progesterone antibody; a milk sample, containing unlabelled progesterone if the cow is pregnant or in dioestrus, is added to each well, together with a fixed quantity of progesterone labelled with an enzyme (usually alkaline phosphatase). After a period of incubation all the contents of the wells are washed away; however, progesterone will remain bound to the antibody and hence the well. A substrate reagent is then added to each of the wells, which, after the second incubation period, reacts with the enzyme-labelled progesterone to produce a colour reaction. The colour can be assessed visually or by a spectrophotometer by comparison with those produced by known standard solutions of progesterone. The amount of labelled progesterone that remains bound to the antibody on the wells is inversely proportional to the amount of unlabelled progesterone in the milk sample. Thus, the higher the concentration of progesterone in the unknown milk sample, the less labelled progesterone will adhere to the wells and hence the lighter the colour reaction. Zero progesterone in the milk sample will result in the most intense colour reaction. The procedure is outlined and illustrated in Figure 3.16. Rapid, solid phase, dipstick methods are likely to become available.

The laboratory-based quantitative tests have a series of progesterone standards that enable a standard curve to be drawn. It is usually recommended that duplicate assays are done for each sample, at least until the operator becomes conversant with the procedure.

There are a few problems in using the assay on the farm. These can be summarised as follows:

- Instructions are not always readily understood by persons not used to laboratory procedures.

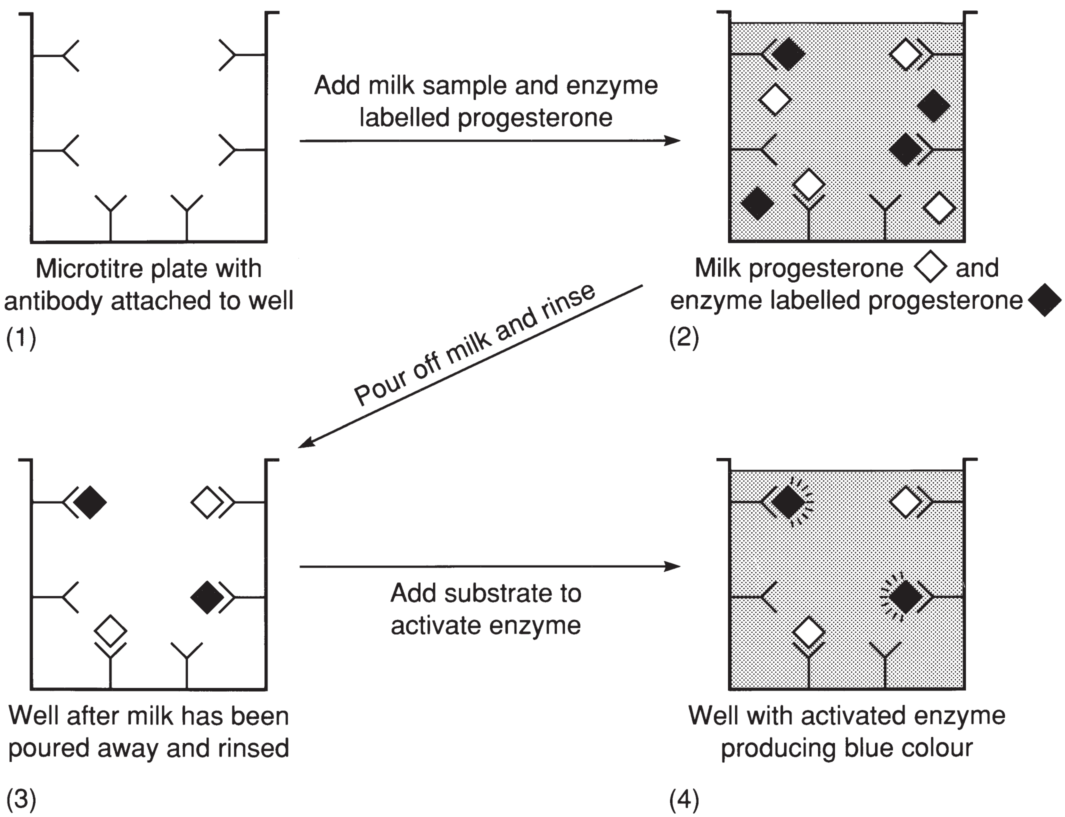


Fig. 3.16 ELISA using a microtitre plate.

- Simple equipment requires a fair degree of manual dexterity.
- Instructions should be closely adhered to, particularly with regard to timing of incubation and quantities of reagents.
- The kits should be kept in a refrigerator at 4°C and should be allowed to warm to room temperature before use; they should not be heated.
- Interpretation of the colour differences can be difficult for some persons.
- Milk samples should be kept at 2–8°C until assayed, and the recommended preservative tablets must be used.

The optimum time for collecting the milk sample is 24 days after service or artificial insemination (Heap et al., 1976). This time interval prevents those cows with a longer-than-average interoestrus interval from giving false positive results; however, in those animals with a shorter-than-average interoestrus interval, false positives will occur. The accuracy of the method in the diagnosis of pregnancy is between 80 and 88% (Heap et al., 1976; Hoffmann et al., 1976; Koegood-Johnsen and Christiansen, 1977); the accuracy of the method for detecting the absence of pregnancy is nearly 100%.

The reasons for false negative results are:

- mistaken identity of the animal either on the farm or in the laboratory
- milk storage problems due to excessive heat or ultraviolet light
- low progesterone production by the CL
- inadequate mixing of milk so that a low fat sample is obtained.

The reasons for false positive results are:

- cows with shorter than average interoestrus intervals, i.e. 18 days. When milk samples are taken 24 days after service or artificial insemination, if the cow is not pregnant she will already be in the early luteal phase of the next cycle
- embryonic death, if it occurs after the day when the milk was collected (see Chapter 24)
- luteal cysts which produce progesterone (see Chapter 22)
- incorrect timing of insemination. Several reports (Hoffmann et al., 1976) have shown that up to 15% of cows are presented for artificial insemination when they are not in oestrus. Thus if a milk sample is taken 24 days after the cow was incorrectly inseminated in early or mid-dioestrus, and the intervening oestrus has not been observed, then she will be in the subsequent dioestrus with a functional CL and elevated milk progesterone concentrations
- pathological prolongation of the life span of the CL; this will be discussed in Chapter 22.

The main advantage of the milk progesterone test is that it identifies those cows that are not pregnant before it is possible to do so by other methods such as rectal palpation, with the exception of the assays of EPF and PSPB, which are still in the developmental stages. A 24-day sample will then enable the herdsperson to anticipate the return to oestrus 42 days after the service if the cow is not pregnant, or enable the veterinarian to examine the animal, if she is a problem, before she returns again. Cows which are found to be pregnant at 24 days should be examined at a later date by transrectal ultrasonography or palpation. The on-farm tests can be used as early as 19 days after service since a low progesterone concentration at this time is highly indicative of non-pregnancy and thus the first return to oestrus can be anticipated (see p. 539). Daily sequential samples can be taken at or around this time, but it is expensive and time-consuming for the herdsperson.

Oestrone sulphate in milk. Oestrone sulphate is quantitatively one of the major oestrogens in the milk of pregnant, lactating cows. During gestation the concentration increases gradually so that after day 105 it is present in the milk of all pregnant animals, whereas in non-pregnant individuals it is low or undetectable; the source of the hormone is the fetoplacental unit. The identification of oestrone sulphate in the milk of a cow at 105 days of gestation, or later, is a very reliable method of pregnancy diagnosis (Hamon et al., 1981). Furthermore, unlike progesterone assays, the precise date of sampling is not required. However, it has limited applications because of the lateness of the time that a positive diagnosis is obtained.

Clinical methods

Transrectal palpation

Palpation of the amniotic vesicle. This method involves the palpation of the amnion towards the end of the first month of pregnancy. It procedes briefly as follows. The bifurcation of the uterine horns is located, then the horns are uncoiled and gently palpated along their entire length between the thumb and middle two fingers. The amniotic sac can be felt as a distinct, round, turgid object 1–2 cm in diameter floating in the allantoic fluid. The vesicle should not be compressed directly but gently pushed backwards and forwards. Some (Rowson and Dott 1963; Ball and Carroll, 1963; Zemjanis, 1971) consider that this technique is dangerous because of the possibility of rupture of the amniotic sac or of the embryonic heart. As with all rectal techniques care is the rule, and excessive pressure and rough handling should be avoided.

Palpation of the allantochorion (membrane slip). This method is dependent upon the facts that in the cow, attachment of the allantochorion to the endometrium occurs only between the cotyledons and the caruncles, and that the intercotyledonary part of the fetal membrane is free.

The method was first described by Abelein (1928) (see Cowie, 1948), who reported that it could be used from the fifth week of gestation. The method is as follows. Identify the bifurcation of the uterine horns, pick up the enlarged, gravid horn between thumb and either index or middle finger just cranial to the bifurcation and gently squeeze and roll the whole thickness of the horn. The allantochorion will eventually be identified as a very fine structure as it slips between the thumb and finger before the uterine and rectal walls are lost from the grasp. It is important in the early stages of pregnancy to grasp the whole width of the horn because as the allantochorion is very thin at this stage the structure that can be more readily identified is the connective tissue band which contains the blood vessels supplying the allantochorion (see Figure 2.1). Fincher (1943) recommended that it should not be used before 40 days of gestation and that it was infallible up to 95 days. The advantage of the method is that it enables the differential diagnosis of pregnancy from mucometra or pyometra. In some cases, particularly after 60 days of gestation, it can be more readily elicited in the non-gravid horn since the tension on the wall is less and this allows it to be grasped more readily.

For the beginner it is a worthwhile exercise to practise it on a fresh gravid genital tract from the abattoir.

Unilateral cornual enlargement. Unless there are twin conceptuses, one in each horn, it is possible to detect a difference in the size of the two horns. This is largely due to the presence of fetal fluids, in particular allantoic fluid, which gives the uterine horn a fluctuating feel with good tone. It can be likened to the feel of a toy balloon which has been filled with water to a point when the wall just starts to stretch. At the same time, if the wall of the horn is squeezed it is noticeable that it is much thinner than that of a non-gravid tract.

In many cases a definite diagnosis of pregnancy can be made on these signs alone. The presence of a CL in the ovary adjacent to the enlarged horn is a useful confirmatory sign; however, a false diagnosis of pregnancy may be made in cases of pyometra, mucometra or incomplete uterine involution (see Chapter 7).

Palpation of the early fetus. At about 45–50 days of gestation the amniotic sac becomes less turgid, and it is sometimes possible to palpate directly the small developing fetus. This should be done with care.

Palpation of caruncles/cotyledons. Caruncles/cotyledons first become recognisable by rectal palpation at 10–11 weeks as roughened elevations when the fingers are passed back and forth over the surface of the enlarged gravid horn. From about 3 months they can be identified as discrete structures in the midline, about 8–10 cm in front of and over the pelvic brim, by pressing down upon the uterine body and base of the horns. In the early stages it is difficult to identify them as distinct, individual structures. The uterus feels as if it has an irregular corrugated surface; it has been likened to palpating a sackful of small potatoes. As pregnancy proceeds, the cotyledons become larger, but once the uterus has sunk into the abdomen between 5 and 7 months, it is sometimes impossible to palpate them, although if substantial pressure is placed on the body of the uterus just cranial to the cervix with the flat portion of the fingertips, as if attempting to palpate the udder transrectally.

Identification of cotyledons is virtually diagnostic of pregnancy, but in the immediate postpartum uterus they can also be felt.

Palpation of the cervix. Evidence of pregnancy can be assumed when there is tension on the cervix. In the non-pregnant or early pregnant cow or heifer the cervix is freely movable from side to side. However, as pregnancy advances the cervix becomes less mobile and it is pulled forwards and downwards over the pelvic brim.

Hypertrophy of the middle uterine artery and development of fremitus. In a non-gravid or early pregnant cow, identification of the middle uterine artery by palpation is usually not possible transrectally. The artery runs in the broad ligament, along a tortuous course, passing downwards, forwards and towards the midline over the pelvic brim close to the junction between pubis and ilium. Usually, it is identified 5–10 cm lateral to the cervix. Inexperienced persons sometimes confuse it with the iliac and obturator arteries, but the middle uterine artery is mobile and it can be encircled within the thumb and forefinger. At some stage during pregnancy it will cease to have the usual pulse, and instead it will becomes a 'thrill' or tremor, which is called fremitus.

There is considerable variation in the time at which the change can first be felt and also when it becomes continuous. The earliest the author has been able to detect it is at 86 days. During the period 100–175 days cases will frequently be met which 'thrill' at first but later pulsate. It is probable that the degree of pressure applied to the artery influences the feeling imparted to the fingers; light pressure detects a 'thrill', whereas a pulse wave is apparent to heavy pressure. The 'thrill' generally becomes continuous after day 175, although cases will be met in which there is distinct pulsation as late as day 200. During the terminal stages of gestation the uterine arteries become greatly hypertrophied and tortuous; they can be distinctly felt, with the thickness of a pencil, with a continuous, tremor-like pulse, laterally situated 2 cm or so in front of the cranial border of the iliac shaft. A difference in size of the two uterine arteries is usually recognisable from about day 100, and this indicates the side of the pregnant horn. Palpation of the middle uterine artery is usually possible in even the biggest cow, and thus is particularly useful in large beef suckler cows. Fremitus in the posterior uterine arteries was detected between 200 and 248 days on the gravid side and between 235 and 279 days on the non-gravid side by Tsolov (1978). He also found that the onset of fremitus was later, the greater the number of times the cow had been pregnant.

Palpation of the late fetus. Palpation of the fetus, either per rectum or by abdominal ballottement, is diagnostic of pregnancy. The ease of palpation depends upon the size of the cow, the degree of stretching of the suspension of the uterus, and the degree of relaxation of the rectum and uterine wall.

Accuracy of pregnancy diagnosis by rectal palpation. The most likely reason for making a false positive diagnosis is subsequent embryonic or fetal death, which is impossible to exclude. Other reasons for false positives are incomplete uterine involution (see Chapter 7), pyometra, mucometra and hydrometra (see Chapter 22), and failure to retract the uterus. The reasons for false negatives are incorrect recording of the date of service or artificial insemination, so that when the cow is examined she is pregnant but a cycle length earlier than expected, and incomplete retraction of the uterus. The latter reason is worthy of further consideration and can be a particular problem in large pluriparous cows with deep abdomens. In order to make a complete examination of the uterus by palpation full retraction is necessary. The inexperienced person may well make a diagnosis of pregnancy because the uterus is out of reach and cannot be palpated. It is important that the diagnosis should be made on the identification of positive signs. It is perfectly permissible to admit uncertainty, to note in writing the changes that can be identified, and to re-examine the animal in 2 or 3 weeks' time.

Induced prenatal death due to rectal palpation. Concern is sometimes expressed that rectal palpation can induce embryonic or fetal death. There have been several studies to evaluate the risks, either by recording if a cow failed to calve having previously been diagnosed as pregnant by rectal palpation or, more recently, in association with milk progesterone assays.

The results have been equivocal, but although it is possible that certain methods and certain individuals may increase the incidence of prenatal death, it is likely that the rectal palpation of cows from 42 days of gestation is a safe and reliable method when performed carefully and skillfully. In those cows where the pregnancy failed, it would probably have occurred irrespective of the procedure used. Furthermore, in experiments where attempts have been made to induce abortion by damaging the fetus at rectal palpation, extensive trauma has frequently been necessary (Paisley et al., 1978).

Vaginal examination. Examination may be manual or visual. In the latter case, an illuminated speculum is used. The condition of the vaginal mucous membrane does not afford definite clinical evidence of pregnancy, for the degree of 'dryness' and blanching which occur during the dioestrous period are very similar to those of pregnancy. It is to the external os of the cervix that attention is directed. During pregnancy the secretion of the cervical glands becomes gelatinous and tough, forming a plug for sealing the canal. In many cases the seal covers or protrudes from the external os. It has developed by day 60.

On manual examination, the finger should be pressed gently into the os. The detection of an adhesive, tenacious secretion rather than a slimy, moist one is strong evidence of pregnancy. With a speculum the seal can sometimes be seen, light brown in colour, covering the os. In many cases, however, the seal occupies the canal only and cannot be detected with certainty.

Ultrasonographic methods

Using the ultrasonic fetal pulse detector, which employs the Doppler principle, it is possible to identify the fetal heart from 6–7 weeks using a rectal probe. Ultrasonic depth analysers (A-mode) have been used to detect pregnancy as early as 40 days. Although a level of accuracy of 85–95% has been achieved in positively identifying pregnant cows, a large percentage of non-pregnant cows (57–87%) were incorrectly diagnosed as being pregnant (Tierney, 1983). Neither of these two ultrasonic methods would appear to have any advantage over rectal palpation with regard to time of examination of accuracy of the diagnosis. Realtime B-mode grey-scale ultrasound scanning is the method of choice for the early diagnosis of pregnancy in the cow. Details of the principle of the technique and the equipment available have been described earlier in this chapter. For an excellent description, the reader is advised to consult Boyd and Omran (1991).

The uterus is imaged transrectally; for early pregnancies a 7.5 MHz linear transducer is required, whereas a 3.5 MHz transducer is preferable for late pregnancies. After insertion of the transducer, both ovaries should be examined to determine the presence of a CL, followed by the right and left horns. Because of the shape of the probe, the diameter of the rectum and the coiled nature of the horns, it is impossible to scan each horn along its length at the same time; thus cross-sectional images of the horns are frequently identified (Figure 3.17f). A tentative diagnosis of an early pregnancy can be made on the identification of a non-echogenic (black) area within the lumen of the uterine horn due to the presence of fetal fluids; however, it must be remembered that fluids other than those associated with pregnancy can give a similar result. Since this occurs first in the horn ipsilateral to the CL of pregnancy, ovarian imaging for the presence of this structure is important. However, the definitive determination of pregnancy is dependent on the identification of an embryo or fetus.

Using a 7.5 MHz transducer, Boyd et al. (1988) were able to confirm pregnancy as early as 9 days, whilst Pierson and Ginther (1984), using a 5 MHz transducer were able to do so at 12 and 14 days in heifers before the blastocyst had elongated (see Chapter 2). By 17 days the blastocyst will have elongated and extended into the contralateral horn; this can usually be easily identified by 26 days.

Experienced persons can accurately diagnose pregnancy, either at the time of, or before, the expected date of return to oestrus in the non-pregnant cyclical animal. This is also true up to 30 days; however, after this stage an accurate and rapid diagnosis can be made relatively easily.

Figure 3.17 shows images at various stages of pregnancy.

The technique can be used to estimate fetal age up to 140 days of gestation (White et al., 1985) following the measurement of a number of different fetal dimensions; of these, the crown–rump

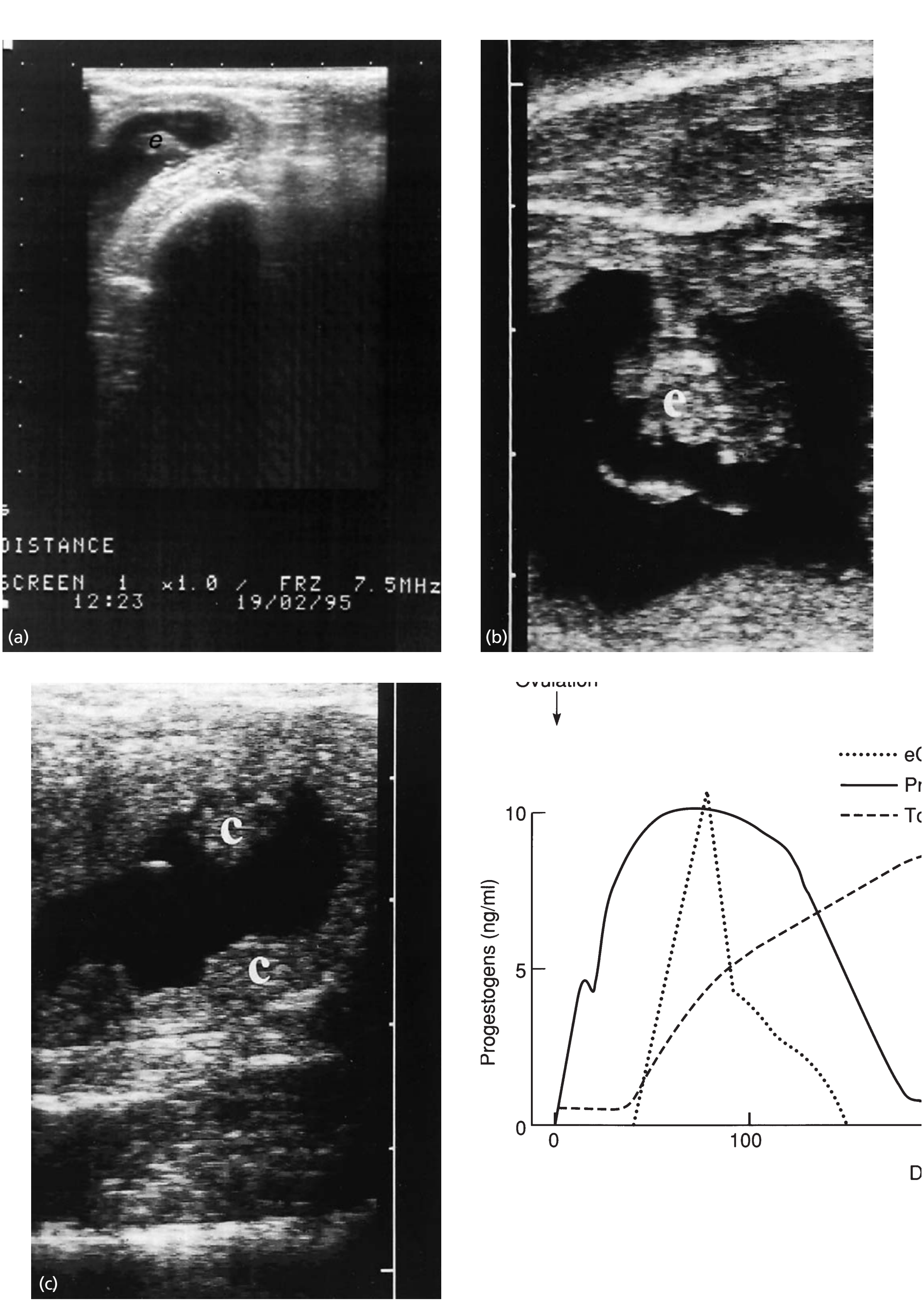


Fig. 3.17 (a)–(d). For caption see opposite.

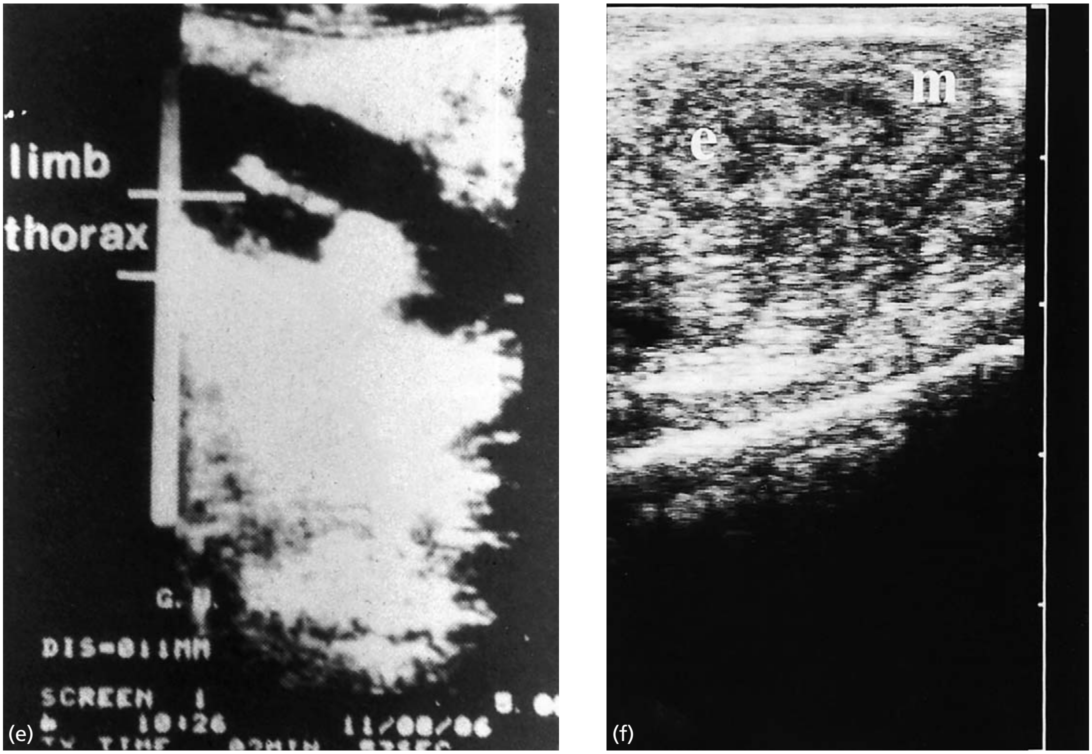


Fig. 3.17 Transrectal B-mode ultrasound images of the gravid uterus of a cow using a 7.5 MHz transducer. (a) At 32 days of gestation. Note embryo, e, surrounded by anechoic (black) area of amniotic fluid. (b) At 38 days of gestation. Note embryo, e. (c) At 38 days of gestation. Note rudimentary caruncles, c. (d) At 41 days of gestation. Note fetus. (e) At 59 days of gestation. (f) Cross-section of non-gravid uterine horn. Note endometrium, e, and muscularis, *m*.

length was least frequently capable of being measured, whereas the trunk diameter was the most readily assessed. In addition, it can also be used to determine the sex of the fetus by assessing the relationship between the genital tubercle and surrounding structures. Thus in the male the genital tubercle migrates towards the umbilicus whereas in the female it migrates towards the tail. The optimum stage for determination is 60–70 days (Stroud, 1996); this experienced author quotes almost a 100% success rate. It is important to stress that the technique requires considerable experience of transrectal ultrasonograpy, and good equipment.

Fetal electrocardiography. Fetal electrocardiography has been noted as a method of pregnancy diagnosis (Larks et al., 1960; Lindahl et al., 1968). It is not applicable before 5 months of gestation, but it might have application for the diagnosis of multiple pregnancies.

Optimum time for diagnosing pregnancy

The aim of pregnancy tests is to identify, as early and as accurately as possible, the absence of pregnancy so that steps can be taken to ensure that the cow is served again and thus ensure an optimum calving pattern (see Chapter 24). However, it is important that inexperienced veterinarians should select a time when they are confident of their accuracy in making the diagnosis, irrespective of the method used.

PREGNANCY AND ITS DETECTION IN THE SOW

Endocrinology

In the non-pregnant sow the plasma progesterone concentration falls rapidly 15–16 days after the previous oestrus, but if conception occurs the CLs persist and the peripheral progesterone concentrations remain elevated at between 30 and 35 ng/ml. Although there is a slight fall to 17–18 ng/ml on day 24, the elevated concentration persists for most of the gestation, decreasing rapidly just before farrowing. The ovaries and CLs are always necessary for the maintenance of pregnancy. The number of embryos present in utero does not influence the progesterone concentration (Monk and Erb, 1974). The minimum concentration of progesterone in the peripheral circulation for the maintenance of pregnancy is about 6 ng/ml (Ellicott and Dzuik, 1973); at lower levels the pregnancy is lost but higher levels do not appear to increase embryonic survival.

Total oestrogen concentrations remain fairly constant during pregnancy but about 2–3 weeks prepartum they begin to increase to about 100 pg/ml, with a sudden surge to values about 500 pg/ml a few days before farrowing. This is followed by a rapid decline after parturition. Oestrone sulphate rises to a peak at 20–30 days of gestation, which is used as a method of diagnosing pregnancy (see later).

Methods of pregnancy diagnosis

Management methods

Traditionally, failure to return to oestrus at 18–22 days after service or artificial insemination has been regarded as a sign of pregnancy. However, the detection of oestrus can be difficult, and it is time-consuming; even the back pressure or riding test, which is generally accepted to be the most reliable (Reed, 1969), is inconsistent. Failure to return to oestrus may be due to a reluctance to show signs, anoestrus or ovarian cysts. It is important to know as soon as possible if a sow or gilt is not pregnant so that she can be served again, treated or culled. A reliable method is also necessary so that breeders can certify that an animal is pregnant before sale. Any technique must be accurate, capable of being used early in gestation and fairly inexpensive.

Clinical methods

Transrectal palpation. This method has been described in detail by Meredith (1976) and Cameron (1977). It is dependent upon palpation per rectum of the cervix, uterus and middle uterine arteries. The details of the method according to Cameron (1977) are as follows.

0–21 days of gestation. The cervix and uterus feel very similar to their state at dioestrus (see Chapter 1). However, during this period the bifurcation of the cornua becomes less distinct and the uterus becomes slightly enlarged, with soft walls. The middle uterine artery increases to approximately 5 mm in diameter towards the third week. It is located as it passes across the external iliac artery (the latter can be identified as it runs along the anteromedial border of the ilium towards the hind leg, ventrally and slightly posteriorly; it is about 1 cm in diameter in the adult sow) running forwards towards the abdominal cavity.

21–30 days of gestation. The bifurcation of the cornua is less distinct, and the cervix and uterine walls are flaccid and thin. The middle uterine artery is 5–8 mm in diameter and more easily identified.

31–60 days of gestation. The cervix feels like a soft-walled tubular structure; the uterus is ill defined and thin-walled. The middle uterine artery has enlarged to about the same size as the external iliac. Fremitus can be first identified at 35–37 days (Meredith, 1976); the pulse pattern can be compared with that of the external iliac artery.

60 days to term. The middle uterine artery is greater in diameter than the external iliac and it has strong fremitus; it now crosses the external iliac artery more dorsally than before. Only towards the end of gestation is it possible to palpate piglets at the level of the cornual bifurcation.

The technique can be performed without the need of much restraint, preferably when the animal is feeding. Unfortunately, it is not possible to perform the technique in gilts because they are too small, and even in large sows a slender arm is advantageous. Cameron (1977) found that between 30 and 60 days of gestation he was 94% accurate in making a diagnosis of pregnancy and 97% accurate in diagnosing non-pregnancy, whilst Meredith (1976) reported an accuracy of 99% and 86%, respectively. The accuracy improves with experience and advancing pregnancy.

Ultrasonographic methods

The use of the fetal pulse detector (Doppler) to diagnose pregnancy in the sow was first described by Fraser and Robertson (1968). The earliest diagnosis made using a rectal probe is about 25 days of gestation. The accuracy of the method is reasonable for the diagnosis of pregnant sows (92–100%) but it is less reliable for non-pregnant sows (25–100%) (McCaughey, 1979).

Ultrasonic amplitude–depth analysis (A-mode ultrasound) has proved to be more reliable. In a study involving 1001 sows using a 2 MHz external transducer probe, Lindahl et al. (1985) reported a 99% accuracy in identifying pregnant sows and a 98% accuracy for non-pregnant sows. These results were obtained between 30 and 90 days of gestation; unreliable results were obtained before 30 days. Similar results were obtained in a smaller sample of 84 sows which were examined between 30 and 64 days after service.

B-mode direct imaging has proved to be very successful in the sow. The transducer probe is applied to the abdominal wall of the standing sow about 5 cm caudal to the umbilicus, to the right of the midline and just lateral to the teats, and is directed towards the caudal abdomen; a coupling medium is always required. In a study of 145 sows, Inabe et al. (1983) reported a 100% accuracy for the diagnosis of pregnancy from 22 days of gestation. Similarly Jackson (1980) reported a 100% accuracy for identifying non-pregnant sows and a 99% accuracy for diagnosing pregnancy from 24 to 37 days of gestation. The small number of errors in this study of 285 sows was due to prenatal death. Using a simple 5 MHz rectal probe a correct diagnosis of pregnancy was made in 10 sows between 12 and 20 days (mean 15.4 days) (Thayer et al., 1985). Obviously, with the development of effective rectal transducer probes, this will be the method of the future as shown in the images obtained at 23 days of gestation (Figure 3.18).

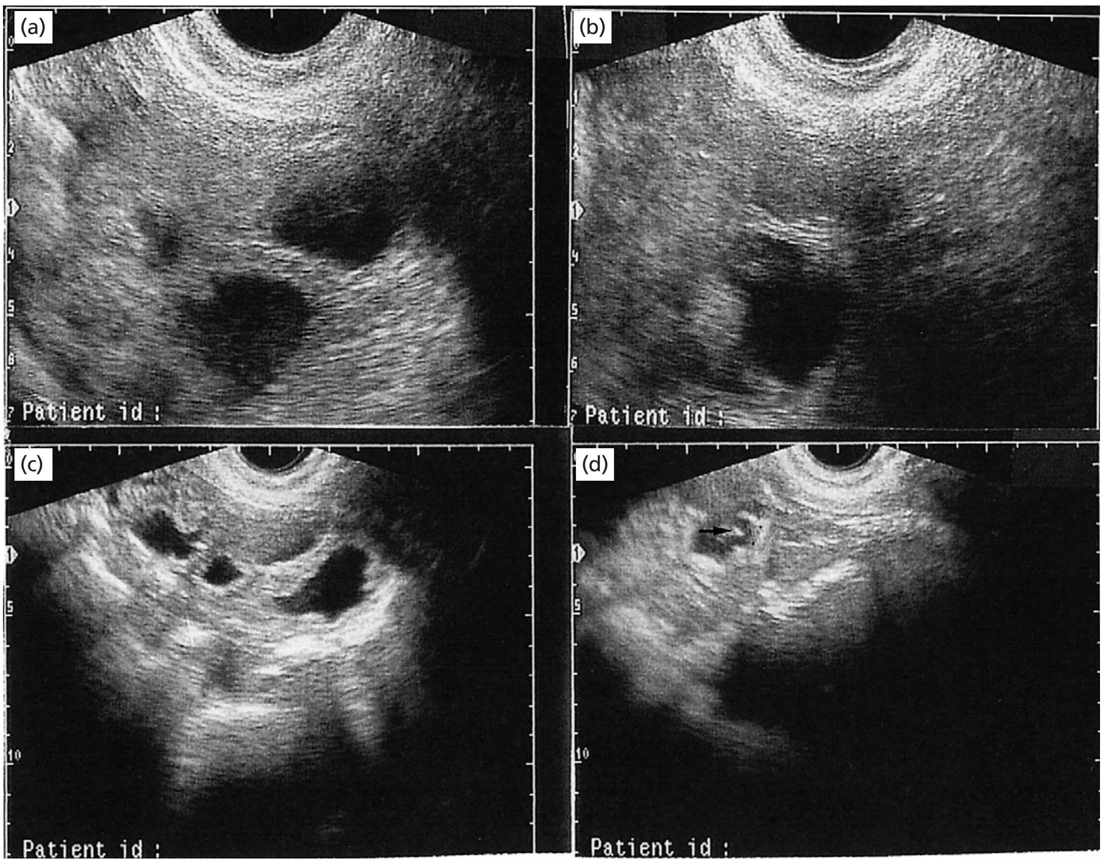


Fig. 3.18 Transrectal B-mode (5 MHz transducer) ultrasound image of a pregnant sow 23 days after service. Note embryos (arrows) surrounded by amniotic fluid (black) (by courtesy of Dr Roy Kirkwood).

Laboratory methods

Vaginal biopsy. Histological assessment of the number of layers of the stratified squamous epithelium of the vaginal mucosa obtained by biopsy (Figure 3.19) can be used as a method of diagnosing pregnancy in the sow (Done and Heard, 1968; Morton and Rankin, 1969). The accuracy of this method between 30 and 90 days of pregnancy is over 90%. Between 18 and 22 days after service it is 97 and 94% for the diagnosis of pregnancy and non-pregnancy, respectively (McCaughey, 1979). There is no doubt that the difference in the histological appearance is greater between oestrus and pregnancy than between dioestrus and pregnancy. The diagnosis depends on the number of layers of vaginal epithelial cells, which in turn relates to the endocrine state; thus during pro-oestrus, when oestrogen is dominant, a rapid proliferation of the stratum germinativum occurs, so that at oestrus there are up to 20 layers (Figure 3.19(a)). From the end of oestrus and

(a)
(b)

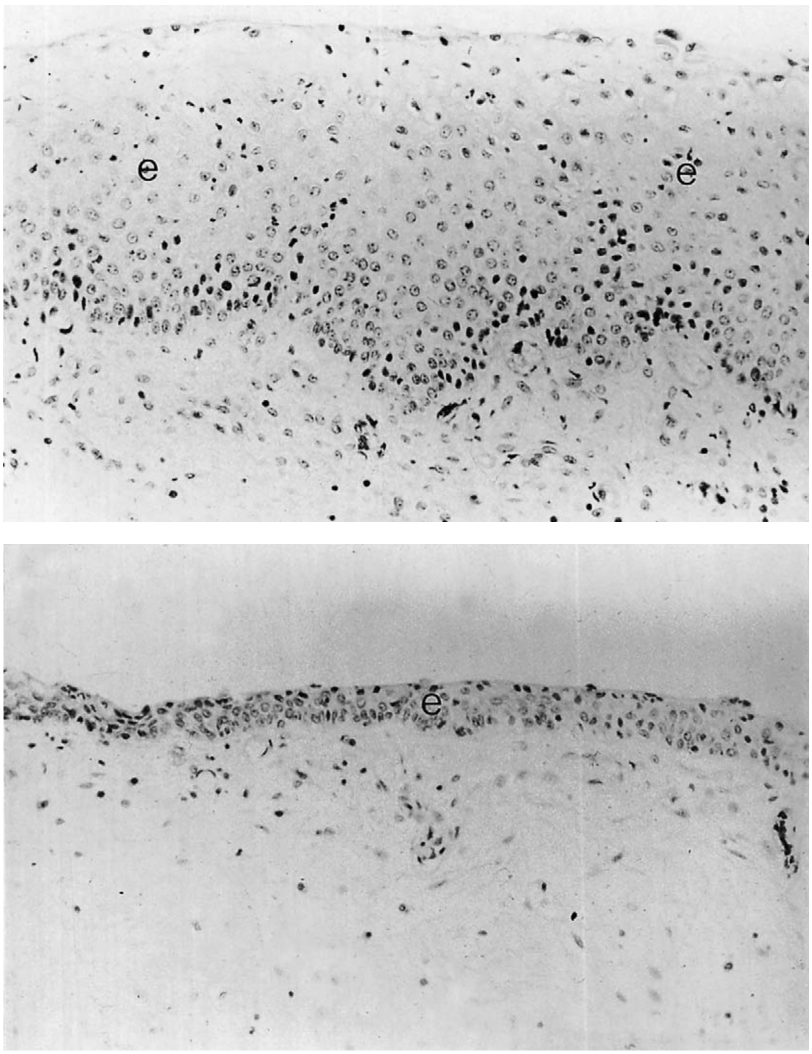
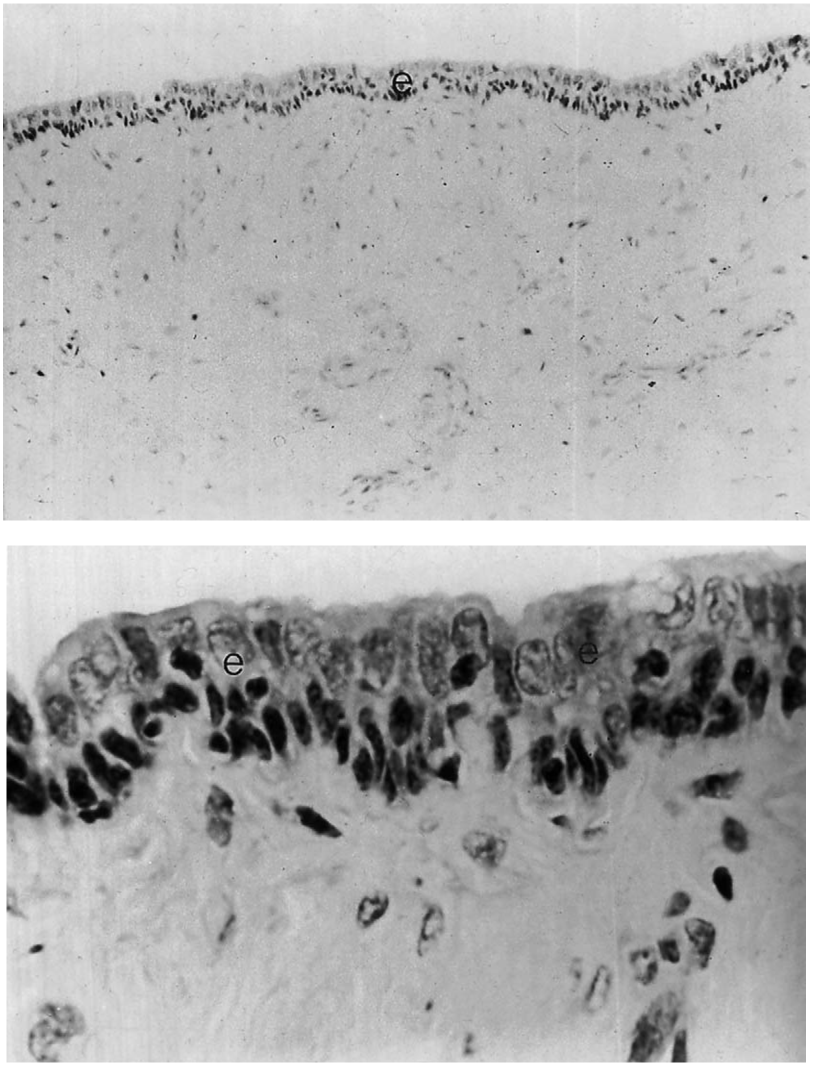


Fig. 3.19 Photomicrographs of sections of sow's vaginal wall biopsies. (a) At oestrus with multilayered epithelium (× 350). (b) During dioestrus (day 10) (× 350). (c) At 32 days of gestation (× 350). (d) At 32 days of gestation (× 1000). Note epithelium (e).

Figs 3.19 (c) and (d), see opposite



(c)

(d)

Fig. 3.19 *continued.*

throughout the luteal phase, when progesterone is dominant, the depth of vaginal epithelium falls, so that by day 11 or 12 there are only three or four irregularly arranged layers (Figure 3.19(b)) and only two or three layers in late dioestrus. With the onset of pregnancy progesterone domination continues, and by day 26 the typical histological picture is two parallel rows of epithelial cells with condensed darkly staining nuclei (Figure 3.19(c) and (d)). This pattern persists until the final 3 weeks of gestation.

Sections taken erroneously from the cervix or posterior vagina are unsatisfactory for diagnosis.

Although the technique is a satisfactory one, the big disadvantage is the cost of the procedure and the time taken to perform the test.

Estimation of plasma progesterone. Since there is a decline in progesterone concentrations in the peripheral blood from about day 16 in non-pregnant cyclical animals (see Figure 1.30), estimation of progesterone concentrations from this time after service would be worthwhile. Meding and Koegood-Johnsen (1978) reported that when sows were bled 16–24 days after service a 96% accuracy of pregnancy diagnosis was achieved; they assumed that values in the plasma ≥7.5 ng/ml were indicative of pregnancy. Because of the irregularity of the interoestrus interval it was more reliable in identifying those sows which were not pregnant. The biggest problem with technique was the difficulty of obtaining blood samples.

Plasma oestrogen assay. Robertson et al. (1978) were unable to detect oestrone sulphate in the blood of non-pregnant cyclic sows and yet in pregnant animals it was detectable from day 20 of gestation. This can therefore be used as a method of diagnosing pregnancy. A small volume of blood sufficient for the assay can be collected from the ear vein; the optimum time for diagnosis, when maximum concentrations of oestrone sulphate are present, is at about 24–28 days.

PREGNANCY AND ITS DETECTION IN THE EWE AND DOE GOAT

Endocrinology

Ewe

In the non-pregnant cyclical ewe, progesterone concentrations in the peripheral blood fall rapidly just before the onset of oestrus (see Figure 1.29). Following conception the CL persists and peak dioestrous values are maintained and gradually increase to about 60 days of gestation, when there is a considerable increase, this rise being due to the placental contribution to progesterone production. Levels remain high until the last week of pregnancy when they decline rapidly to 1 ng/ml at parturition. The concentration of progesterone is significantly higher in multiple pregnancies (Basset et al., 1969) since it has been calculated that in late pregnancy the placenta produces five times as much progesterone as the ovary (Linzell and Heap, 1968). Maximum progesterone concentrations in the peripheral blood of ewes with a single lamb were 3.78 ng/ml between days 105 and 110, with twins 5.09 ng/ml between days 125 and 130, and with triplets 9.18 ng/ml between days 125 and 130 (Emady et al., 1974) (Figure 3.20).

Oestrogen concentrations in the peripheral circulation remain low throughout gestation. A few days before parturition they start to rise then suddenly increase to about 400 pg/ml at the time of lambing, followed by a rapid fall (Challis, 1971).

Prolactin concentrations fluctuate during pregnancy between 20 and 80 ng/ml; towards the end, however, they start to increase and reach a peak of

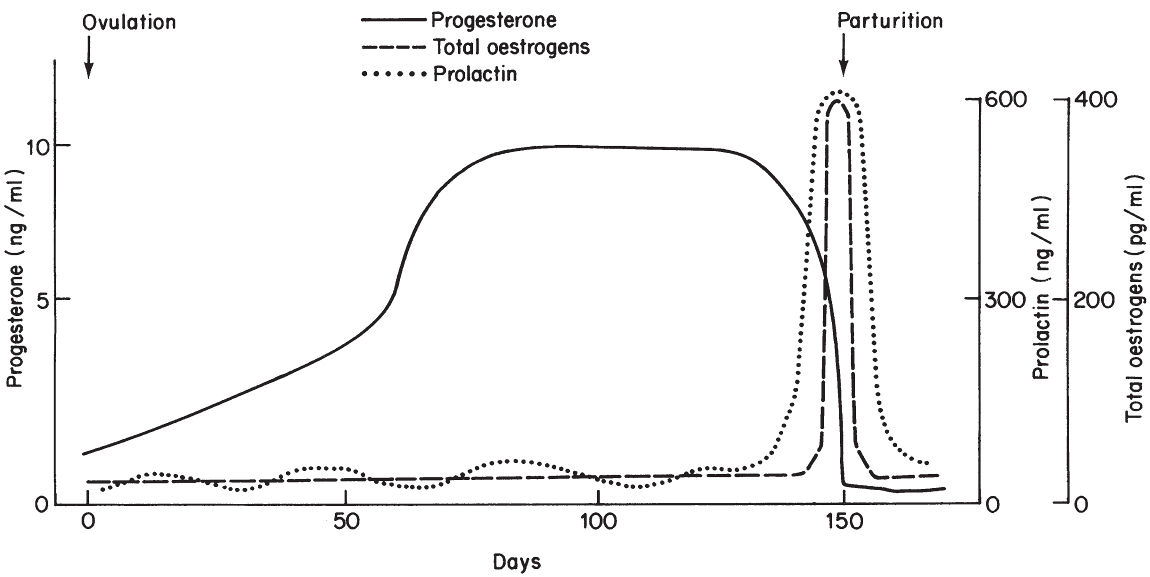


Fig. 3.20 Schematic representation of the trends in hormone concentrations in the peripheral circulation of the ewe during pregnancy and parturition.

between 400 and 700 ng/ml on the day of lambing (Davis and Reichert, 1971; Kann and Denamur, 1974).

Placental lactogen has been detected in maternal plasma from 48 days of gestation; it reaches a maximum by about 140 days, gradually decreasing until lambing. It has been identified in trophoblast tissue from 16–17-day blastocysts (Martal and Djiane, 1977). The role of this hormone is still unclear; it may have a role in the luteotrophic complex of the pregnant ewe and also in controlling fetal growth and mammary development.

Bilateral ovariectomy after 55 days will not result in abortion because by this stage of gestation the placenta has taken over the major role of progesterone production. However, it should be remembered that the CLs persist for the duration of the pregnancy and regress only at the time of parturition (see Chapter 6).

Doe goat

As in the ewe, progesterone concentrations in the peripheral blood and milk decline around the time of oestrus; thus sampling approximately 21 days after service or artificial insemination will enable a distinction to be made between non-pregnancy and pseudopregnancy or pregnancy. In the latter, they increase until a plateau is reached, and then decline rapidly a few days before parturition. Total oestrogens in the peripheral circulation are much higher than those recorded in the ewe. They increase gradually from 30 to 40 days of gestation, reaching a peak value of over 600 pg/ml just before parturition (Challis and Linzell, 1971). Prolactin remains low during pregnancy but rises rapidly just before parturition.

Bilateral ovariectomy at any stage of gestation will result in loss of the pregnancy; thus, extra-ovarian sources would appear to be unable to produce sufficient progesterone for the maintenance of pregnancy.

Methods of pregnancy diagnosis in the ewe

There are a large number of published methods of diagnosing pregnancy in the ewe, many of which are historical, and these have been reviewed in detail (Richardson, 1972). The number and variety of methods point to the fact that there was not a simple, accurate and inexpensive clinical method of diagnosing pregnancy in the ewe until the advent of B-mode ultrasonography, which is without doubt the method of choice.

Management methods

Traditionally the method used by shepherds is the observation that ewes, which have been marked by a 'keeled' or 'raddled' ram, fail to be marked again within 16–19 days. This is a sufficiently reliable sign of pregnancy for most purposes, but subsequent embryonic death will reduce its accuracy, and 20–30% of pregnant ewes will show oestrus during early pregnancy. It is important to ensure that the raddle crayon is changed regularly every 16 days, that it is sufficiently soft to produce a mark and that the colour sequence allows easy colour identification.

Beyond 100 days of gestation the fetus may be palpated through the abdominal wall, and development of the udder is then obvious in primipara. The best way to ballotte the fetus is to have the ewe standing normally and to lift the abdomen repeatedly immediately in front of the udder; the fetus can be felt to drop on to the palpating hand.

Ultrasonographic methods

The fetal pulse detector (Doppler) has been used to diagnose pregnancy in ewes, and two types of probe are available. The external probe is applied to the skin surface of the abdomen just cranial to the udder. The fleece in this region is sparse and with transmission gel applied to the end of the probe it is slowly moved over the surface. The ewe can be restrained either standing or sitting on her haunches. Characteristic sounds indicate the presence of the fetal heart ('tack, tack, tack') or vessels ('swish, swish, swish'); the frequency greatly exceeds that of the mother's heart rate, except in late gestation when the fetal heart rate is reduced. Between 40 and 80 days of gestation the accuracy of detection is no better than 60% (Hulet, 1968; Richardson, 1972). However, after 80 days, with a reasonable amount of practice, it is over 90%

accurate and it takes an average 3 or 4 minutes per ewe to make a diagnosis.

Using a rectal probe Lindahl (1970) reported an accuracy of 97% between 35 and 55 days after mating. There are a large number of reports giving similar results from as early as 20–25 days. The rectal probe is safe and easy to use and requires limited restraint of the ewe. With the fetal pulse detector the diagnosis of false positives should be virtually nil, the only source of error being the confusion of the maternal pulse sounds with those of the fetus. However, false negatives are always a possibility, since there is a limit to the amount of time that the search for confirmatory sounds can be made.

The external probe can also be used between 80 and 100 days to differentiate between single and multiple pregnancies, although the accuracy in identifying the precise number of fetal lambs is poor.

A B-mode ultrasound sector transducer probe, using the transabdominal approach, has proved to be an accurate and rapid method of not only differentiating pregnant from non-pregnant ewes but also accurately determining fetal numbers (Figure 3.21). The cost-effectiveness of such a procedure is obvious since it is possible not only to eliminate barren ewes but also to adjust feeding levels to accommodate the number of lambs. Not only does this save on feed cost but also reduces the chances of pregnancy toxaemia occurring. Pregnancy can be detected as early as 30 days, although the optimum time to differentiate fetal numbers is 45–50 days. White et al. (1984), using a 2.25, 3 or 3.5 MHz transducer, examined a total of 1120 ewes 36–90 days after tupping. The fleece was shorn on the abdomen of each ewe extending some 20 cm cranial to the udder and across the whole width. The ewe was restrained on her back and, using vegetable oil as a coupling medium, the abdomen was scanned. A positive diagnosis of pregnancy based upon the imaging of a fluid-filled uterus and placental material, especially caruncles/cotyledons, could be made quite quickly. Care was taken to examine the limits of the uterus so that fetal numbers could be detected accurately. An experienced person could examine on average 75 ewes per hour. It is possible to examine the ewe in a sitting position. An experienced person was over 99% correct in differentiating pregnant from non-pregnant ewes and 98.9% in identifying fetal numbers. The commonest source of error was failing to identify the third fetus in ewes with triplets. Inexperienced persons soon developed a high level of accuracy. Commercially, B-mode ultrasound scanning is done with the ewe in the standing position in an elevated restraining crate, with the transducer probe placed on the hairless area just cranial and lateral to the udder.

Laboratory methods

Vaginal biopsy. The method is similar to that reported for the sow where the stratified squamous epithelium of the vaginal mucosa is sensitive to the hormonal changes that occur during the oestrous cycle and pregnancy (Richardson, 1972).

Milk and plasma progesterone. Pregnancy can be diagnosed on the fact that in the pregnant ewe the CLs persist and hence peripheral progesterone concentrations will remain elevated at 15–18 days after mating. In lactating ewes it is also possible to determine the progesterone levels in milk. Plasma and milk progesterone values in pregnant sheep 18–22 days after mating were similar (3.7 ng/ml), whereas in non-pregnant ewes they were 1 ng/ml. Lambing results showed a similar accuracy of 82 and 84% (Shemesh et al., 1979).

Rosette inhibition titre (RIT) test. This is an established test for determining the immunosuppressive potential of antilymphocyte serum which has been applied to determining the presence of an 'early pregnancy factor' (EPF) in ewes. In ewes which were subsequently found to be pregnant, the factor could be demonstrated as early as 24 hours after mating (Morton et al., 1979). The RIT test is time-consuming and difficult to maintain; according to Sasser and Ruder (1987), the development of a radio-immunoassay or ELISA for EPF should provide a more reliable method.

Clinical methods

Radiography. Both dorsoventral and lateral radiographs can be taken. Richardson (1972) recommends the use of a 12 × 15 medium-speed film with a grid voltage of 80–90 kV and maximum current of 100 mA. Using an exposure time of

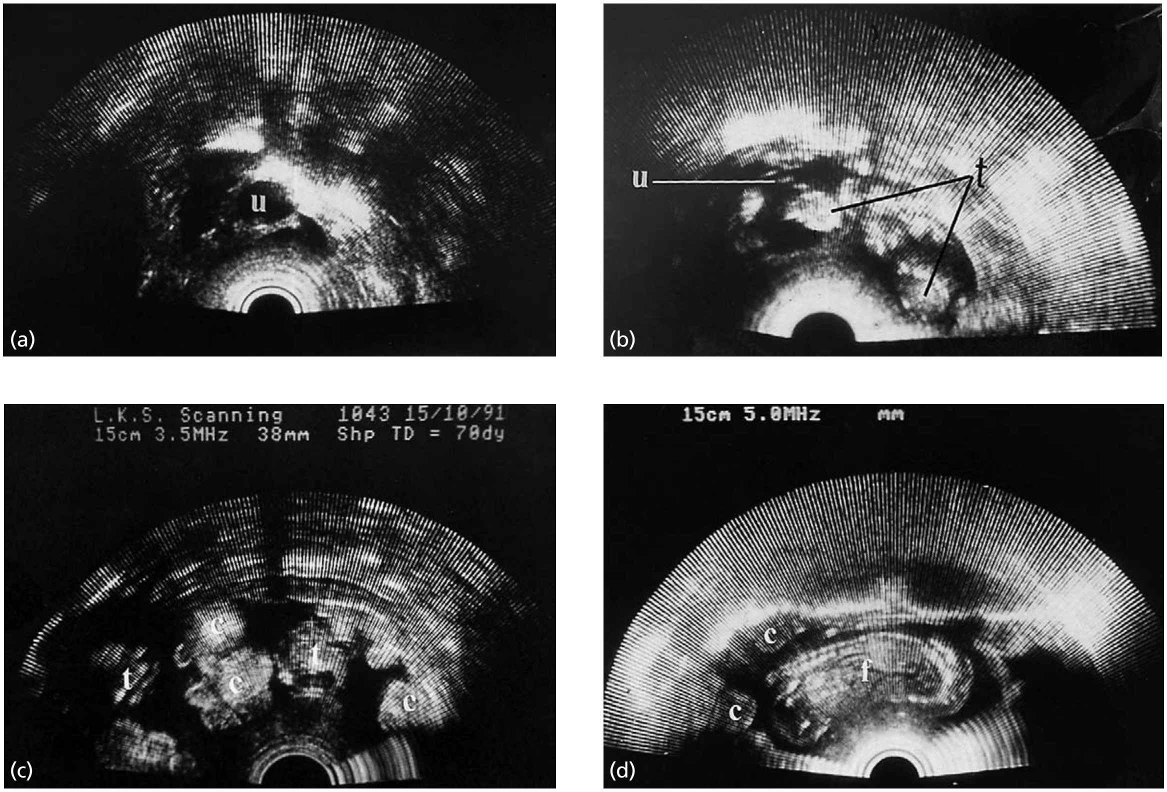


Fig. 3.21 Transabdominal B-mode ultrasound images of the ewe's pregnant uterus and its contents using 3.0 or 5.0 MHz sector transducer. (a) At approximately 35 days of gestation. At this stage it is possible to identify fluid-filled sections of the uterine horns (*u*). In the absence of an embryo or fetus it is only indicative of a possible pregnancy. (b) At approximately 55 days of gestation. Note twin fetuses (*t*) surrounded by non-echogenic (black) amniotic fluid. The umbilical cord can be seen attached to the left fetus (*u*). (c) At approximately 70 days of gestation. Note twin fetuses (*t*) and cotyledons (c). (d) At approximately 80 days of gestation. Note the large single fetus (f) in which the spine can be identified; also cotyledons (c). (e) At approximately 81 days of gestation. Note the single fetus (*f*) with ribs (*r*) and heart (*h*) (courtesy Dr P. J. Goddard).

0.3–0.5 seconds and depending on the dorsoventral dimension of the ewe, fetuses were detectable from 70 days of gestation. The overall accuracy of the method in detecting pregnancy increases with advancing gestation: 52% between 66 and 95 days to 100% after 96 days (Richardson, 1972).

The accuracy in detecting fetal numbers is not so great. Ardran and Brown (1964) quote 22% between 51 and 70 days, 79% between 71 and 90 days and 87% between 91 and 110 days. The method is also useful for estimating the gestational length from 110 days by the measurement of the fetal long bones.

Although the technique is reliable, it is expensive and time-consuming and hence is not practicable for normal farming enterprises.

Other diagnostic methods

Palpation of caudal uterine artery. Identification of enlargement of the caudal uterine artery has been reported as a fairly reliable method of diagnosing pregnancy; the technique requires patience and skill. The arteries can be palpated per vaginam as they run outside the arterior vaginal wall at the 10 o'clock and 2 o'clock positions (Richardson, 1972).

Peritoneoscopy. Phillipo et al. (1971) obtained 91% accuracy of pregnancy detection between 17 and 28 days by means of direct inspection of the uterus and ovaries with a laparoscope, using general anaesthesia.

Methods of pregnancy diagnosis in the goat

Many of the methods which have been described above for the ewe are also applicable to the goat. Using the fetal pulse detector a reliable diagnosis of pregnancy is possible at 50 days with an abdominal probe and 25 days with a rectal probe. Excellent results have also been obtained with B-mode direct imaging from about 30 days of gestation.

The milk progesterone test has been used extensively since most parous goats are lactating at the time of mating. Whole milk samples are collected from the bucket after thorough mixing on or about the day of mating and, in the absence of return to oestrus, 22 and 26 days later (Holdsworth and Davies, 1979). The accuracy for identifying goats that were not pregnant was 100%; however, some false positive results were obtained due to pseudopregnancy, ovarian cysts and elevated progesterone values at oestrus. It is likely, however, that the method will become routine in goat husbandry.

Oestrone sulphate is produced by the fetoplacental unit; its presence in plasma or in milk is a positive indication of pregnancy, thus enabling differentiation from pseudopregnancy. Although it has been shown that oestrone sulphate concentrations from 30 days of gestation exceed those in non-pregnant individuals (Heap et al., 1981), the earliest optimum time is 50 days or later after service (Chaplin and Holdsworth, 1982).

PREGNANCY AND ITS DETECTION IN THE DOG

Endocrinology

The bitch has a prolonged luteal phase with persistence of the CLs for 70–80 days in the non-pregnant animal. Progesterone concentrations in the peripheral circulation of pregnant bitches are similar to those of non-pregnant individuals and for this reason, unlike in other species, cannot be used to diagnose pregnancy. Concannon et al. (1975) obtained mean maximum values of 29 ng/ml for pregnant and 27 ng/ml for non-pregnant bitches. However, there was a lot of individual variation, with peak values obtained between 8 and 29 days after the LH peak in pregnant bitches and between 12 and 28 days in non-pregnant bitches. There is some evidence that at the time of implantation (17–21 days), or just after implantation, progesterone concentrations increase, due possibly to the effect of a placental gonadotrophin (Jones et al., 1973). From about 30 days of gestation there is a gradual decrease in progesterone so that by about day 60 values of 5 ng/ml are obtained, followed by a sudden decline just before parturition, to zero just afterwards. In the non-pregnant bitch there is no rapid fall; low levels of progesterone persist. The number of days in which values ≥1 ng/ml were obtained were 68 days in non-pregnant compared with 63.8 days in pregnant bitches (Concannon et al., 1975).

Total oestrogen values are slightly higher in the pregnant bitch than in the non-pregnant bitch, with some evidence of an increase at the time of implantation (Concannon et al., 1975). They remain fairly constant during the rest of gestation (20–27 pg/ml), before declining 2 days prepartum to non-pregnant values by the day of parturition (Figure 3.22).

Although prolactin concentrations increase during the first half of the luteal phase in both pregnant and non-pregnant bitches, there is a much greater rise in the second half of the former. The gradual rise during pregnancy ends with a sudden surge during the rapid decline in progesterone which occurs 1–2 days before whelping (De Coster et al., 1983; McCann et al., 1988).

Relaxin can be detected in the peripheral circulation of pregnant labrador and beagle bitches at

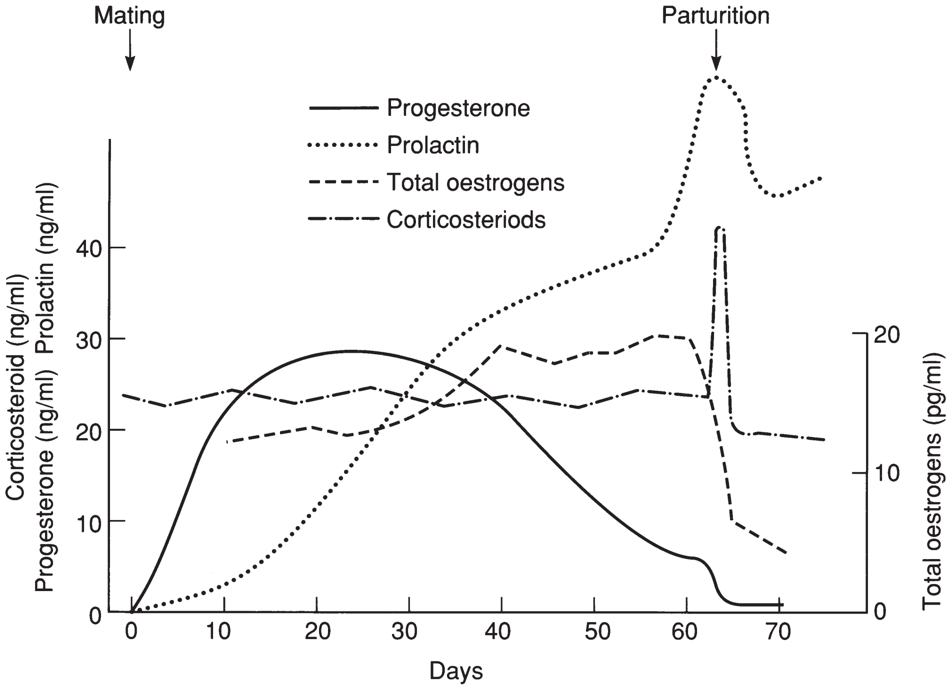


Fig. 3.22 Schematic representation of the trends in hormone concentrations in the peripheral circulation of the bitch during pregnancy and parturition.

20–30 days of gestation, where it was absent in non-pregnant bitches at all stages of the reproductive cycle in non-pregnant animals (Steinetz et al., 1989).

The ovaries of the bitch are necessary for the maintenance of pregnancy; even their removal as late as 56 days resulted in abortion (Sokolowski, 1971).

The average gestation length in the bitch is normally quoted as 63–64 days, but the interval from first mating to whelping can vary from 56 to 71 days. However, if gestation length is measured from the time of the pre-ovulatory LH peak it is very constant at between 64 and 66 days (Concannon et al., 1983).

Methods of pregnancy diagnosis

Since the bitch is not polycyclic, pregnancy cannot be anticipated by a failure to return to oestrus. There is some evidence that pregnancy does increase the interoestrous interval and causes greater regularity of oestrus (Anderson and Wooten, 1959). It is also generally accepted that the period of oestrus ends more quickly after conception.

The main problem in diagnosing pregnancy in the bitch is that overt pseudopregnancy is very common, the degree varying from individual to individual.

The deposition of abdominal and subcutaneous fat during pregnancy is often marked. It is a storing of fat for the subsequent lactation, for it is generally lost again during the period of nursing. The gravid uterus and its contents cause no appreciable increase in body weight during the first 5 weeks. From this point body weight rapidly increases according to the number of fetuses. The increase will vary from 1 kg in a 5 kg bitch to 7 kg or more in one of 27 kg, but by the time body weight has become a guide there are other very definite signs of pregnancy (Figure 3.23). In multiple pregnancy, abdominal distension becomes progressive and obvious from the fifth week onwards, but in animals gravid with one or two fetuses only, particularly when the bitch is large or very fat, distension may not be noticeable.

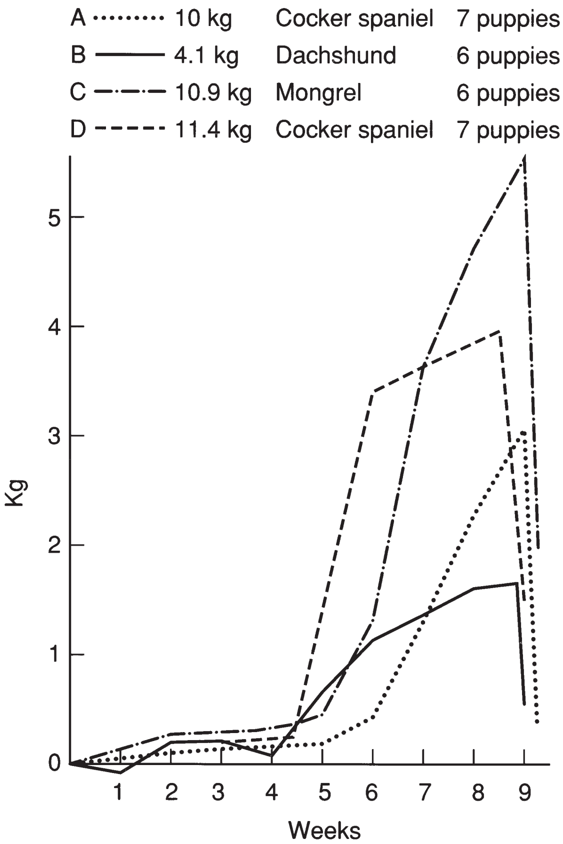


Fig. 3.23 Increase in body weight in bitches during pregnancy.

There are several causes of abdominal distension in the bitch which must be differentiated from pregnancy. The most important is pyometra, which occurs during pseudopregnancy; others are ascites, peritonitis with effusion, splenic enlargement and neoplasia of the liver, abdominal lymphatic glands or uterus.

Clinical methods

Mammary glands. Characteristic changes occur in the mammary glands. Unfortunately, similar but less definite changes may occur during pseudopregnancy. These changes are more easily recognised in primigravida. At about day 35, in unpigmented skins, the teats become bright pink, enlarged and turgid; they protrude. This condition persists until about day 45, when the teats become larger still but softer and tumefied. They may become pigmented. Appreciable hypertrophy of the glands commences at day 50. It progresses until at term the mammary glands comprise two parallel, enlarged and oedematous areas with a depression between them, extending from the pelvic brim to the anterior part of the chest. A watery secretion can generally be expressed from the teats 2–3 days before parturition. The onset of milk secretion coincides with parturition. In multigravida, mammary hypertrophy commences about 7 days before term and in some cases milk can be expressed from the teats several days before parturition.

Abdominal palpation. The ease and accuracy of abdominal palpation will depend upon the following factors:

- the size of the animal: the smaller, the easier
- its temperament: whether palpation is resisted
- the period in gestation at which examination is made
- the number of fetuses in utero
- whether the bitch is of normal size or grossly fat.

Days 18–21. At this stage the embryos represent a series of tense, oval distensions in the cornua, about 12 mm long by 9 mm broad. In small bitches which can be readily manipulated it may be possible to state approximately the number present. Those situated in the posterior parts of the cornua are most easily felt; if only one or two, situated anteriorly, are present they may be missed. In large or fat bitches it is improbable that embryos will be detected at this stage. Care must be taken not to confuse faeces in the colon with fetuses.

Days 24–30. This is the optimum period for the early diagnosis of pregnancy. By day 24 these distensions have become spherical in outline, from 6 to 30 mm in diameter. They remain tense and are easily recognised (Figure 3.24). Sometimes there is variation in size, the posterior ones being rather smaller than those in front. The embryonic units maintain this spherical form until about day 33.

Days 35–44. The constricted portions of the cornua between the embryonic units progressively dilate, the distensions become elongated and lose much of their tenseness. At this period the uterus

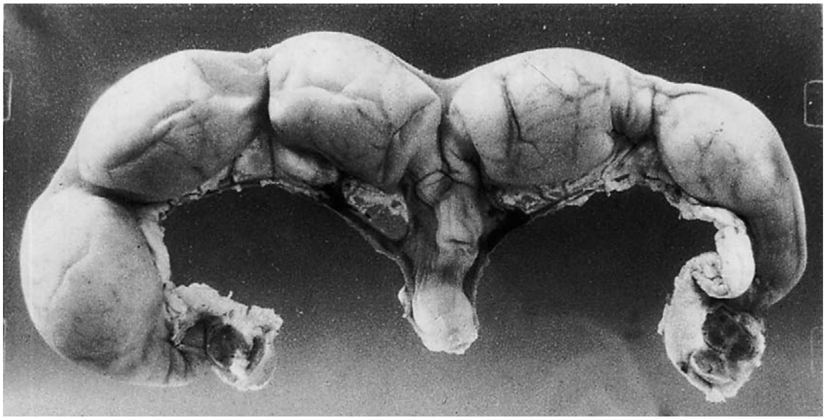


Fig. 3.24 Uterus and ovaries of a bitch, pregnancy of about 30 days. Note five conceptual swellings.

comes into contact with the abdominal wall, and in the animal pregnant with multiple fetuses abdominal distension is commencing to be visible. Nevertheless, palpation of the fetuses themselves is not yet possible and as the uterus itself has lost much of its tension positive diagnosis may be difficult, particularly in those pregnant with one or two only.

Days 45–55. During this stage increase in the size of the fetuses is rapid. At day 45 it may be possible to detect the caudally situated ones between the fingers; in a 9 kg bitch they are approximately 63 mm long and 12 mm broad. It is during this stage that the disposition of the uterus in the abdominal cavity changes. In animals pregnant with multiple fetuses, each cornua represents an elongated cylinder, 38–51 mm in diameter and 228–300 mm long. Caudally, they extend into the uterine body, which has by this time become dilated. Each horn is in two segments: the caudal, which lies on the abdominal floor and passes forwards to the margins of the liver, and the cranial, which lies dorsal and lateral to it, with its long axis directed backwards towards the pelvis. In the last stages the uterus almost entirely fills the abdomen.

Days 55–63. During this period there should be no difficulty in diagnosing pregnancy provided the bitch allows manipulation of the abdomen. The size of the fetuses is such that they can readily be detected. High in the flank the one occupying the apex of the cornua will be felt, while in the midline just in front of the pelvic brim is the one with its extremity in the uterine body. If manipulation is resisted, digital examination per rectum is helpful. The bitch's foreparts should be raised and the uterus pressed backwards towards the pelvic inlet by pressure on the abdomen. The presented part of the caudal fetus will be detected beneath the finger. In big or fat bitches pregnant with one or two only, doubt may still exist, although by this time the mammary glands afford valuable confirmatory evidence.

Accuracy. The accuracy of abdominal palpation is sometimes questioned, particularly in differentiating pregnancy from overt pseudopregnancy. Apart from Hancock and Rowlands (1949), and more recently Allen and Meredith (1981), there have been no reports on the assessment of the accuracy of this method. The latter authors found that although pregnancy was detected at 21–25 days of gestation, the accuracy was only 52% compared with 87% from 26 to 35 days. However, in the case of the correct diagnosis of bitches that did not subsequently whelp, the accuracies were 92 and 73%, respectively, for the same gestational ages. The optimum time for accuracy is between 26 and 35 days of gestation. It is important for a high level of accuracy that the interval from mating to examination is known, since assessment of whether a bitch is pregnant or not is dependent upon the identification of conceptual swellings of a size comparable with the gestational age of the bitch.

Radiography. Radiography is a particularly useful diagnostic aid in the terminal stages of pregnancy, especially in the obese dog where a differential diagnosis from pseudopregnancy is required or in bitches with a single puppy that may have suffered prolonged gestation. It is also very valuable in dystocia cases to disclose the presence of retained puppies and the disposition of a presenting puppy.

In most cases a single radiograph with the bitch in lateral recumbency will suffice, although in an attempt to identify fetal numbers and fetal presentation more accurately a dorsoventral view may be useful. An intensity of less than 100 mA, voltage between 65 and 90 kV and a speed of 0.15–0.03 seconds should be adequate, depending on the size of the bitch and the amount of body fat (Royal et al., 1979). In interpreting radiographs, three points require identification: firstly, displacement of the intestinal mass by the early gravid uterus; secondly, identification of the uterus; and, thirdly, the presence of fetal skeletons. It is possible to see fetal sacs as early as 23–25 days of gestation (Royal et al., 1979). At the end of 6 weeks there may be evidence of fetal skeletons, with the skulls identifiable by 45 days. At the end of 7 weeks it is normally possible to identify the whole fetal skeleton. The technique is not reliable in the identification of fetal numbers. The accuracy of radiographic diagnosis is very much dependent upon the quality of the radiograph that is obtained (Figure 3.25).

Ultrasonographic methods

Using the Doppler method with an external transducer probe placed on the abdominal wall adjacent to the mammary glands, fetal heart sounds were detected as early as 29 days of gestation (Helper, 1970), although in all the 25 bitches confirmed to be pregnant the fetal hearts were heard by 32 days; they were consistently twice the maternal heart rate. Sounds associated with placental blood flow were heard in some bitches as early as 25 days, although it was sometimes difficult to distinguish them from maternal sounds. Helper reported an accuracy of 100%. Riznar and Mahek (1978) found that the earliest time a positive diagnosis could be made was at 44 days. They stressed its value in late gestation, 61–70 days after mating, especially as a method of confirming the presence of live or dead puppies. Using two different instruments, Allen and Meredith (1981) found a low level of accuracy between 25 and 35 days of gestation, which improved with advancing gestational age to reach 100% with one instrument between 43 and 64 days.

Fig. 3.25 Radiograph of the abdomen of a pregnant bitch near term. Note the fetal skeletons.

A-mode ultrasound depth analysers have been employed to diagnose pregnancy using an external transducer probe. Smith and Kirk (1975) were able to detect pregnancy as early as 18 days after mating; these authors stressed the importance of not scanning too far caudally because of the chance of reflections from a full urinary bladder. Using a similar instrument with a 2.25 MHz transducer probe, the earliest correct positive diagnosis of pregnancy was made at 26 days (Allen and Meredith, 1981). From 32 days to term a level of accuracy of about 90% was obtained for the correct diagnosis of pregnant and non-pregnant bitches.

As with other species, B-mode ultrasound has been clearly demonstrated to be the most accurate method of diagnosing pregnancy in the bitch (Figure 3.26). Furthermore, with the development of improved transducers it is likely to become more accurate and become a widely used diagnostic tool. Using a 2.4 MHz linear array transducer probe placed on the abdominal wall, positive identification of pregnancy at a high level of accuracy was achieved 28 days after natural mating or artificial insemination (Bondestam et al., 1984). Of 77 bitches that whelped, only one was incorrectly diagnosed as being non-pregnant, an accuracy of 99.3%, and she expelled a macerated fetus, whilst all 58 that were diagnosed non-pregnant failed to give birth to puppies. Earlier identification of the conceptus has been reported at 14 days (Tainturier and Moysan, 1984) and 21 days (Taverne, 1984). However, the former workers suggested that it was preferable to wait until 20 days.

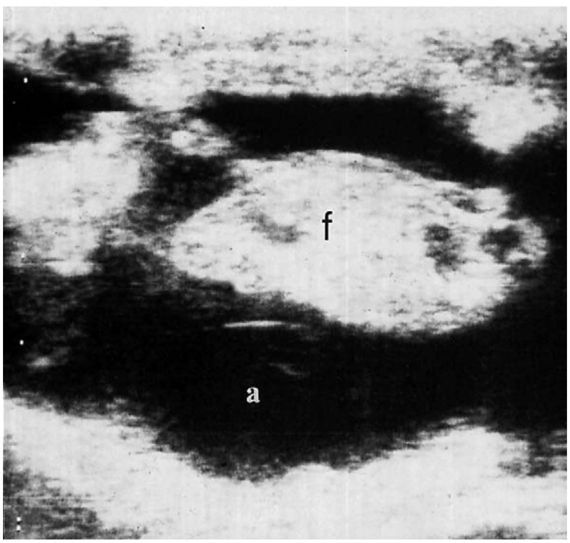


Fig. 3.26 Ultrasound image of the abdomen of a gravid bitch 32 days after plasma LH surge. Note the fetal puppy in transverse section (f) surrounded by amniotic fluid (a).

The accurate estimation of litter size has proved to be difficult, especially in larger breeds of dog. A figure of 40% accuracy at 29 days after mating and 83.3% accuracy from 50 days to term has been reported (Bondestam et al., 1984).

It is generally unnecessary to clip the hair of dogs, even those breeds with long coats, provided that the hair is parted and plenty of coupling gel is used.

Laboratory methods

Measurement of serum proteins. Gentry and Liptrap (1981) observed a three-fold rise in serum fibrinogen concentrations during pregnancy, with peak values occurring 4–5 weeks after mating. Since this phenomenon did not occur at the corresponding stage of the luteal phase in non-pregnant bitches, it can be used as a method of detecting pregnancy. Eckersall et al. (1993) have reported an acute phase response in pregnant bitches as demonstrated by the rise in serum C-reactive protein (CRP) in mid-gestation. This is likely to be produced in response to the implantation of the embryo causing tissue damage. There are always dangers that false positives may arise if there is infection and inflammation elsewhere.

Measurement of hormones in the peripheral circulation. Whereas persistent elevated progesterone concentrations in the peripheral circulation are used to detect pregnancy in the polyoestrous species, they are of no value in the bitch because of the prolonged luteal phase (pseudopregnancy) in non-pregnant animals. Relaxin concentrations are elevated from 20–30 days of gestation, whereas they are not in the non-pregnant animal; kits are now commercially available.

PREGNANCY AND ITS DETECTION IN THE CAT

Ovulation occurs 23–30 hours after mating (Concannon et al., 1980), and serum progesterone

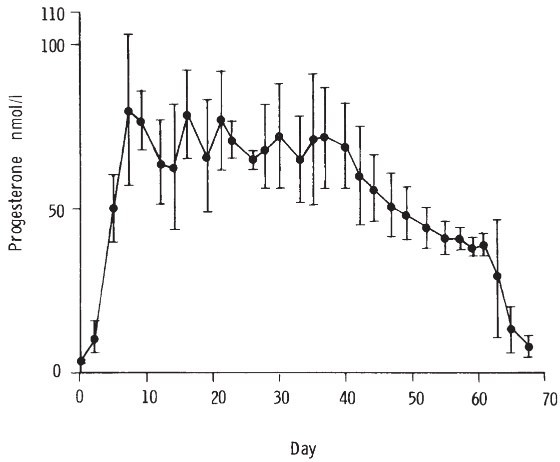


Fig. 3.27 Mean (±SD) plasma concentrations of progesterone throughout pregnancy (four cats). Day 0 represents the day of mating (by courtesy of T. J. Gruffydd-Jones).

concentrations rapidly increase from the baseline of under 10 nmol/l to reach peak values of around 100 nmol/l between the first and fourth weeks of pregnancy (Verhage et al., 1976) (Figures 3.27 and 3.28).

Cats are unusual in that queens may continue to display oestrous behaviour and accept mating, even though ovulation may have occurred and there is significant production of progesterone (Stabenfeldt, 1974).

At 3–4 weeks of pregnancy, hyperaemia of the teats occurs. This is particularly prominent in maiden queens. It is a progesterone-dependent phenomenon and is also seen in pseudopregnancy. There is conflicting evidence concerning the relative roles of the CLs and fetoplacental units in the synthesis of progesterone during pregnancy. Whereas Scott (1970) and Gruffydd-Jones (1982) have reported the maintenance of pregnancy following ovariectomy after 45–50 and 49 days, respectively, Verstegeøn et al. (1993a) have reported abortion following ovariectomy at 45 days of gestation. Progesterone concentrations gradually fall from their peak values during the first month of pregnancy, the fall becoming precipitous during the last 2 days prior to parturition. There may be a slight pre-parturient increase in oestrogen concentrations but this declines just before parturition.

Relaxin is produced by the placenta during pregnancy and is thought to contribute to its maintenance by inhibiting uterine activity. It appears during the third week of pregnancy, with concentrations declining just before parturition (Stewart and Stabenfeldt, 1985). Prolactin is produced during the last third of pregnancy and concentrations decline as weaning takes place (Banks et al., 1983).

The average gestation length is 63–65 days but is reported to vary between 59 and 70 days. The fact that ovulation may not necessarily occur after the first mating may partly explain this considerable variation.

The modal litter size for non-pedigree cats is four with a range of three to seven but there is variation in litter size between pedigree breeds. Oriental breeds tend to have larger litters, sometimes in excess of 10 kittens, whilst pedigree longhair queens tend to have smaller litters, often of only two or three kittens. Singleton litters are unusual and this may reflect fetal resorption due to inadequate fetoplacental endocrine contribution to the maintenance of pregnancy.

A proportion of queens will display oestrous behaviour whilst pregnant. Some of these cats will mate, and this may lead to superfetation. Free-living queens may mate with several competing toms during oestrus and hence superfecundity is common.

Methods of pregnancy diagnosis

Cats lend themselves particularly well to pregnancy diagnosis by abdominal palpation. This is most satisfactorily performed 16–26 days after mating when conceptuses are readily identifiable as individual turgid spherical swellings. Pregnancy may be confirmed as early as 13 days after mating, but, at this stage, the conceptuses may be confused with faecal boluses. After 6 weeks, the conceptual swellings increase markedly in size, elongating and merging, thus making palpation more difficult. However, by this stage, there will usually be significant abdominal enlargement.

B-mode ultrasound enables pregnancy diagnosis to be confirmed by demonstration of an enlarged uterus as early as the first week of pregnancy and, more reliably, by identifying the gestational sacs from the second week (Davidson et al., 1986)

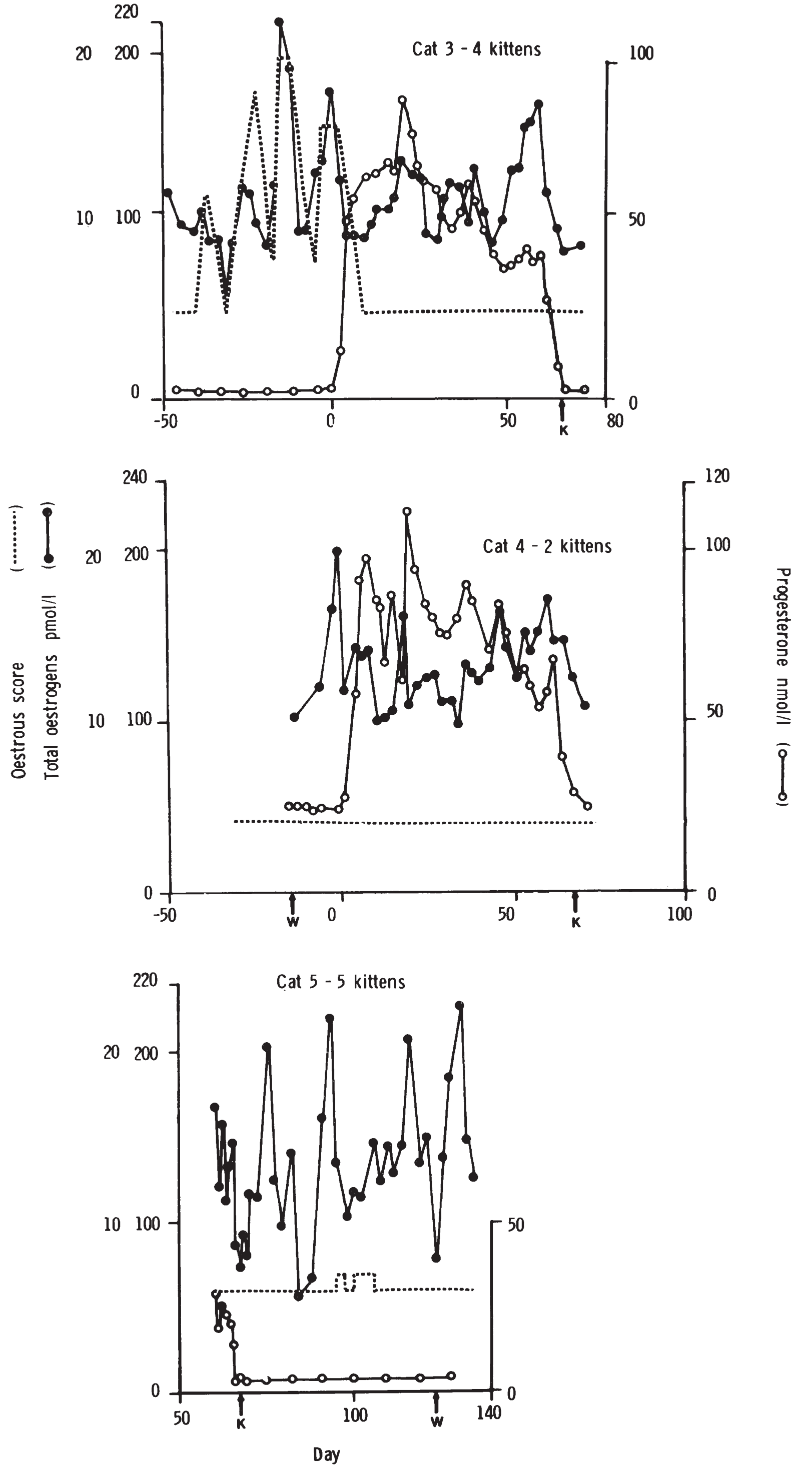


Fig. 3.28 Plasma concentrations of total oestrogens (○——○) and progesterone (●——●), and oestrous scores (–––––) throughout oestrus, pregnancy and lactation in individual queens. Day 0 represents the day of mating; K, the day of kittening; W, the day of weaning (by courtesy of T. J. Gruffydd-Jones).

Fig. 3.29 Ultrasound image of the abdomen of a gravid queen approximately 35 days after mating (7.5 MHz sector transducer). Note the fetus (arrowed).

(Figure 3.29). Fetal cardiac activity can be detected from the third week onwards to assess fetal viability.

PREVENTION AND TERMINATION OF PREGNANCY

In all domestic species there will be occasions when it will be desirable to either prevent pregnancy occurring or terminate it prematurely. Such occasions may follow an unintended mating (misalliance), where pregnancy and parturition may present a severe risk to the dam's health, or where the owners of the animal do not want the pregnancy to continue.

Mare

The management and treatment of twinning are discussed in detail in Chapter 26. If the pregnancy needs to be curtailed for this and other reasons, such as mismating, the treatment of choice is $PGF_{2\alpha}$ or an analogue administered after the CL has become responsive to the hormone, i.e. 4 days after ovulation (see Chapter 1) and before the formation of the endometrial cups, i.e. about 35 days. Therefore, it is preferable to treat approximately 10–15 days after mating. Alternatively, intrauterine infusion of 250–500 ml of physiological saline during the same period will also be effective, since as well as a physical effect in flushing out the conceptus, it also stimulates the release of endogenous $PGF_{2\alpha}$.

Cow

Pregnancy can be terminated from 4–5 to 100 days after ovulation with $PGF_{2\alpha}$ or an analogue, and even up to 150 days many cows will respond. After 150 days, the placenta is the major source of progesterone for the maintenance of pregnancy, until about 270 days of gestation; $PGF_{2\alpha}$ alone is not effective. During this period, either long-acting corticosteroids alone, or in combination with $PGF_{2\alpha}$ are required (see Chapter 6).

Large doses of oestradiol benzoate can terminate pregnancy up to about 150 days by stimulating endogenous $PGF_{2\alpha}$ release; however, it is not as effective as using the hormone directly.

Doe goat

Pregnancy can be terminated at any stage with $PGF_{2\alpha}$.

Ewe

$PGF_{2\alpha}$ is effective in terminating pregnancy after day 4 and before day 12. During the days 12–21 period, there will be no response because of the protective effect of oIFN-τ which ensures the survival of the CL. There is also some suggestion of another refractory period between days 25 and 40. After 45–55 days, the CL is no longer the main source of progesterone for the maintenance of pregnancy, and at this stage corticosteroids will be necessary to terminate pregnancy (see Chapter 6).

Sow

Pregnancy can be terminated at any stage with $PGF_{2\alpha}$.

Bitch

Pregnancy can be prevented in the bitch by the strategic use of oestrogens during the first 5 days after mating. They exert their effect by interfering with the transport of the zygotes from the uterine tube to the uterine horns, probably by causing oedema of the endosalpinx and thus a temporary tubal occlusion. For many years, oestradiol benzoate was used as a single intramuscular or subcutaneous injection at a dose rate of 5–10 mg. Signs of oestrus are prolonged, and it is not advisable to repeat treatment if a mating occurs again, since there is evidence that it predisposes to cystic endometrial hyperplasia (pyometra) if the treatment is repeated. Oral diethylstilboestrol and ethinyl oestradiol treatment has also been used. More recently the use of lower doses of oestradiol benzoate 0.01 mg/kg administered on the third, fifth and possibly seventh days after mating has been advocated since it is possibly a safer option.

Attention has also focused on the termination of pregnancy at a later stage. Natural $PGF_{2\alpha}$ rather than analogues has been found to be effective when administered at a dose rate of 150–270 μg/kg subcutaneously twice daily consecutively on days 10–14 after the bitch has entered metoestrus or pregnancy, as confirmed by exfoliative vaginal cytology (see Chapter 1) (Romagnoli et al., 1993). Earlier reports in which pregnancy was terminated at a later stage, namely 25–30 days, resulted in unacceptable side-effects (Lein et al., 1989). At present, it is doubtful if $PGF_{2\alpha}$ should be used for this purpose.

Recently a dopamine agonist, cabergoline, which also inhibits prolactin secretion and hence indirectly withdraws the luteotrophic support for the CLs, has been used to terminate pregnancy in bitches. It has been used at a dose rate of 1.65 μg/kg subcutaneously for 5 days at 25–40 days of gestation (Onclin et al., 1993). Unlike the prolactin inhibitor, bromocriptine, it does not have unpleasant side-effects, but since a resultant uterine inertia (see Chapter 10) may result in retention of puppies which become macerated (see Chapter 4), it is not without dangers. Most commonly, carbergoline is administered orally daily (5 μg/kg), whilst cloprostenol (5 μg/kg) is given parenterally on alternate days. Abortion/resorption usually follows 10 days after treatment; however, termination of an unwanted pregnancy in the bitch should be closely monitored, particularly with sequential transabdominal B-mode ultrasound.

Cat

Oestradiol cypionate by intramuscular injection at a dose rate of 125–250 μg within 40 hours of mating has been shown to be effective in preventing pregnancy, probably by interfering with the normal transport of the zygotes within the uterine tubes (Herron and Sis, 1974). Similarly, injections of diethylstilboestrol have been used. However, there are little data on possible side-effects, and such treatments should only be used in exceptional circumstances. A single 5 mg dose of megestral acetate is used by some within 1 day of mating.

Recently, both $PGF_{2\alpha}$ and the dopamine agonist cabergoline have been shown to be fairly effective in causing abortion; however, the former has unpleasant side-effects (Verstegen et al., 1993b). Regimens using carbergoline and low-dose cloprostenol appear to be most promising (Onclin and Verstegen, 1997).

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4 Abnormal development of the conceptus and its consequences

The early development of the conceptus has been described in connection with the development of the fetal membranes (see Chapter 2). A number of factors can influence embryonic development. The conceptus may be exposed to harmful agents during the pre-attachment, embryonic or fetal stages of development, and vulnerability to these agents varies with these different stages. For example, during the pre-attachment stage the embryo is very resistant to teratogens and the zona pellucida is an efficient barrier to many viruses. By contrast, the embryonic stage, with rapid cell growth and differentiation, is most susceptible to teratogens. Furthermore, each organ has a critical period of development. For example, the palate, cerebellum and urogenital systems develop relatively late in the fetal period. It should also be remembered that the membranes are part of the conceptus and so any impairment to their development will affect the fetus.

Embryonic or fetal death, together with the birth of abnormal offspring, represent a considerable biological and economic waste.

FERTILISATION FAILURE AND EMBRYONIC/FETAL LOSS

Fertilisation rates in domestic animals are generally very high. Under normal circumstances one would expect approximately 90% of ova shed to be fertilised. However, a high proportion of the ova shed fail to develop to full-term offspring. In some instances as many as 65% are lost during embryonic and fetal development (Table 4.1). Commercially, this loss is of great economic importance.

Table 4.1 Embryonic wastage in domestic animals

Species	*Wastage (%)*	*Reference*
Cattle	45–65	Ayalon (1981)
Pig	30–50	Scofield (1976)
Sheep	20–30	Edey (1969)
Horse	15–24	Ball (1993)

Detection of embryonic/fetal loss

In polyoestrous species embryonic loss can be suspected when there is an irregular extension of the interoestrous period. However, this will be an underestimate of total loss because it will not detect that which is occurring early on, before the maternal recognition of pregnancy and the resultant extension of the life of the corpus luteum (see Chapter 3). Furthermore, in polytocous species, like the pig, embryos may be lost without termination of pregnancy.

More accurate estimations of embryonic loss can be made by slaughtering at different times during gestation and correlating the number of embryos with the number of corpora lutea. However, this method requires the sacrifice of the animal and hence the loss of the pregnancy. A non-invasive method is preferable, but one such as the per rectum examination of the fetus has the disadvantage that it can be carried out only in the larger domestic animals. Furthermore, since the pregnancy can be palpated only relatively late, early embryonic loss goes undetected. More recently, ultrasonic scanning, such as Doppler, A-mode and realtime B-mode techniques, has allowed the very early detection of pregnancy and embryonic loss in a non-invasive manner.

Time of embryonic loss

The various techniques for estimating embryonic loss have shown that most occurs very early in gestation. That which occurs before fetal maternal recognition, and therefore does not involve the elongation of the life of the corpus luteum, is referred to as early embryonic death (EED). Loss after the life of the corpus luteum has been extended is termed late embryonic death (LED). In mares, most loss occurs between days 10 and 14 post-service, in beef cattle it is before day 15 post-service, and in dairy heifers losses plateau after about day 19 post-service. In sheep most losses occur between days 15 and 18 post-service. In the pig, there appears to be two critical stages of embryonic loss, i.e. when the blastocyst begins to expand on day 9 and around implantation at day 13 post-service.

Table 4.2 Some teratogenic agents in ruminants

	Cattle	*Sheep*	*Goats*
Viruses			
Akabane virus	+	+	+
Bluetongue virus	+	+	+
Border disease virus	−	+	−
Bovine viral diarrhoea virus	+	+	−
Cache valley virus	−	+	−
Rift valley fever virus	+	+	+
Wesselbron virus	+	+	−
Plants			
Veratrum californicum	−	+	−
Lupins	+	+	−
Others			
Hyperthermia	+	+	−
Iodine deficiency	+	+	−

Causes of embryonic/fetal loss

Prenatal development is a continuous process of tissue differentiation, organogenesis and maturation. The process is complex, and critical periods of development occur at different times in the different species. Agents causing embryonic loss are therefore highly variable.

Broadly speaking, embryonic loss may be due to either genetic or environmental factors or a combination of the two. The exact effect of each factor depends upon when, during gestation, it is encountered and how it exerts its influence.

Environmental factors causing embryonic/fetal loss

Environmental factors include climate, nutrition, stress, ovulation rate, failure of the normal fetomaternal recognition factors, uterine conditions, hormones, infectious agents, and teratogens. Some infectious agents causing embryonic or fetal loss in the different species are described in Chapters 23, 25, 26, 27 and 28. Teratogenic agents are discussed below, and some are shown in Table 4.2. The remaining environmental causes of embryonic or fetal loss are discussed under separate species headings.

Genetic causes of embryonic/fetal loss

Genetic factors causing embryonic loss include single-gene defects, polygenic abnormalities and chromosomal anomalies. A few single-gene mutations are lethal and result in the death of the conceptus. If the gene is dominant, a single copy may be sufficient to cause death, whilst in other instances it is only the homozygous state that is lethal (e.g. the dominant Manx gene (M) in the cat). Recessive genes only exert their effect in the homozygous state.

Not all genetic defects are lethal. Some abnormal fetuses survive to term, which is biologically and economically wasteful. Therefore, carrier animals should be eliminated from the breeding programme wherever possible. Traditional methods of test mating to identify animals which are carriers of a recessive gene (backcross to the recessive) are laborious and in some cases not justified on welfare grounds. However, new molecular genetic techniques have enabled the development of relatively simple blood tests for carriers of some recessive genes. The list of genes that can be recognised is growing as the genetic maps for the different species are expanded (see Bowling and Ruvinsky, 2000; Fries and Ruvinsky 1999; Piper and Ruvinsky, 1997). Databases on inherited disorders in domestic animals are available on the Internet, e.g.:

- http://www.angis.org.au/Databases/BIRX/mis/
- http://www.angis.org.au/Databases/BIRX/ocoa/
- http://www.angis.org.au/Databases/BIRX/omia/

Some genetic and congenital abnormalities in domestic animals are shown in Tables 4.3–4.9. (For reviews, see: pig – Woollen, 1993; sheep – Dennis, 1993; dog – Stockman, 1982, 1983a,b; Robinson, 1990; cat – Robinson, 1991.)

Table 4.3 Genetic abnormalities in cattle (recessive conditions)

Abnormality	*Breed*
Achondroplasia	Holstein Friesian
Amputates	Holstein Friesian
Oedematous calves	Ayrshire
Tibial hemimelia	Galloway
Arthrogryposis	Charolais (linked to production characteristics?)
Hip dysplasia	Charolais
Familial ataxia	Charolais
Hairless condition	Many
Factor XI deficiency	Holstein Friesian
Syndactyly	Holstein Friesian
DUMS[a]	Holstein Friesian
Weaver	Brown Swiss
Arachnomelia	Brown Swiss
Spinal muscular atrophy	Brown Swiss
α-Mannosidosis	Aberdeen Angus
BLAD[b]	Holstein Friesian

[a] DUMS, deficiency of uridine monophosphate synthesis
[b] BLAD, bovine leucocyte adhesion deficiency

Table 4.5 Some inherited defects in sheep

Abnormality	*Probable genetic cause*
Agnathia	Lethal recessive
Brachygnathia	Recessive (also teratogens)
Arthrogryposis	Recessive (also teratogens)
Inguinal hernia	Recessive
Atresia ani	Recessive
Cryptorchidism	Recessive
Bilateral cystic renal dysplasia	Dominant
Neuraxonal dystrophy	Recessive
Entropion	Unknown
Cataracts	Dominant in New Zealand Romney
Split eyelid	Unknown
Photosensitivity (hyperbilirubinaemia)	Recessive
Micropthalmia/anopthalmia	Recessive
Cerebellar ataxia	Recessive
Muscular dystrophy	Recessive
Goitre	Recessive (also nutritional)
Dwarfism	Recessive

Table 4.4 Common hereditary defects in pigs

Abnormality	*Progency with defect (%)*		*Probable genetic basis*
	Large White	*Landrace*	
Congenital tremors	0.02	0.05	Possibly sex-linked or recessive
Congenital splay leg	0.14	1.43	Recessive, may be sex-linked
Porcine stress syndrome (malignant hyperthermia)	–	–	Recessive genes linked to genes for lean carcass. Mainly in Pietrain
Inguinal (scrotal) hernia	0.44	0.71	Recessive
Atresia ani	0.25	0.32	Recessive, 50% penetrance
Cryptorchidism	0.09	0.23	Recessive, incomplete penetrance
Cleft palate	–	–	Recessive
Pityriasis rosea	0.09	0.42	Unknown
Umbilical hernia	0.13	0.07	Recessive
Intersex	0.06	0.08	Unknown
Dermatosis vegetans	–	–	Recessive
Inherited thick forelegs	–	–	Recessive
Crooked tails	–	–	Dominant
Microphthalmia	–	–	Dominant, with low penetrance
Epitheliogenesis imperfecta	–	–	Recessive
Arthrogryposis	–	–	Recessive (dominant in Large White)
Cerebrospinal lipodystrophy	–	–	Recessive
Bilateral renal hypoplasia	–	–	Recessive
Renal cysts	–	–	Dominant

Table 4.6 Some inherited defects in goats

Abnormality	*Probable genetic cause*
Myotonia congenita	Unknown
β-Mannosidosis	Recessive
Intersexuality	Recessive (associated with the polling gene which is dominant)
Afibrinogenaemia	Incompletely dominant
Anotia/microtia	Incompletely dominant
Udder hypoplasia	Polygenic
Extra teats (polythelia)	Polygenic
Achondroplasia	Incompletely dominant

It was once thought that chromosomal abnormalities might be an important cause of loss of the conceptus because, in humans, it was found that approximately 50% of spontaneously aborted fetuses had a chromosomal abnormality (Lauritsen et al., 1972). However, investigations in domestic animals have shown that probably less than 10% of pre-implantation losses are caused by gross chromosomal abnormalities (Table 4.10).

Certain specific chromosomal abnormalities – for example, reciprocal translocations – do result

Table 4.7 Some genetic defects in dogs

Abnormality	*Breed*	*Possible cause*
General		
Elbow dysplasia (ununited anconeal process)	Many breeds	Polygenic, multifactorial
Hip dysplasia	German shepherd dogs, Labrador retrievers and others	Polygenic, multifactorial
Von Perthes's disease	West Highland whites, miniature and toy poodles	Autosomal recessive but possibly polygenic
Giant axonal neuropathy	German shepherd dogs	Autosomal recessive
Progressive axonopathy	Boxer dogs	Autosomal recessive
Scottie cramp	Scottish terrier	Autosomal recessive
Cryptorchidism	Many	?
High uric acid secretion	Dalmatians	Autosomal recessive
Dermoid sinus	Rhodesian ridgeback	Autosomal recessive?
Deafness	Dalmatians	Polygenic?
Clotting factor deficiencies		
Factor VII deficiency	Beagles	Autosomal recessive
Haemophilia A (factor VIII deficiency = classic haemophilia)	Many	X-linked recessive
Haemophilia B (factor IX deficiency = Christmas disease)	Cairn terrier, St Bernard, America cocker spaniel, French bulldog, Scottish terrier, Old English sheepdog, Shetland sheepdog, Alaskan malamute, Black and tan coonhound	X-linked recessive
Von Willebrand's disease	Scottish terrier, Chesapeake Bay retriever Many other breeds	Autosomal recessive Autosomal dominant
Ocular defects		
Progressive retinal atrophy	Many breeds	Dominant or recessive depending upon type and breed
Hereditary cataract (HC)	Many breeds	?
Collie eye anomaly (CEA)	Collies	?
Cataract	Many	Many different types and genetic causes
Entropion/ectropion	Many	Polygenic
Merle eye	Many	Dominant gene affecting coat colour and tapetum formation. The homozygote is more seriously affected

Table 4.8 Some hereditary defects in cats

Abnormality	*Probable genetic cause*
Oesophageal stenosis	Recessive?
Cataract	Recessive?
Chediak–Higashi syndrome	Recessive
Cutaneous asthenia	Dominant
Deafness in white cats	Dominant?
Episodic weakness	Recessive
Flat-chested kitten syndrome	Recessive
Four ears	Recessive (semilethal?)
Gangliodosis GM_1	Recessive
Gangliodosis GM_2	Recessive
Haemophilia A	Sex-linked recessive
Haemophilia B	Sex-linked recessive
Hageman factor deficiency	Incomplete dominant
Hairlessness (hypotrichosis)	Recessive
Hydrocephalus	Recessive
Hyperoxaluria	Recessive
Hyperchylomicronaemia	Recessive
Mannosidosis	Recessive
Manx taillessness	Dominant (lethal in homozygous state)
Meningoencephalocoele	Recessive
Mucopolysaccharidosis I	Recessive
Mucopolysaccharidosis VI	Recessive
Neuroaxonal dystrophy	Recessive
Polydactyly	Dominant
Porphyria	Dominant
Progressive retinal atrophy	Two forms, one recessive, one dominant
Spheroid lysosomal disease	Recessive
Sphingomyelinosis	Recessive
Umbilical hernia	Unknown

Table 4.9 Some inherited defects of horses

Abnormality	*Probable genetic cause*
Cryptorchidism	Recessive?
Haemophilia	Sex-linked recessive
Combined immunodeficiency (CID)	Recessive
Primary agammaglobulinaemia	Sex-linked recessive?
Aniridia	Dominant
Hereditary ataxia	Recessive
Occipito-atlanto-axial Malformation (OAAM)	Recessive
Torticollis	Recessive
Atresia coli	Recessive
Overshot/undershot jaw	Unknown
White foal syndrome	Recessive
Epitheliogenesis imperfecta	Recessive
Umbilical/inguinal hernia	Unknown
Lethal dominant white	Dominant
Cerebellar abiotropy	Recessive?
Hereditary multiple exostosis	Dominant
Ulnar/tibial malformation	Recessive

Table 4.10 Incidence of chromosomal abnormalities in pre-implantation embryos of domestic animals (data from King, 1990)

Species	*Abnormalities (%)*
Sheep	6.6
Cattle	10.4
Pig	5.0
Horse	0

in reduced litter sizes because of embryonic death. These are discussed separately below.

CHROMOSOME ABNORMALITIES AS A CAUSE OF INFERTILITY AND EMBRYONIC DEATH IN DOMESTIC ANIMALS

It is now well recognised that chromosomal abnormalities play an important role in infertility in some species. The chromosome complement can be determined from any dividing cell. The most common cell type used is peripheral blood lymphocytes. A *heparinised* blood sample is required, and this should be sent for analysis as soon as possible after collection. The process depends upon obtaining dividing cells after a 2- or 3-day culture in a simple tissue culture medium with suitable supplements. After this short-term culture, the cells are inhibited from completing their division by the addition of a spindle blocker to the medium. The effect of this is to accumulate cells at mitotic metaphase. The cells are then fixed and dropped on to slides, which makes the chromosomes spread out so that they can be analysed.

Whilst blood is the most convenient source of dividing cells, longer-term fibroblast cultures can be established from almost any tissue, e.g. skin and peritoneum. Direct preparations (i.e. without any culture) can be made from tissue that is normally rapidly dividing, such as bone marrow, but this is difficult and often painful to obtain and the preparations tend to be of a poorer quality.

Various differential staining techniques enable chromosomes to be individually identified and small abnormalities to be detected. The simplest staining technique (conventional staining) reveals the chromosomal number and morphology, whilst differential staining techniques identify either areas of highly repetitive DNA sequences (C banding) or bands of euchromatin and heterochromatin (G banding and R banding). Molecular genetic techniques, involving fluorescent in situ hybridisation (FISH), can be used to identify specific DNA sequences or whole chromosomes ('chromosome paints'). The chromosome spreads can be photographed, cut out and arranged in an agreed order to construct a *karyotype* (Figure 4.1). The normal diploid chromosome number in our domestic species is shown in Table 4.11.

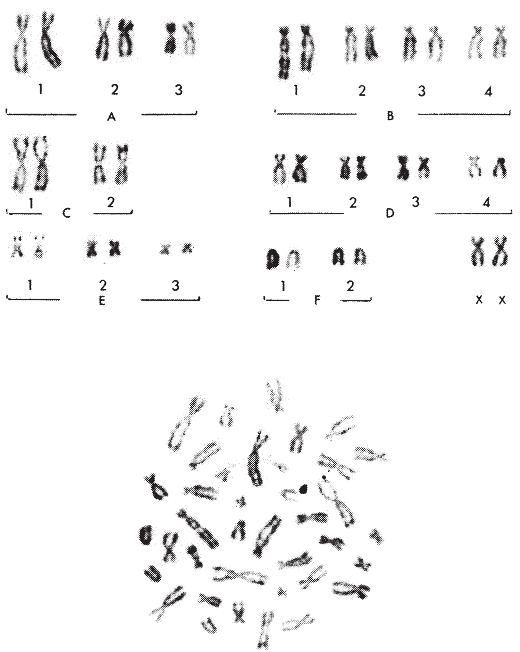

Fig. 4.1 Karyotype and spread from a female domestic cat, *Felis cattus*. $2n = 38$.

Table 4.11 Chromosome number in domestic animals

Species	*Chromosome number*
Cattle	60
Sheep	54
Goat	60
Horse	64
Donkey	62
Pig	38
Swamp buffalo	48
River buffalo	50
Bactrian camel	74
Cat	38
Dog	78

Chromosome abnormalities may be numerical (e.g. aneuploidy or polyploidy) or structural and may occur in the sex chromosomes (X or Y) or the non-sex chromosomes, which are called autosomes. Furthermore, an individual may have more than one cell line and therefore be a mixaploid.

Aneuploidy

Aneuploidy is when the chromosome number is almost diploid but there are one or two chromosomes too many or too few. Aneuploidy arises if there is non-disjunction during meiosis so that the chromosomes do not separate in a balanced fashion. X chromosome aneuploidy in females (XO – Turner's syndrome, and XXX – triple-X syndrome) results in infertility because two X chromosomes are required for normal meiosis in the embryo. Deviation from the normal number results in oocyte atresia during embryonic development.

Extra X chromosomes in the male (XXY, i.e. Klinefelter's syndrome) result in infertility because the extra X interferes with spermatogenesis at puberty. Animals with Klinefelter's syndrome are phenotypic males but with small testes and are azoospermic and sterile.

Aneuploidy of the autosomes results in either too many (trisomy) or too few (monosomy) copies of a particular chromosome and its associated genes. The outcome of this depends upon the genes involved. Either the individual has developmental abnormalities or, if these are not compatible with life, there is embryonic death.

Polyploidy

This is when there are whole multiples of the haploid (i.e. half the diploid) chromosome number in excess, e.g. triploidy is three times the haploid number, and tetraploidy is four times the haploid number. Polyploidy arises when there is a failure of the block to polyspermy or if there is retention of the first or second (or both) polar bodies during oogenesis.

Structural abnormalities

Problems caused by structural chromosomal abnormalities will depend upon whether genetic material has been lost (deletions) or just rearranged (insertions, inversions and translocations). In the case of deletions, carriers of the anomaly may have developmental abnormalities, depending upon which genes have been lost, which may cause embryonic death. With rearrangements, balanced carriers of the anomaly are phenotypically normal, but problems arise during meiotic prophase because the chromosomes have problems at pairing. This often results in non-disjunction and the production of chromosomally unbalanced gametes, which, if they participate in fertilisation events, produce unbalanced zygotes (trisomies or monosomies). Such unbalanced embryos are usually not viable.

Different abnormalities are found at different frequencies in the different species. The more common anomalies in each species are discussed below, but for a more comprehensive description the reader should consult McFeely (1990).

Horses

Chromosomal anomalies are an important cause of infertility in mares. Between 50 and 60% of mares with gonadal dysgenesis have an abnormal chromosome complement. The commonest chromosomal abnormality in mares is X chromosome aneuploidy, e.g. XO or XXX. Another common anomaly is XY sex reversal, i.e. the animal presents as a phenotypic mare but is, in fact, a genetic male. It is difficult to assess the incidence of such anomalies since there have been few random studies of mares. However, in a study of 204 mares presented at stud for breeding, Nie et al. (1993) found one mare was 65,XXX, one was 63,XO/64,XX and one was a 64,XY sex-reversed male.

XO mares are usually small for their age and some have poor body conformation. They usually fail to show any signs of oestrus, and the ovaries are small, fibrous and underdeveloped. The uterus is small and often described as infantile. All XO mares to date have been infertile. In women, the infertility is due to accelerated oocyte loss during fetal development and ovarian atresia in the prepubertal period. A similar mechanism is believed to be involved in the horse.

XXX mares often present with the same clinical history as XO mares. Others have shown irregular cyclic activity, but all have been infertile. In humans, some XXX women have been fertile, although with a reduced fertility. Since it is only infertile mares that are usually examined, it may be that some XXX horses are fertile and so are never presented for examination.

XY sex reversal is not uncommon in the horse. Most cases have been seen in Arabs and thoroughbreds, but this may merely reflect the common breeds examined. All are phenotypically female but are genetic males. The clinical signs are variable. Some animals fail to show signs of oestrus at all whilst others have irregular but strong oestrous behaviour. Some animals have had small, undifferentiated gonads whilst others have had apparently normal ovaries with follicular activity. Most are infertile, but a very few have been fertile and at least one has produced an XY 'filly'.

In some cases the mare may be a *mixaploid* and have normal as well as abnormal cells, e.g. XO/XX, XX/XXX, XO/XX/XXX or XO/XY. In animals with a very low incidence of abnormal cells in an otherwise normal XX population, diagnosis of the condition can be difficult.

The fertility in mixaploid animals with a normal XX cell line is difficult to predict because, although most have been infertile, some have produced a foal. If the animal is actually showing oestrus and follicles are palpable on the ovary it is probably advisable not to declare the animal infertile.

Diagnosis of a chromosomal cause of a fertility problem cannot be made on the basis of the clinical history alone because animals with a normal karyotype present with the same history and clinical findings.

Few studies have been carried out on *stallions* with a fertility problem. Two colts have been reported with autosomal trisomies (Power, 1990). One was a cryptorchid (Power, 1987) and the other had small testes (Klunder et al., 1989). Structural abnormalities have also been found in chromosome 1 in a stallion with a history of early embryonic death in mares which he covered (Long, 1996).

Cattle

The commonest chromosomal abnormality in cattle is a structural anomaly known as a centric fusion translocation. Two chromosomes fuse together near the centromere, resulting in a reduction in the chromosome number but little or no loss in genetic material (Figure 4.2). Over 30 different centric fusion translocations have been reported in cattle, and the commonest of these is the 1/29 translocation which is found in a large number of breeds throughout the world (Table 4.12). Heterozygosity (i.e. carrying one copy) for a centric fusion translocation results in a drop in fertility, the extent of which depends upon which translocation is being carried. Animals heterozygous for the 1/29 translocation have a reduced fertility of the order of 5%. The drop in fertility is due to non-disjunction at meiosis and the pro-

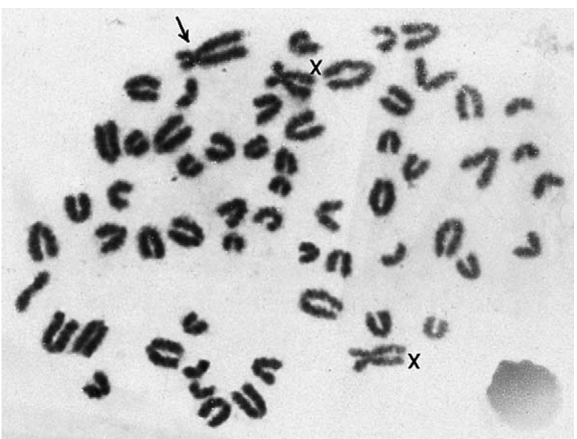

Fig. 4.2 Chromosome spread from a cow, heterozygous for the 1/29 centric fusion translocation. $2n = 59$. The arrow indicates the translocation chromosome.

Table 4.12 Cattle breeds carrying the 1/29 centric fusion translocation

Breed	*Incidence (%)*
Barrosa	65.1
Bauole	
Blonde d'Aquitaine	14.2–21.6
British White	65.6–78.8
Brown Atlas	
Brown Mountain	
Brown Swiss	0.2
Charolais	1.9
Chianina	13.6
Corsican	40.0
Czechoslovakian Red Pied	
De Lida	8.2
Fleckvieh	3.1
Galicia	11.8
Gascons	
German Red Pied	
Grauviel	
Hungarian Grey	3.8
Japanese Black	
Kuri	
Limousin	6.0
Marchigiana	18.9
Maremmana	
Maronesa	49.5
Modicana	6.5
Montebeliard	2.2
Nguni	10.2
Norwegian Red	
Ottenese	
Pisa	
Podolian	
Red Poll	19.0
Romagnola	22.5
Russian Black Pied	
Santa Gertrudis	
Siamese	
Simmental	3.0
Swedish Red and White	13.4
Vosgienne	
Zamora	24.0

duction of chromosomally unbalanced gametes. The resultant unbalanced embryos undergo early embryonic death. The reduced fertility is manifested as a small increase in services per conception in the female and a lower non-return rate in the male. Carriers of some of the other centric fusion translocations have an even greater reduction in fertility, e.g. 46% with the 25/27 translocation in the Alpine Grey.

Artificial insemination organisations in many European countries now screen their bull population, and animals carrying translocations are not used. Many countries will not allow the importation of animals carrying such translocations.

A number of other chromosomal anomalies have been reported in cattle but these are much less common. Some phenotypically abnormal calves have been born with autosomal trisomies, a few cases of infertility in the bull have been due to the XXY Klinefelter syndrome, and XXX infertile cows have been found.

Chromosomal analysis is used in the diagnosis of *freemartins* because, in this condition, the fusion of the placental blood vessels not only allows the Mullerian inhibition substance (MIS) and testosterone from the male to affect the female development, but also allows the mixing of haemopoietic precursor cells and so leads to the establishment of male and female cells in the blood of each twin. This can be detected by a simple chromosome analysis at any time after birth, and therefore freemartinism can be predicted (Long, 1990). Diagnosis is easier if blood samples from both the suspect freemartin and the male twin are analysed. This is because sometimes there is a very low number of male cells in the female, but if that is so, there will be an equally low number of male cells in the male, if placental anastomosis has taken place. Examination of the male saves considerable time in these cases. However, diagnosis is possible using samples from the female alone. No samples are required from the sire or dam.

There is some debate as to the fertility of males, born twin to females. Some surveys have shown reduced non-return rates (Cribiu and Popescu, 1982), poor sperm freezability (Switonski et al., 1991) or poor sperm concentration and motility in the ejaculate (Dunn et al., 1979) of chimeric bulls. However, other surveys have shown no reduced fertility (Gustavsson, 1977), and a normal semen picture (Gustavsson, 1977; Jaszczak et al., 1988) in such animals. There is no obvious reason for the contradictions in these findings.

Pigs

In the pig, reciprocal translocations are the most common chromosome anomalies. These are structural abnormalities where parts of chromosomes are exchanged with little or no loss of genetic material. Carriers of reciprocal translocations are phenotypically normal but have problems at meiosis and produce unbalanced gametes. This leads to a reduced litter size or even infertility. In Sweden, 50% of boars culled for small litter sizes were found to be heterozygous for a reciprocal translocation. Of some concern is the fact that most of these translocations appear to have arisen de novo and were not inherited. This implies that there is something in the pig's environment that is inducing new translocations. Approximately 45 different reciprocal translocations have been identified in the pig to date.

Sheep

Cases of infertility in the ram have been due to the XXY Klinefelter syndrome. Centric fusion translocations are found in New Zealand Romney, Perendale and Drysdale sheep in New Zealand and in the Romney Marsh breed in Britain. However, unlike in cattle, centric fusion translocations have not been associated with a reduction in fertility in sheep. Reciprocal translocations have been reported in sheep with reduced fertility but these are rare (Glahn-Luft and Wassmuth, 1980; Anamthawat-Jonsson et al., 1992).

In a survey of barren ewes, XX/XY (freemartinism) was the commonest finding (Long et al., 1996). The XO (Turner's syndrome) may be another cause of barren ewes, since one or two cases have been reported.

Goats

Centric fusion translocations have been found in Saanen and Toggenburg goats, but the effect on fertility is difficult to assess because of the confounding factor of the intersex gene associated with polledness in this species. Nevertheless, it does seem that heterozygosity for a centric fusion translocation is associated with reduced fertility in the Saanen breed.

The intersex gene is an autosomal recessive which is linked to the dominant gene for polledness. Thus, females that are homozygous for the polling gene are also homozygous for the intersex gene and are intersexes. Males that are homozygous for the polling gene are usually normal. However, 10–30% have been infertile due to tubular blockage in the head of the epididymis. This intersex gene in goats is thought to be similar to the *Sxr* gene in mice which causes testicular development in genetic females.

Cats

Chromosomal abnormalities are not common in the cat except in association with the tortoiseshell coat colour in the male. Many of these are XXY Klinefelter cats, and they are always sterile. However, the mixaploid animals which have a normal XY male cell line may be fertile. Fertility presumably depends upon whether the normal XY cell line is found in the testis. Fertile male tortoiseshell cats will breed as if they were normal XY toms, and will pass on the coat colour genes in a normal Mendelian fashion. Whilst male tortoiseshell cats are not common they are probably less rare than is believed. A survey of 9816 cats in Britain, of which 4598 were males, revealed 20 male tortoiseshell cats (Leaman et al., 1999).

Since there is no reason to suppose that these chromosomal anomalies are only found in tortoiseshell cats they are probably responsible for fertility failure in other cats but have gone undiagnosed because very few cats are examined cytogenetically.

The XO condition has been reported and is therefore a possible cause of infertility in the female.

Dogs

Chromosomal abnormalities have not been associated with infertility in the dog except in one case of Klinefelter's syndrome and one case of prolonged pro-oestrus in a bitch with X chromosome monosomy (77,XO). Centric fusion translocations have been reported, but there have been no consistent clinical findings. Their influence on fertility has not been investigated.

Mules and hinnies

The mule is a cross between a female horse and a male donkey, and the hinnie is a cross between a female donkey and a male horse. The males of both crosses show abnormalities of chromosome pairing at the pachytene stage of meiotic prophase, and little or no mature spermatozoa are produced. Thus the males are infertile. Females are also affected during the fetal development of the germ cells, and most oogonia die as they are entering meiosis. However, sometimes a mature follicle is present in the adult, and, rarely, confirmed foalings have been reported in both mules and hinnies.

CONGENITAL ABNORMALITIES AND TERATOGENS

Congenital abnormalities are abnormalities that are present at birth (Figure 4.3). They may be caused by genetic factors or some other agent. A teratogen is an agent that can induce abnormalities in a developing conceptus. Teratogenic agents may not kill the developing conceptus, but many of the abnormalities they induce are incompatible with life.

Teratogens have their major effect during the embryonic stages. Before this, during pregastrulation, the conceptus is relatively resistant to the effects of teratogens, and after this, at the fetal stage, it is only the late-developing systems such as the palate, cerebellum and parts of the cardiovascular and urogenital system that are affected. A teratogen may be a drug, hormone, chemical, gamma irradiation, trace element, variations of temperature, or an infectious agent (particularly viruses – see Oberst (1993) for a review). For example, in the pig, swine fever (hog cholera) virus will produce neurological abnormalities such as demyelination, cerebellar and spinal hypoplasia, hydrocephalus and arthrogryposis. Table 4.2 shows some of the known teratogenic agents in

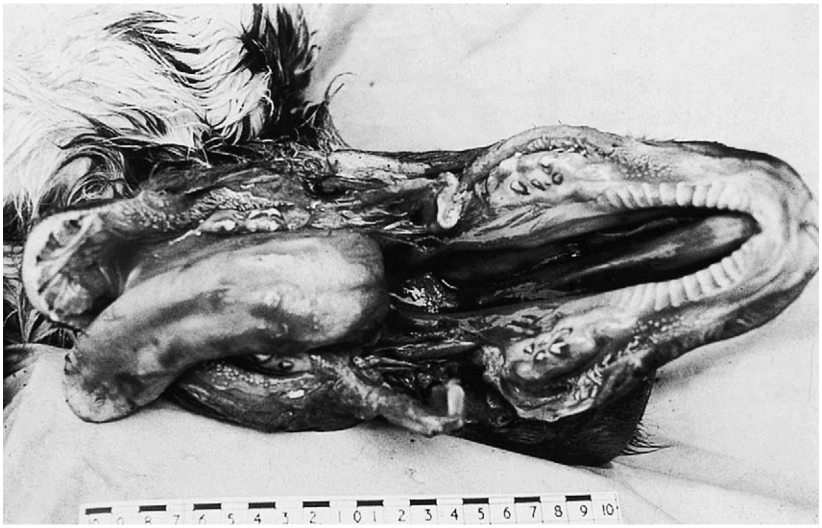

Fig. 4.3 Extreme example of cleft palate in a calf.

ruminants. In the dog, some common pharmacological agents such as corticosteroids and griseofulvin are known teratogens, and care must be exercised in their use in pregnant bitches. In the cat, the panleucopenia virus will cause teratogenic effects in pregnant queens.

Congenital abnormalities may cause obstetrical problems. For example, *perosomus elumbis*, which occurs in ruminants and swine, is characterised by hypoplasia or aplasia of the spinal cord which ends in the thoracic region. The regions of the body, including the hind limbs, which are normally supplied by the lumbar and sacral nerves, exhibit muscular atrophy, and joint movement does not develop. The rigidity of the posterior limbs may then cause dystocia.

Schistosoma reflexus, another abnormality common in ruminants and swine, has as the main defect acute angulation of the vertebral column such that the tail lies close to the head (Figure 4.4). The chest and abdominal cavities are incomplete ventrally so that the viscera are exposed. Again such cases may cause dystocia (see Chapter 16).

Another strange entity is that known as *amorphous globosus*, acardiac monster or fetal mole (Figure 4.5). These are spherical bodies, attached to the fetal membranes of a normal fetus. They are formed from connective tissue surrounded by skin and may be of a different sex to that of the normal twin. Since there is not usually any gonadal development they do not pose a threat of freemartin development.

Double monsters (Figures 4.6 and 4.7), which are found in a number of species, will present as absolute fetal oversize. Other examples of causes of fetal oversize are hydrocephalus (Figures 4.8 and 4.9) and accessory limbs (Figure 4.10).

Some of the congenital anomalies that are of importance in veterinary obstetrics have a genetic cause. For example, achondroplasia (dwalf calves) (Figure 4.11) and amputates (otter calves) (Figure 4.12) in Friesians, double muscling and arthrogryposis (Figure 4.13) in the Charolais, and oedematous calves in the Ayrshire breed (Figure 4.14) (see Table 4.3). Leipold and Dennis (1986) described rectovaginal constriction in Jersey cattle, due to a simple autosomal recessive, as an important cause of severe dystocia. Affected animals required episiotomy or caesarean section at parturition.

Some congenital abnormalities, such as spastic paresis (Figure 4.15), resolve in time.

EMBRYONIC/FETAL LOSS IN THE DIFFERENT SPECIES

Pigs

Ovulation rate is not usually a limiting factor in productivity in the pig but, in general, as ovulation

Fig. 4.4 *Schistosoma reflexus* in a calf.

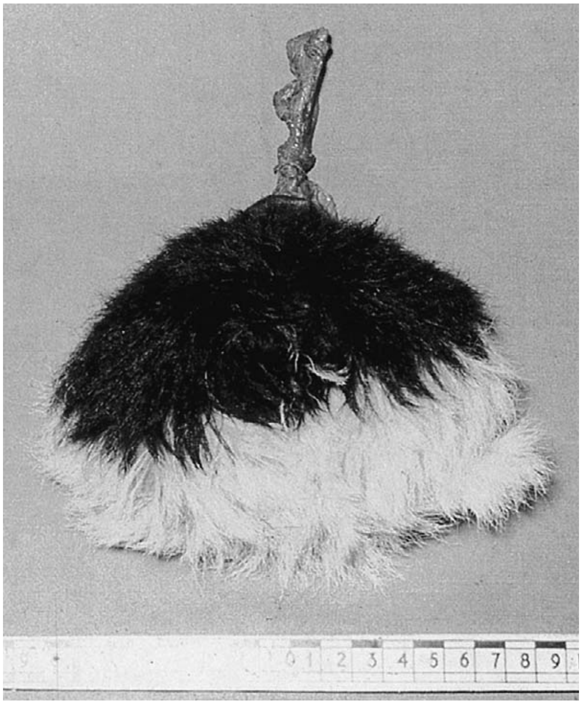

Fig. 4.5 *Amorphous globosus* in the cow.

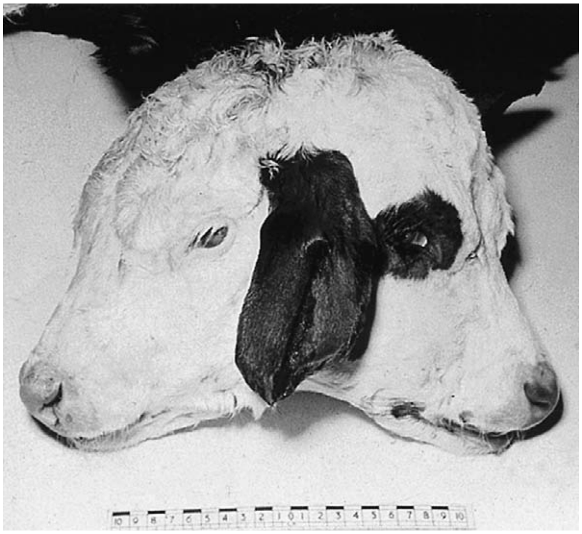

Fig. 4.6 Double-headed calf.

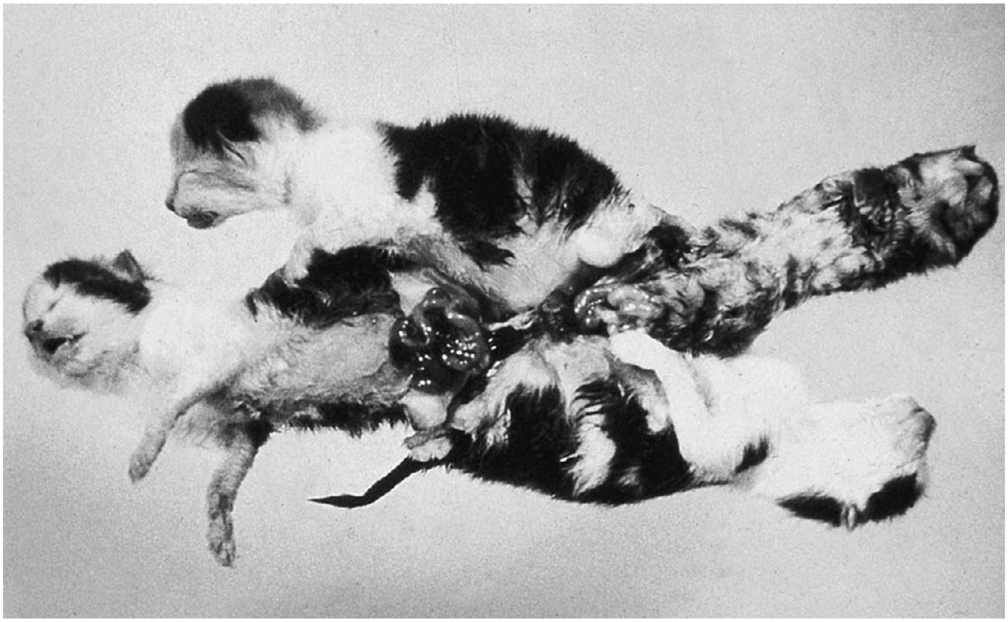

Fig. 4.7 Litter of four kittens joined in the pelvic region.

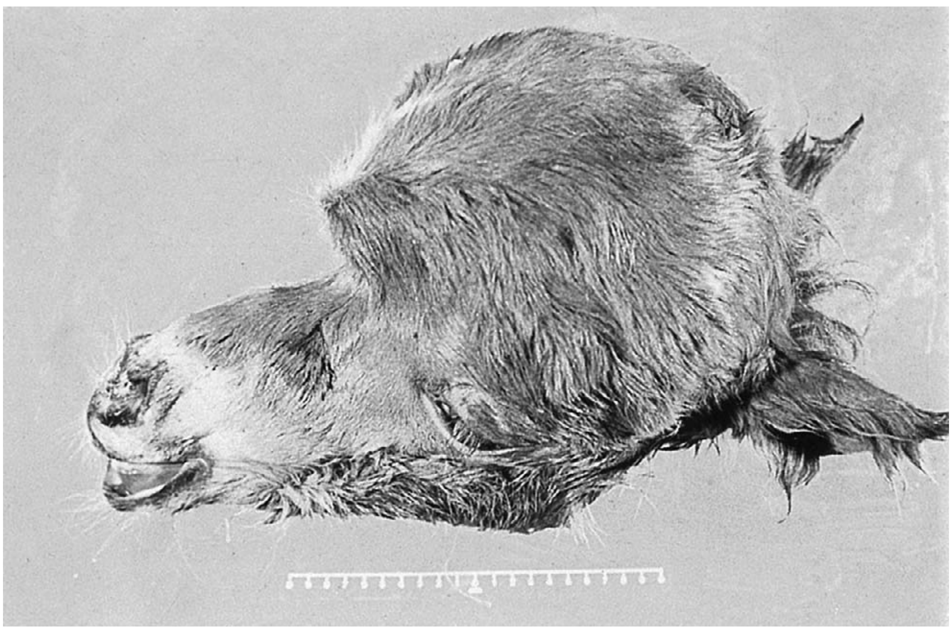

Fig. 4.8 Hydrocephalus in a foal fetus.

Fig. 4.9 Hydrocephalus in a live-born calf.

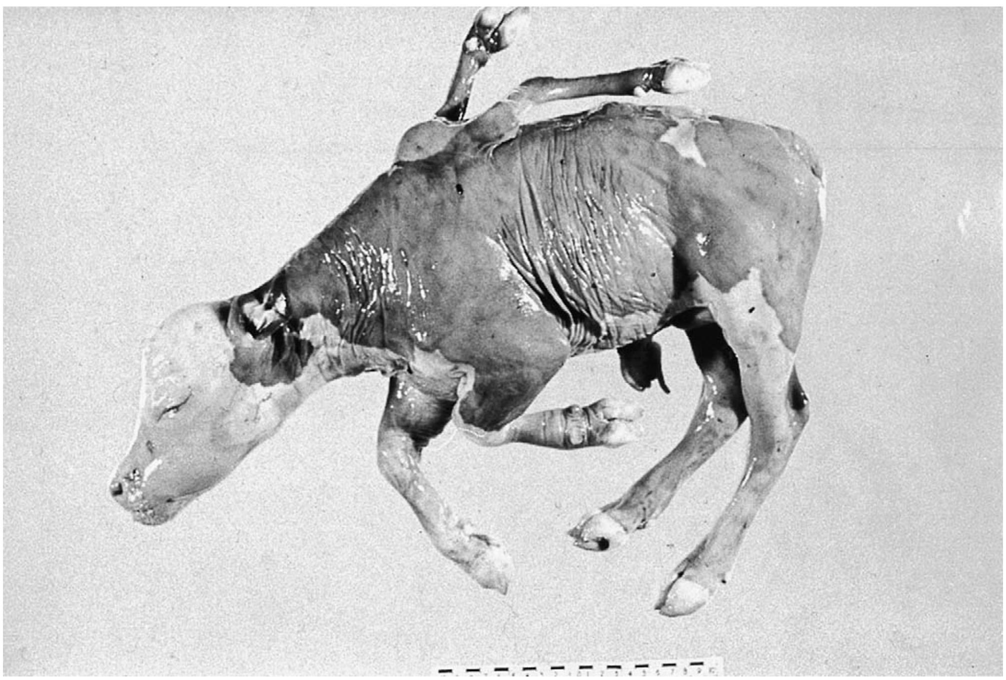

Fig. 4.10 Calf embryo with accessory front limbs.

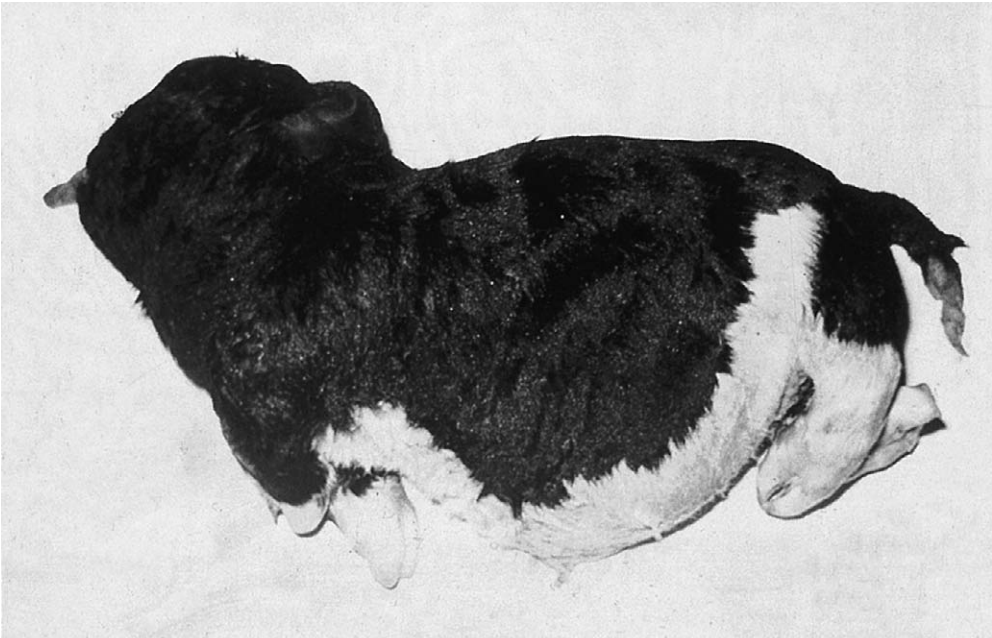

Fig. 4.11 Achondroplasia in a calf.

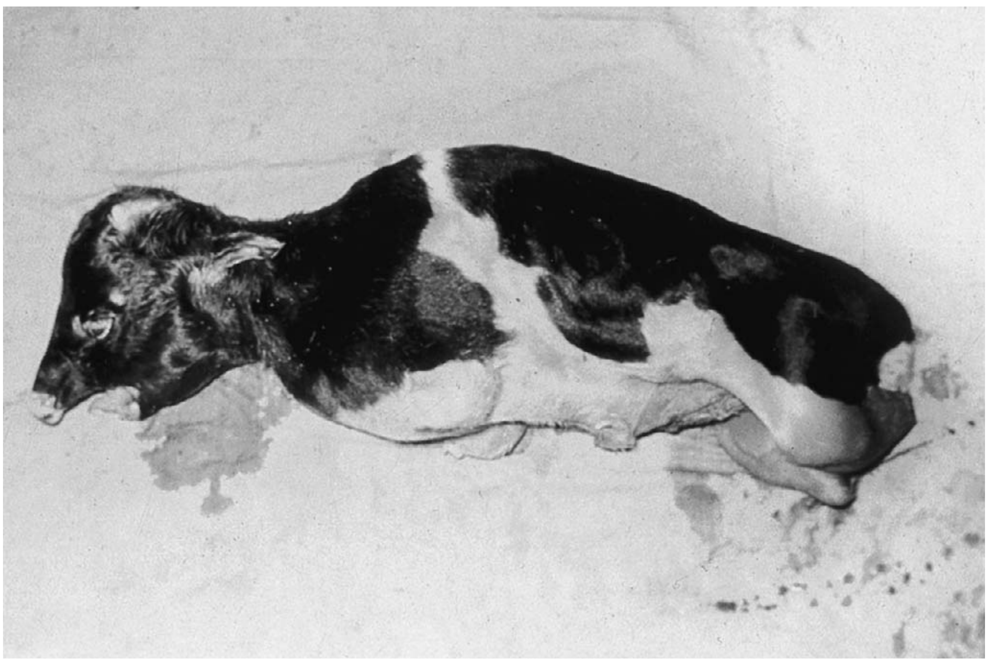

Fig. 4.12 Amputate (otter) calf.

Fig. 4.13 Arthrogryposis in a calf.

Fig. 4.14 Oedematous Ayrshire calf.

Fig. 4.15 Spastic paresis in a calf.

rates increase, the embryo survival rate decreases. This can be demonstrated in gilts, where the ovulation rate can be artificially increased but embryo survival rate decreases.

Even if early embryonic death does not occur with high ovulation rates, a problem may arise later in pregnancy with competition for uterine space. It has been suggested that a higher fetal death rate exists when there are more than five fetuses per horn, with those embryos in the middle of the horn being smaller (Perry and Rowell, 1969).

Apart from the above intrinsic factors, extrinsic factors such as nutrition and stress play an important part in embryonic loss in the pig. For example, it is well documented that high energy levels after service result in reduced embryo survival. Stress, associated with extremes of temperature, or certain management systems such as sow stalls or tethers, is also known to result in increased embryo mortality. Other husbandry policies, such as lactation length, also affect embryonic death rates, and lactation lengths of less than 3 weeks produce a marked rise in embryonic mortality (Varley and Cole, 1976), presumably due to a poor uterine environment.

Some infectious causes of embryonic loss and abortion in the pig are discussed in Chapter 27.

Cattle

Considering the size and importance of the dairy industry in Western countries there is actually relative little hard experimental data about the causes of embryonic loss in cattle, most of which occurs between days 8 and 16 after insemination.

The timing of insemination is important. Insemination too late in the oestrous period leads to ovum ageing and embryonic death. (In laboratory species, ovum ageing has been shown to result in chromosomal abnormalities.) Artificial insemination during pregnancy will induce loss, either through mechanical trauma to fetal membranes or the introduction of infection. Specific infectious agents causing loss are described in Chapter 23. Cows conceiving too soon after calving have a higher embryonic loss rate, and this is presumed to be due to a poor uterine environment.

Nutritional causes such as β-carotene, selenium, phosphorus and copper deficiencies have all been implicated in embryonic loss, but unequivocal data are not available. High intakes of crude protein, in particular rumen-degradable protein (RDP), have been associated with reduced fertility. This is said to be due to the toxic effects of blood urea or ammonia on the embryo.

Stress, e.g. heat stress, has also been shown to result in embryonic loss (Thatcher and Collier, 1986). A high rate of increase in milk yield and high milk yield per se in early lactation are negatively correlated with fertility and this could be considered a metabolic stress.

Horses

The commonest cause of embryonic loss in mares is twin conceptions (see Chapter 26). Competition for placental space usually results in one fetus growing more slowly than the other, and the smaller fetus, with a smaller placenta, dies. Death of one fetus often results in the loss of the second. Other intrinsic factors which are thought to be related to embryonic loss in the mare include oviductal secretions, embryonic vesicle mobility and uterine environment. Since the mare's embryo is at a more advanced stage whilst still in the uterine tube, the environment may be relatively more important in this species than others. In addition, the embryonic vesicle remains free in the lumen of the uterine horn much longer in the mare than in other species, and the degree of mobility of this vesicle is thought to be important in maternal recognition of pregnancy (Ginther, 1985; McDowell et al., 1985). Greater mobility enhances the suppression of luteolysis and results in higher levels of progesterone (Ginther et al., 1985). As regards the uterine environment, recurrent endometritis and post-service infection lead to perivascular fibrosis, and this is a common cause of embryonic and fetal death between 40 and 90 days of gestation (see Chapter 26). Increased maternal age has also been associated with increased embryonic loss, but this may merely reflect increased chronic uterine pathology.

Other factors such as lactation and service at the foal heat also result in higher embryonic death rates, although the latter may be due to lactational stress. Stress, due to transportation, is thought to cause embryonic losses in the mare. However, recent studies failed to confirm this even though transport did result in raised plasma ascorbic acid levels, which have been associated with prolonged stress (Baucus et al., 1990). Nutritional stress, in the form of restricted energy intake, does increase embryonic loss.

Infectious causes of loss are discussed in Chapter 26.

Sheep

Nutrition, specifically energy level, is known to affect embryonic survival in sheep in a complex manner. Low body condition at mating is detrimental to embryo survival, irrespective of post-mating nutrition. However, in ewes that lose weight post-service, embryo mortality is increased. Prolonged, moderate undernutrition has more effect on ewe lambs than adult ewes. Nutritional energy may exert its effect via peripheral blood progesterone levels since there is an inverse relationship between food intake and progesterone levels. Other nutrients important in embryo survival are vitamin E and selenium.

Certain plants, such as kale and *Veratrum californicum*, will cause embryonic death. The latter is also a teratogenic agent (see Table 4.2).

The effects of nutrition may be exaggerated or confounded by differences in ovulation rate since losses have often been reported to be disproportionately high in twin ovulations. In breeds with very high ovulation rates (i.e. litter bearers), the embryonic death rate rises proportionately, but this is probably due to limitations of uterine space.

High environmental temperature, particularly in the first week after mating, has been shown experimentally to increase the embryonic death rate dramatically. This could be important in climatic heat waves. However, if there is diurnal variation, as would occur naturally, the loss is much lower.

Physiological stress, such as that produced by overcrowding or handling of sheep, also increases embryonic loss. This may be due to excess secretion of progesterone by the adrenals and/or raised corticosteroid levels (Wilmut et al., 1986).

The age of the ewe is also important, since there is some evidence that ewe lambs have a higher incidence of embryonic loss than mature ewes (Quirke and Hanrahan, 1977).

Infectious causes of fetal loss are discussed in Chapter 25.

Goats

Goats are particularly susceptible to non-infectious fetal loss, and this is particularly true of the Angora breed. Losses are also common in poorly fed animals of any breed. Another reported cause of fetal loss is dosing with anthelmintics such as carbon tetrachloride and phenothiazine.

Infectious causes of abortion in goats are discussed in Chapter 25.

Dogs

Very little information is available on the non-infectious causes of embryonic loss in the dog. In some instances whole litters are resorbed. It used to be thought that this was due to progesterone insufficiency, but there is no real evidence for this proposition. Furthermore, high levels of exogenous progesterone may cause abnormal sex differentiation in the male puppies.

The commonest infectious cause of fetal loss is *Brucella canis*. This organism is not, however, found in Britain. Canine herpes virus can also cause fetal death and mummification. In this condition, infection of the fetus can be transplacental in pregnant bitches, but more commonly the neonate is infected during passage through the birth canal at parturition (see Chapter 28).

Cats

Very little information is available on the non-infectious causes of embryonic loss in the cat. Cats are susceptible to the loss of embryos due to stress, and care should be taken not to distress a pregnant queen. The infectious causes of embryonic loss include feline viral rhinotracheitis (FVR) and feline panleucopenia virus (FPV), which cause abortion, fetal mummification and stillborn kittens, and feline leukaemia virus (FELV), which causes fetal resorption and abortion and is also responsible for producing the fading kitten syndrome (see Chapter 28).

SEQUELAE TO EMBRYONIC OR FETAL DEATH

Following early embryonic death the embryonic tissues are usually *resorbed*, and the animal returns to oestrus if there is no other conceptus in the uterus. If death occurs before there has been maternal recognition of pregnancy the oestrous cycle is not prolonged. If it occurs after recognition has taken place, the oestrous cycle will be prolonged.

If death of the embryo is due to an infection then, even although the embryonic material may be absorbed, a *pyometra* may follow. In cattle this condition is characterised by persistence of the corpus luteum, closed cervix and pus accumulation in the uterine body and horns. It is a particular characteristic of infection with *Tritrichomonas fetus* (see Chapter 23).

If fetal death occurs after ossification of the bones has begun, complete resorption of fetal material cannot take place. Instead, *fetal mummification* occurs. The commonest form of mummification is *papyraceous mummification*, where the fetal fluids are resorbed and the fetal membranes become shrivelled and dried so that they resemble parchment (hence the name). The uterus contracts on to the fetus, which becomes twisted and contorted. In polytocous species, if mummification occurs in only some embryos, this does not interfere with the continuation of the pregnancy of the live fetuses. The mummified fetus is simply expelled at parturition.

Mummification is very common in the pig, and is a particular characteristic of infection with the SMEDI viruses. It is also seen in large litters as a consequence of uterine overcrowding and placental insufficiency. In the cat, fetal mummification is not uncommon in large litters, and is again assumed to be due to uterine overcrowding. In the dog, fetal mummification is a characteristic of canine herpes virus (CHV) infection. In the ewe, fetal mummification may be seen with twins and/or triplets when one of the embryos has died. In the mare, mummification is rare and is always associated with twin pregnancies. If twinning does occur, one of the fetuses usually develops more slowly than the other and is smaller. The small fetus usually dies, and if the other fetus survives and the pregnancy is maintained, the dead fetus will mummify and be delivered at term with the live foal (Figure 4.16).

In cattle, fetal mummification occurs with an incidence of 0.13–1.8% (Barth, 1986) and *haematic mummification* is the norm. In this condition the fetal fluids are resorbed but the fetus and membranes are surrounded by a viscous, chocolate-coloured material. It was once thought that the colour was due to pigments from the blood and that the condition was due to caruncular haemorrhage (hence the name) which resulted in fetal death. However, the haemorrhage is thought now to be an effect of fetal death, rather than the cause. Various theories have been put forward as to the aetiology of the condition. It has been suggested that there is a genetic cause, particularly since the condition

Fig. 4.16 Mummified foal (f) with attached placenta (p), twin to a normal foal.

appeared to be more common in Channel Island breeds (Jersey and Guernsey) and occurred with a high frequency in a particular family of British Friesians (Logan, 1973). Torsion of the umbilical cord has been suggested as the primary cause of fetal death, but this has not been a consistent finding in haematic mummies. On the other hand, the condition has been induced hormonally using oestradiol and trembolone acetate (Gorse, 1979), which suggests that a hormonal anomaly may be the cause.

Haematic mummification can occur following fetal death at ages ranging from 3 to 8 months of gestation. Since there is no fetal signal for the onset of parturition the corpus luteum is retained and the 'pregnancy' will be maintained for an unpredictable time. The condition is often only diagnosed when the cow is examined because of a *prolonged gestation* period. Treatment of choice is the induction of abortion by luteolysis using prostaglandins. The fetus is normally expelled in 2–4 days. The prognosis for further breeding is good since there has been no intrinsic damage to the reproductive tract. However, care needs to be taken that the mummified fetus is indeed expelled, otherwise a possible sequel is *fetal maceration*. Fetal maceration can occur in any species, but it is described most frequently in cattle. It occurs as a consequence of the failure of an aborting fetus to be expelled, due perhaps to uterine inertia. Bacteria enter the uterus through the dilated cervix, and by a combination of putrefaction and autolysis the soft tissues are digested, leaving a mass of fetal bones within the uterus. Sometimes these become embedded in the uterine wall and are difficult to remove other than by hysterotomy (Figure 4.17). Under these circumstances a chronic endometritis ensues and there is severe damage to the endometrium. The animal should therefore be sent for slaughter.

Prolonged gestation need not always be related to fetal death. It is, for example, a characteristic of anencephalic pregnancies (Figure 4.18), in which, due to the absence of the fetal pituitary, normal parturition cannot be initiated (see Chapter 6). If the fetus is alive it continues to grow, and so the result of prolonged gestation is absolute fetal oversize, which leads to *dystocia*.

Other sequelae to fetal death are *abortion* and *stillbirth*. Abortions are often caused by infectious agents, and these are dealt with elsewhere (see Chapters 23 and 25–28). Stillbirths may occur because of developmental anomalies incompatible with life.

DROPSY OF THE FETAL MEMBRANES AND FETUS

Three dropsical conditions of the conceptus may be seen in veterinary obstetrics: oedema of the placenta, dropsy of the fetal sacs and dropsy of the fetus. They may occur separately or in combination.

Oedema of the placenta

This frequently accompanies a placentitis: for example, *Brucella abortus* infection in cattle. It does

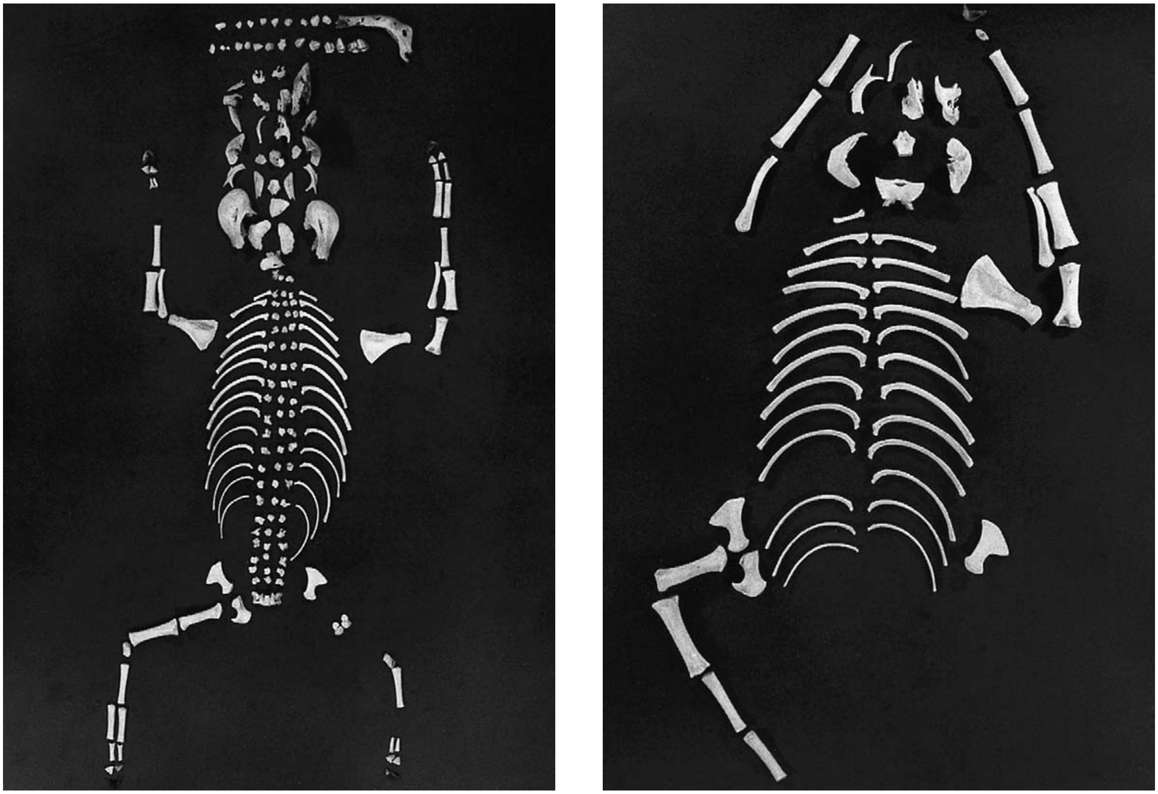

Fig. 4.17 Fetal bones removed from the uterus of cow following maceration.

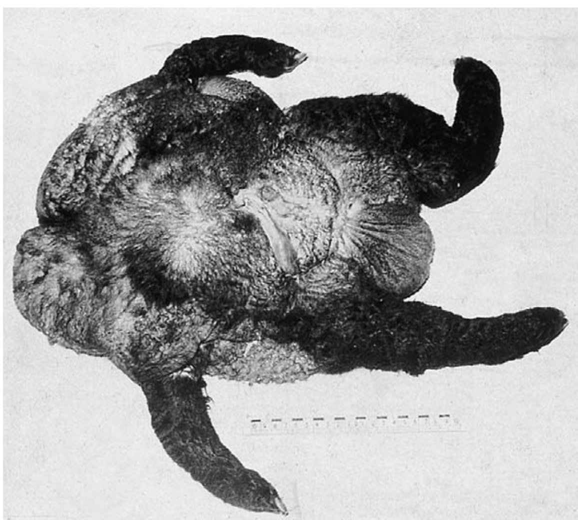

Fig. 4.18 Anencephalic lamb.

not cause dystocia but is frequently associated with abortion or stillbirth.

Dropsy of the fetal sacs

Both the amniotic and allantoic sacs can contain excessive quantities of fetal fluid (see Chapter 2); when this occurs it is referred to as hydramnios or hydrallantois, depending on which sac is involved. Hydrallantois is much more common than hydramnios, although the latter is always seen in association with specific fetal abnormalities such as the 'bulldog' calf in the Dexter. Although dropsy of the fetal sacs is essentially a bovine condition, Vandeplassche (1973) has seen 48 equine cases in which pluriparous mares of 10–20 years of age showed very rapid onset of the condition between 7 and 9 months of gestation. A few cases have been recorded in sheep, associated with either twins or triplets, in which the excess of fluid – amounting to about 18.5 litres – was in the amniotic sac. It has also been reported in the dog involving all fetuses in a litter.

Apart from the hereditary cases of hydramnios which accompany the Dexter 'bulldog' calf, and which may occur as early as the third or fourth month, most instances of dropsy of the fetal sacs of cattle are seen in the last 3 months of gestation. The cause is not known. Arthur (1957) found the number of functioning cotyledons was abnormally low – the non-pregnant horn usually was not participating in placental formation – and there was a compensatory accessory caruncular development in the pregnant horn. Histologically, there was a non-infectious degeneration and necrosis of the endometrium and, as already observed, the fetus was undersized. Normally, in cattle, there is a markedly accelerated production of allantoic fluid at 6–7 months of gestation, and it is suggested that, where placental dysfunction exists, this increase may become uncontrolled and lead to massive accumulation. It is also frequently associated with twins.

All cases of hydrallantois are progressive, but they vary in time of clinical onset (within the last 3 months of pregnancy) and in their rate of progression. The essential symptom is distension of the abdomen by the excess of fetal fluid (Figure 4.19). The later in gestation the condition occurs, the more likely it is that the cow will survive to term, whereas if the abdomen is obviously distended at 6 or 7 months, the cow will become extremely ill long before term. The volume of allantoic fluid varies up to 273 litres, and such large amounts impose a serious strain on the cow and greatly hamper respiration and reduce appetite. There is gradual loss of condition, eventually causing recumbency and death (Figure 4.20). Occasionally, the animal becomes relieved by aborting. The less severely affected reach term in poor condition and, because of uterine inertia (often accompanied by incomplete dilation of the cervix), frequently require help at parturition.

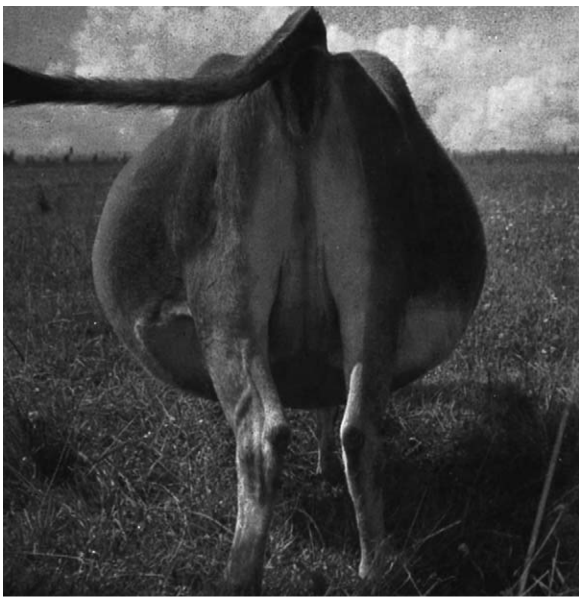

Fig. 4.19 Jersey cow with the distended 'pear-shaped' abdomen typical of hydrops allantois.

The diagnosis of bovine hydrallantois is based on the easily appreciable fluid distension of the abdomen, with its associated symptoms, in the last third of pregnancy. Confirmation may be obtained by the rectal palpation of the markedly swollen uterus and by the failure to palpate the fetus either per rectum or externally.

The *treatment* of hydrallantois calls for a realistic approach and a nicety of judgement. Cases that have become recumbent should be slaughtered. Where the animal is near term, a one- or two-stage caesarean operation is indicated. With both methods it is imperative that the fluid is allowed to escape slowly, so as to prevent the occurrence of hypovolaemic shock associated with splanchnic pooling of blood. Since hydrallantois is frequently seen in twin pregnancies in cows, it is particularly important to search the grossly distended uterus for the second calf.

Cases of hydrallantois which calve, or are delivered by caesarian operation, frequently retain the placenta and, owing to tardy uterine involution, often develop metritis. This may lead to a protracted convalescence and delayed conception.

By using a synthetic corticosteroid (dexamethasone or flumethasone) in conjunction with oxytocin, Vandeplassche (1973) reported improved therapy for hydrallantois. About 4 or 5 days after an injection of 20 mg of dexamethasone or 5–10 mg of flumethasone the cervix relaxes and the cow is given oxytocin by means of an intravenous drip for 30 minutes. Of 20 cows so treated, 17 recovered. In respect of the management of equine hydrallantois, the same writer advises that affected mares enter a spontaneous abortion phase but fail to expel the

Fig. 4.20 Same cow as in Figure 4.19 after slaughter showing the greatly enlarged uterus.

fetus because of uterine atony. Vandeplassche breaks the allantochorion to release its fluid (commonly amounting to 100 litres). The mare is then given oxytocin by intravenous drip, and when the cervix has relaxed sufficiently the fetus is manually withdrawn. The placenta itself is markedly oedematous, and retention is prevented by further administration of oxytocin.

Dropsy of the fetus

There are several types of fetal dropsy, and those of obstetric importance are hydrocephalus (see Figures 4.8 and 4.9), ascites and anasarca. The form of the fetus and the degree of obstetric hazard are determined by the location and amount of the excess of fluid. Dystocia is due to the increased diameter of the fetus.

Hydrocephalus

Hydrocephalus involves a swelling of the cranium due to an accumulation of fluid which may be in the ventricular system or between the brain and the dura. It affects all species of animals and is seen most commonly by veterinary obstetricians in pigs, puppies and calves (see Figures 4.8 and 4.9).

In the more severe forms of hydrocephalus there is marked thinning of the cranial bones. This facilitates trocarisation and compression of the skull so as to allow vaginal delivery. Where this cannot be done, the dome of the cranium may be sawn off with fetotomy wire or a chain saw. If the fetus is decapitated there is still the difficulty of delivering the head. Caesarean section may be performed, but there is no merit in obtaining a live hydrocephalic calf; however, this operation, may be necessary in severe cases affecting pigs and dogs, and in cattle when the calf is presented posteriorly or when hydrocephalus is accompanied by ankylosis of the limb joints.

Fetal ascites

Dropsy of the peritoneum is a common accompaniment of infectious disease of the fetus and of developmental defects, such as achondroplasia (see Figure 4.11). Occasionally, it occurs as the only defect. Aborted fetuses are often dropsical; when the fetus is full-term, ascites may cause dystocia. This can usually be relieved by incising the fetal abdomen with a fetotomy knife.

Fetal anasarca

The affected fetus is usually carried to term, and concern is caused by the lack of progress in second-stage labour. This is due to the great increase in fetal volume caused by the excess of fluid in the subcutaneous tissues, particularly of the head and hind limbs. In the case of the head, there is so much swelling that the normal features are masked and the resultant appearance is quite grotesque. It is an interesting point that an undue proportion of these anasarcous fetuses are presented posteriorly, in which case the enormous swelling of the presenting limbs is very conspicuous. There is frequently an excess of fluid in the peritoneal and pleural cavities with dilatation of the umbilical and inguinal rings as well as hydrocoele. The substance of the fetal membranes is also oedematous and occasionally there is a degree of hydrallantois.

SUPERFECUNDATION

Superfecundation is the condition in which offspring from two sires are conceived contemporaneously. Owing to the number of ova shed and their longevity, as well as to the length of oestrus and the promiscuous mating behaviour of the species, superfecundation is most likely in the bitch. The phenomenon is suspected when mating to two dogs of different breeds is known to have occurred, and the suspicion is heightened when the litter shows marked dual variation in colour pattern, conformation and size. Corroboration of superfecundation by inspection of the progency is valid only when pure-bred partners are involved. It is likely that the majority of alleged cases are due simply to genetic variation in the offspring of impure parents.

Superfecundation has been reported when a mare gave birth to twin horse and mule foals, and when a Friesian cow delivered twin Friesian and Hereford calves.

SUPERFETATION

Superfetation is the condition that arises when an animal already pregnant mates, ovulates and conceives a second fetus or second litter. It is not uncommon for a cow to be mated when pregnant, but no evidence is available that ovulation occurs in a cow carrying a live fetus. Ovulation does occur in pregnant mares, and in this species superfetation is theoretically possible. Superfetation is suspected when fetuses of very different size are born together or when two fetuses, or two litters, are born at widely separated times. Apparently authentic cases have been described in which two normally mature fetuses, or litters, have been delivered at times corresponding in gestation length to two widely separated and observed matings.

In general there is considerable doubt about superfetation; however, Vandeplassche et al. (1968) have produced convincing proof that it does occur in the double parturitions of sows. They investigated 12 cases of double porcine parturition following a single mating, and in two of them they explored the uterus and ovaries by laparotomy after the second farrowing. They concluded that double parturition followed a single mating at which an excessively large number of eggs were fertilised and which later distributed themselves normally throughout both uterine horns. Instead of the more usual subsequent reduction of the litter size by embryonic death, the embryos in the cranial halves of both cornua remained unimplanted in a state of 'embryonic diapause' for periods varying from 4 to 98 days, after which they were reactivated and implanted, thus constituting a spontaneous superfetation in the cranial parts of the horns. The embryos which implanted in the caudal parts of the horns underwent a normal gestation and parturition; a second parturition at variable intervals occurred when the piglets from the delayed implantation reached maturity. Vandeplassche and his co-workers believe that the cases of double parturition in pigs and in other species which follow mating at separate oestrous periods are also due to embryonic diapause rather than to superfetation; also that occasional cases of prolonged gestation may be due to the same cause.

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5 Prolapse of the vagina and cervix

Typically, prolapse of the vagina and cervix (CVP) is a disorder of ruminants normally in late gestation. Occasionally it is seen after parturition and rarely it occurs unconnected with pregnancy or parturition. It less frequently occurs in pigs. It can be recognised by the protrusion of varying parts of the vaginal wall and sometimes the cervix through the vulva so that the vaginal mucosa is exposed. In some bitches, hyperplasia of the vaginal mucosa occurs at oestrus and may protrude through the vulva. This is sometimes referred to as vaginal prolapse, although such a description is incorrect and is not comparable with the condition in other species (see Chapter 28). Chronic prolapse during pregnancy in the bitch requiring hysteropexy has been described (Memon et al., 1993).

SHEEP

Incidence and cost

In so far as sheep are concerned, the disorder is much more common than in other species, and thus is of real economic importance. Average incidences are 0.53%, 0.46% and 0.98%, which may rise to 20% and 46% in some flocks (Edgar, 1952; Bosse et al., 1989; Low and Sutherland, 1987, respectively). Financial cost is substantial. For example, Hosie (1989) calculated that in the UK each case cost £41.08; this was based on the average cost of replacing a breeding minus the value of a culled affected ewe, lamb mortality and the cost of treatment. In addition, there is the unquantifiable welfare implications. The financial costs of the disorder are based on the following assumptions:

- Some affected ewes will die.
- If not treated some will abort.
- Some will have reduced fertility.
- Ewes may be culled because of the possibility that prolapse may recur.
- The stillbirth rate and neonatal mortality are increased.
- There is a greater chance of dystocia in affected ewes.

Classification of severity

The disorder is normally easily recognised, although sometimes the prolapsed organ can be confused with the allantochorion as it protrudes from the vulva before it ruptures. The severity of the disorder varies, and a number of different classifications have been used. Probably the simplest is that used by Bosse et al. (1989), which is as follows (see Figures 5.1 and 5.2):

- Stage 1, in which the vaginal mucosa protrudes from the vulva when the ewe is recumbent, but disappears when she stands.
- Stage 2, in which the protruding vaginal mucosa remains visible even when the ewe stands; the cervix is not visible.
- Stage 3, in which the vagina protrudes, and the cervix is visible.

Other systems of classification have been used which also take into account the duration of the prolapse, its size and the other organs contained within the prolapsed organ; Cox (1987) uses the terms mild, moderate and severe. The bladder is most frequently involved as it becomes reflected to occupy the vesicogenital peritoneal pouch. This can be followed by complete or partial constriction of the urethra causing urinary retention; the uterine horns and intestines can also be involved. Realtime ultrasonography can be used to diagnose the contents of the prolapse (Scott and Gessert, 1998).

Fig. 5.1 Moderate, early prolapse of the vagina in a ewe.

Fig. 5.2 Severe prolapse of the vagina in a ewe.

Causes and pathogenesis

Much of the evidence relating to the cause of the disorder is anecdotal. The following are said to be predisposing factors (Noakes, 1999):

- hormonal excesses and imbalances
- hypocalcaemia
- large fetal load (twins or triplets)
- fat condition
- thin condition
- inadequate exercise
- short tail docking
- bulky food (root crops)
- excess dietary fibre
- dietary oestrogens and their precursors
- sloping terrain
- vaginal irritation
- previous dystocia
- inherited predisposition.

Irrespective of the alleged predisposing factors, there are three requirements for CVP to occur (McLean, 1956). They are:

- The vaginal wall must be in a state where it can be readily everted, and the vaginal lumen must be large.
- The vulva and vestibular wall must be relaxed.
- There must be a force which can displace the vaginal wall, causing it to become everted.

Recent studies (Ayen et al., 1998) have attempted to measure the changes in the compliance of the vaginal wall (which will be related to the ease with which the wall can be everted) and the size of the vaginal lumen, ‘the capacity of the vagina’ (which will assess the ease with which the everted vagina can be expelled to the exterior). There are considerable between-animal variations in these parameters, which explains why CVP occurs in some ewes but not others. The compliance of the vaginal wall was greater during the second than the first pregnancy, and this supports the observation that CVP is less frequently identified in primigravid than in plurigravid ewes. Both endogenous and exogenous oestradiol and progesterone can influence the compliance of the vaginal wall and the capacity of the vagina. The vaginal wall is comprised of collagen and smooth muscle, so both parameters will be influenced by either the amount, or the distribution, or the type of collagen present. There is no evidence for the former (Ayen and Noakes, 1998), but extrapolation from work relating to genitourinary prolapse in humans suggests that it may well be a change in the collagen type which reduces the mechanical strength of the vaginal wall (Jackson et al., 1996).

Clinical signs and progression of the disease

The clinical signs are obvious; the only possible confusion might be the protruding allantochorion. Prolapse occurs most frequently in the last 2–3 weeks of gestation. The only distinction that needs to be made is the severity. In sheep a severe prolapse with heavy straining is not well tolerated, and fatalities from shock, exhaustion and anaerobic infection are common. Abortion, or premature delivery, often of a dead fetus, may be followed by a rapid maternal recovery.

White (1961) first described a fatal condition of heavily pregnant ewes in which the intestines become prolapsed through a spontaneous rupture of the dorsal or lateral wall of the vagina. This has been shown to be associated with vaginal prolapse, although why it should occur is not entirely clear. More recently, a study involving the Norwegian Dala breed has questioned this theory and has presented information which suggests that it might be due to a number of reasons, including uterine torsion (Mosdol, 1999).

Treatment

Treatment of ewes with CVP is often done without any consideration for the welfare of the animal. It is relatively easy for veterinarians to reduce pain and discomfort during replacement by using caudal epidural anaesthesia. The technique which is described in Chapter 12 should be used for all but the mildest prolapses; it also makes the replacement much easier to perform.

In sheep, the perineal wool, or string fastened to it, may be tied across the vulva; large safety pins have sometimes been used. For retaining the prolapsed vagina of ewes, Fowler and Evans (1957) and Jones (1958) first described the use of a stainless steel stay which, in the form of the letter 'U', is placed in the vagina. The emerging ends are bent at right angles and fitted with 'eyes', which are securely fastened with string on either side to the wool of the gluteal region. This type of prolapse retainer has been improved by the development of a plastic spoon which is fastened in the same way as the 'U'-shaped device, or in association with a harness made from baling twine or nylon strapping (Figure 5.3). Bühner's method, which is described in detail in cattle below, is equally applicable to ewes with prolapsed vaginas; a suture of monofilament nylon can be laid by means of a large half-curved, cutting suture needle, although a small Bühner's needle is preferable.

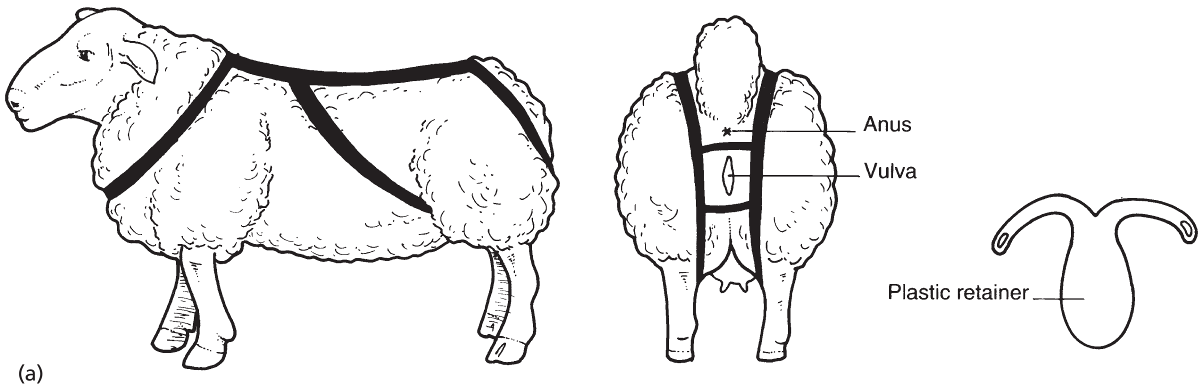
The early replacement and retention of the prolapse is very important to prevent trauma and to ensure that the ewe maintains pregnancy to term.

In so far as genetic aspects are concerned, it would seem unwise to breed from animals which have shown vaginal prolapse. There is little doubt that by adopting this culling policy over the years, stock owners have exerted a large measure of control over the condition. Although the recurrence rate is variable, ranging from 72% to 3.6% (Bossé et al., 1989; Stubbings, 1971), the author found an 18% rate in a small study involving eleven ewes.

CATTLE

Causes and pathogenesis

The exact cause of the disorder has not been ascertained but several factors are generally believed to play a part. Cattle of the beef breeds, particularly Herefords, Simmental and Charolais, are most commonly affected. Woodward and



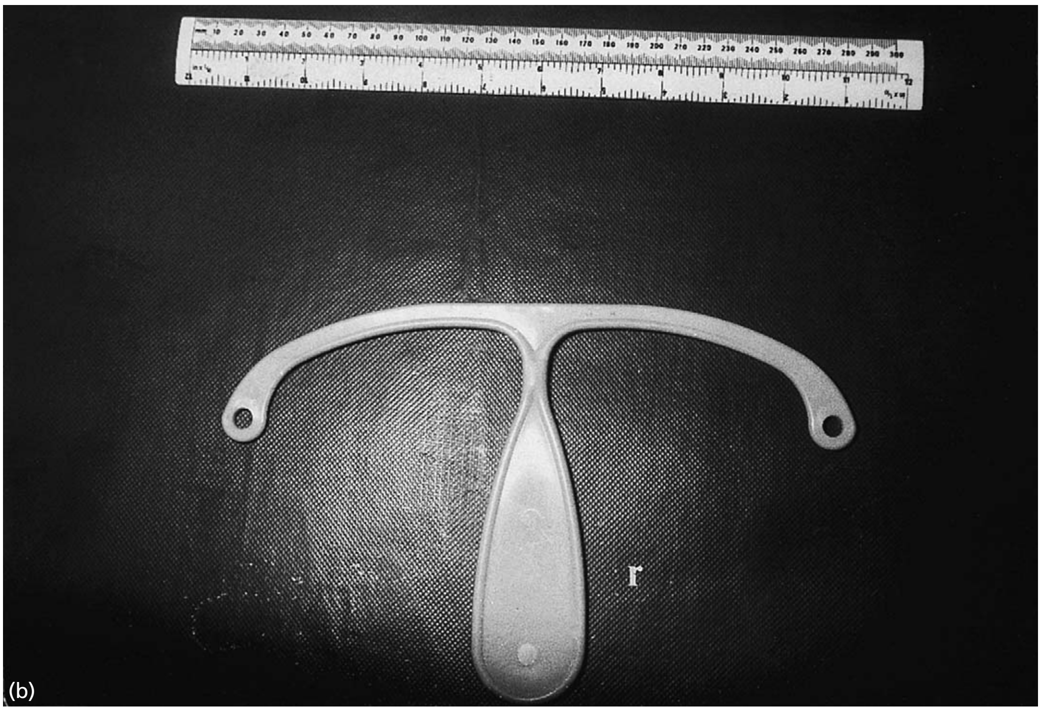


Fig. 5.3 (a) The positioning of a harness (constructed of baling twine or nylon strapping) in a ewe together with a plastic retainer. The latter is not always used if pressure from the harness on the perineum is sufficient to retain the prolapse. (b) The plastic retainer (r). This can be used without the harness, and can be attached to the wool using tapes.

Queensberry (1956) recorded 1.1% of vaginal prolapses in 7859 pregnancies in Hereford cattle in the USA, and it has been suggested that in them the anatomical anchorage of the genital tract is less efficient than in other animals. An excessive deposition of fat in the perivaginal connective tissue and ligamentous relaxation may increase the mobility of the vagina. Both these effects might be due to a state of endocrine imbalance, in which oestrogenic hormones predominate; the administration of stilboestrol has been shown to soften the suspensory ligaments of the genital tract. Where oestrogenic substances are present in inordinate amounts in the diet, as in subterranean clover pastures of Western Australia (Bennetts, 1944), or in mouldy maize and barley which are considered to have a high oestrogen content, this can result in a high incidence of prolapse. When heifers are fed these in their diet they may show vulvovaginitis with oedema of the vulva, relaxation of the pelvic ligaments, tenesmus and vaginal prolapse (Koen and Smith, 1945; McErlean, 1952). Predisposition to CVP is inherited, as shown by its greater frequency of occurrence in beef cattle. Mechanical factors, such as the increasing intra-abdominal pressure of late pregnancy and gravity, acting through the medium of a sloping byre floor when cattle are tethered, are considered significant (McLean and Claxton, 1960).

Postparturient prolapse of the vagina of cattle is usually due to severe straining in response to vaginal trauma, or infection, following a serious dystocia. Vaginal contusion at parturition, followed by *Fusobacterium necrophorum* infection, exerts a high degree of irritation with frequent exhausting expulsive efforts.

Clinical signs and progression of the disease

Initially the lesion involves a protrusion of the mucous membrane – more particularly of the floor – of that part of the vagina which lies just cranial to the urethral opening. In severe cases the whole of the anterior vagina and cervix may protrude. The further from parturition that the disease begins, the more serious it is likely to become because advancing pregnancy tends to accentuate the condition. In cattle most cases are seen in the last 2 months of gestation. In the mildest cases the lesion appears only when the cow is recumbent; when the animal rises the prolapse recedes. The tendency is, however, to a progressive degree of prolapse and, in time, a larger bulk protrudes and does not disappear in the standing position. Now the dependent tissue, with its circulation impeded, is prone to injury and infection. The resultant irritation causes expulsive straining efforts. This increases the degree of prolapse, and a vicious circle is established. Eventually the whole of the vagina, cervix and even the rectum may become everted (Figures 5.4–5.6). Thrombosis, ulceration and necrosis of the prolapsed organ, accompanied by toxaemia and severe straining, lead to anorexia, rapid deterioration in bodily condition and occasionally death.

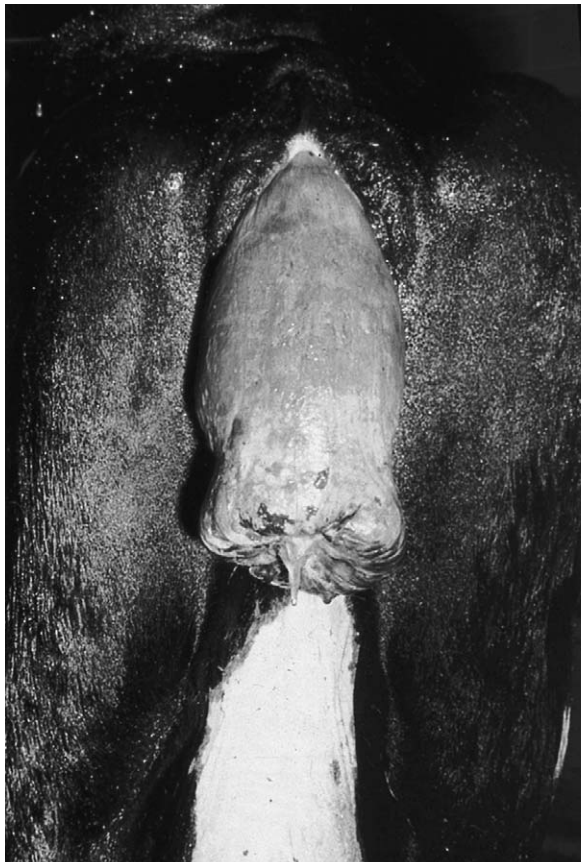


Fig. 5.4 Prolapse of vagina in a Friesian cow.

Treatment

If prompt attention is given, simple measures often succeed. The aim is to arrest the process by early replacement and retention of the prolapsed portion. Caudal epidural anaesthesia (see Chapter 12) is indicated both to obviate straining and to desensitise the perineum for suturing. The everted mass is washed clean using plain water or a mild non-irritant antiseptic and replaced gently with the palms of the hand, being careful not to cause trauma to the inflamed and sometimes fragile tissue. It is retained by tape or stout nylon sutures which cross the vulva and are inserted in the perineal skin. Quill sutures tied over rubber tubing are best. In cases where the vagina has suffered

Fig. 5.5 Prolapse of vagina and cervix (as indicated by the finger).

Fig. 5.6 Prolapse of vagina, cervix and, because of persistent straining, the rectum.

little damage and especially where parturition is imminent, such measures are usually sufficient, particularly when, as is possible with dairy cows, the patient can be stalled on a forward slope. But where there is much irritation from trauma and infection with consequent vigorous straining, the retaining sutures may be dislodged and prolapse recur. Straining may be controlled by caudal epidural anaesthesia, and although xylazine will prolong its effect (see Chapter 12), it is not practicable to provide continuous anaesthesia by this means. Perineural injection of the pudic nerves has the same effect, and the same disadvantage. Tenesmus has been prevented for several days to a week or more by the induction of artificial pneumoperitoneum.

For cows showing recurrent prolapse and which are remote from parturition, and also for postpartum cases, Roberts (1949) has suggested a method of almost complete surgical occlusion of the vulva by a technique which is really an extension of Caslick's plastic operation for preventing vaginal aspiration (Figure 5.7). Under caudal epidural or local infiltration anaesthesia, strips of mucous membrane, 1.2 cm wide, are dissected from the upper three-fourths of each vulval lip. The denuded areas are then approximated by means of fine nylon sutures, and a few mattress sutures of tape or stout nylon are deeply placed across the vulva to protect the coapted lips from the effects of straining. First-intention healing should occur, and the suture line must be incised when parturition is imminent.

Farquharson (1949), who saw hundreds of cases of vaginal prolapse in Hereford range cattle in Colorado, successfully applied a technique of

Fig. 5.7 Chronic prolapse of the cervix. This was treated by using Roberts's modification of Caslick's operation.

submucous resection, or 'reefing' operation, on the prolapsed organ. The object of the operation, which should not be performed later than 3–4 weeks from term, is to excise the protruding mucosa – which forms the bulk of the everted mass – and then approximate the cut edges. Proximal and distal encircling incisions through the mucous membrane are made near the urethral opening and the cervix respectively, and the intervening mucosa, in the form of a crescent, is removed by blunt dissection through the oedematous submucosa. In order to control haemorrhage and to facilitate suturing, it is best to perform the circumferential dissections in separate segments; as the resection of each segment is completed, so the cut edges are coapted with continuous catgut or other absorbable sutures. The operation is performed under posterior epidural anaesthesia. Subsequent parturition and conception are not affected, and the cure is permanent. An alternative approach is to prevent displacement of the cervix caudally by anchoring it using non-absorbable sutures or nylon tape to the prepubic tendon or the sacrosciatic ligaments (Winkler, 1966). Although the author of the paper describes the technique as being done under caudal epidural anaesthesia, in the author's experience, whilst the approach to the sacrosciatic ligament is possible using this approach, attachment to the prepubic tendon requires general anaesthesia.

In the author's experience the best means of retaining the replaced vagina is the technique described by Bühner (1958) (Figure 5.8). It entails the placing, by use of a special needle, of a subcutaneous suture of nylon tape around the vulva. To facilitate introduction of the large needle two 'stab' incisions are made (under epidural anaesthesia previously induced to aid replacement of the vagina) in the midline; the upper one is midway between the dorsal commissure of the vulva and the anus, while the lower one is immediately beneath the ventral vulval commissure. The needle is inserted into the lower incision and gradually passed subcutaneously up the right side of the vulva until its point emerges through the upper incision, whereupon the needle is threaded with a double length of nylon tape. While one end of the tape is firmly held, the loaded needle is pulled downwards until free of the lower incision when it is unthreaded, thus leaving a length of tape protruding from each incision. The needle is now inserted again into the lower incision and passed subcutaneously up the left vulval labium. When its point emerges the needle is threaded, then pulled backwards and outwards from the lower aperture and unthreaded. The tape now encircles the vulva subcutaneously and its two ends hang from the lower incision. These ends are tied with a simple knot with such a degree of tightness that four fingers can be inserted flatwise

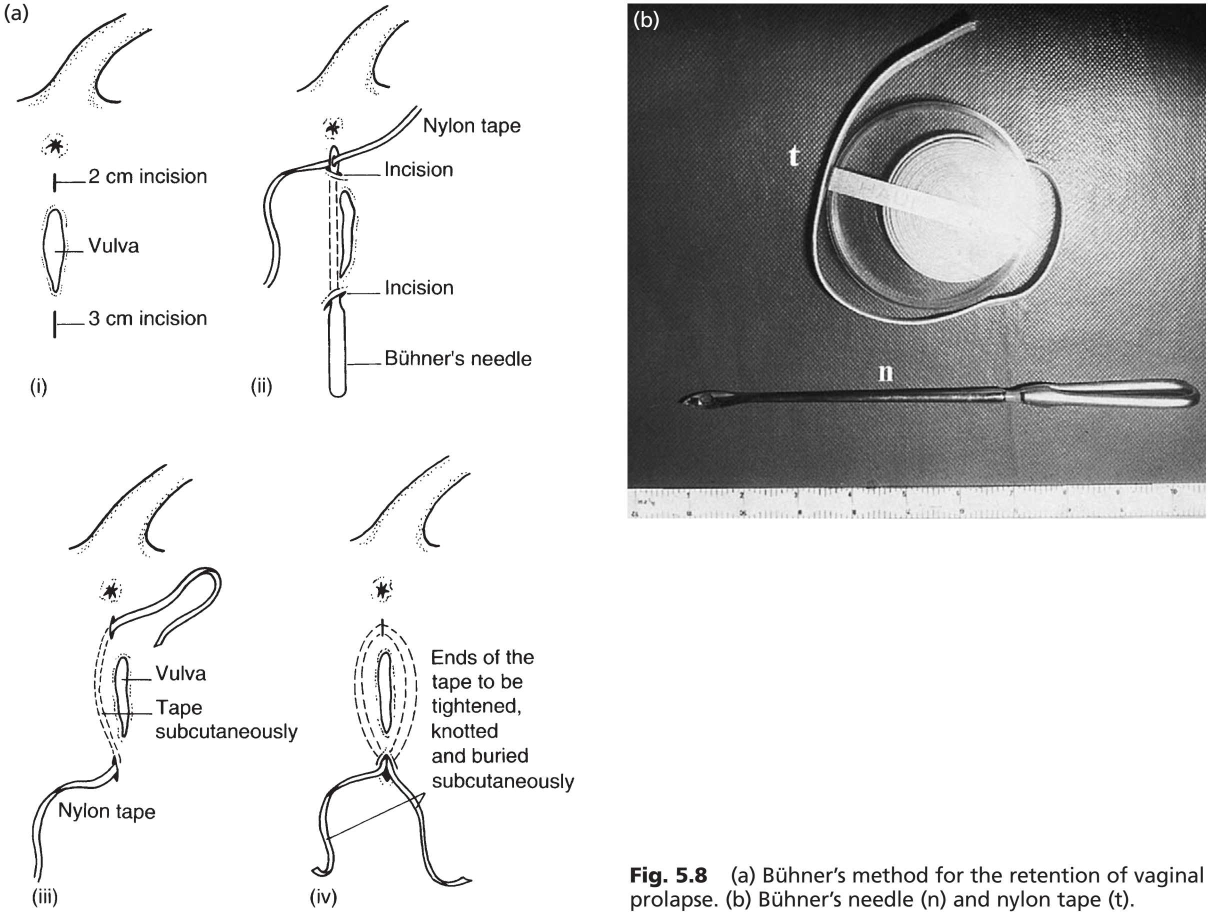


Fig. 5.8 (a) Bühner's method for the retention of vaginal prolapse. (b) Bühner's needle (n) and nylon tape (t).

up to their second joints into the vulva. The upper incision is closed with a couple of sutures of fine monofilament nylon while the lower incision can be either left open or sutured according to the cow's proximity to parturition. The suture causes practically no tissue reaction; the vulval labiae are not damaged by it, and it can remain in situ for months, until the cow is on the point of calving, when the knot should be cut so as to release the thread and allow the vulva to dilate for the birth of the calf.

PIGS

Vaginal prolapse occurs in gilts at oestrus, and also after the feeding of mouldy cereals supposedly due to a high oestrogen content.

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6 Parturition and the care of parturient animals

PARTURITION

It is essential for the veterinarian to be perfectly familiar with the normal course of parturition in domestic species in order to be able to differentiate between physiological and pathological birth. The appropriate intervention at the correct time can increase the likelihood of a successful outcome, by ensuring that both mother and offspring survive.

Initiation of parturition

Parturition is one of the most fascinating of biological processes, for although its physiology is explicable and its associated endocrine changes have been fairly well established, the factors which initiate birth and thereby terminate pregnancy after a constant length of gestation for a given species are still imperfectly understood. The modern concept, which is firmly based on experimental studies and clinical observations, is that the fetus exerts an overriding control on the length of gestation and that the mother can influence the time of birth only within narrow limits.

The uterine musculature is the key component of labour, and the essential physiological change between gestation and birth is a liberation of the contractile potential of the myometrium; the factors involved in this transformation are neural, humoral and mechanical.

Of the humoral factors, the most important is the reversal of those mechanisms which are necessary for the maintenance of pregnancy, in particular the removal of the progesterone block, which ensures that, during this phase of the animal's reproductive life, the myometrium is largely quiescent.

The mechanisms that are responsible for the initiation of parturition vary slightly between species. Initially much of the experimental work to determine them was done in the ewe. This, together with circumstantial evidence obtained from cattle, sheep, goats and humans, in which it was observed that prolonged gestation was usually associated with abnormalities of the fetal brain and adrenal, supported the hypothesis first advocated by Hippocrates that the fetus is responsible for controlling the time when parturition occurs.

The mechanisms are fairly well defined in sheep, cattle, goats and pigs, but in the horse, dog and cat there are a number of important areas which are not well understood. Since much of the work has been reported in sheep it is proposed to describe in detail the mechanisms in this species and subsequently to indicate those differences which have been identified in other species.

Ewe

Parturition in the ewe occurs as result of activation of the fetal hypothalamus–pituitary–adrenal (HPA) axis; the areas of the hypothalamus involved in this process are the paraventricular nuclei. The fetal HPA axis is similar to the adult HPA axis, except that the fetal brain is still developing in late gestation and the former communicates with the latter via the placenta. There is still uncertainty about the mechanisms responsible for the activation of the fetal hypothalamus. A number of theories have been proposed. These are:

- maturation of the fetal hypothalamus which might result in the development of critical synapses in the paraventricular nucleus, allowing an increase in fetal neuroendocrine function
- ability of the hypothalamus to respond to the effects of placental hormones
- fetal stressors such as hypoxia, hypercapnia, changes in blood pressure and blood glucose (Wood et al., 1999).

It is also postulated that placentally-derived hormones such as oestrogens, progesterone, PGE_2 or corticotrophin-releasing factor (CRF) may also act on the hypothalamus.

Let us consider the interactions between the pituitary and adrenal cortex. Before 120 days of gestation, much of the cortisol present in the circulation of the fetal lamb is derived from the ewe via transplacental transfer. During the last 20–25 days of gestation, there is a dramatic rise in fetal cortisol concentrations, which reach a peak 2–3 days before birth, thereafter declining 7–10 days postpartum. The source of the increase in fetal cortisol is the fetal adrenal, which is due to both an increase in the size of the organ in relation to total body weight, and an increase in its sensitivity to adrenocorticotrophic hormone (ACTH) as a result of accelerated processing of ACTH from pro-opiomelanocortin (POMC); maternal cortisol concentrations only rise around the time of parturition (Wood, 1999). At the same time, the binding capacity of the fetal plasma increases, thus reducing the amount of free cortisol in the fetal circulation and thereby reducing the negative feedback effect on the secretion of ACTH by the fetal pituitary.

In sheep fetal pituitaries, the 'fetal' corticotrophs are replaced by smaller stellate cells, the so-called 'adult' corticotrophs, around 125 days of gestation, which might reflect an increased potential for ACTH secretion (Antolovich et al., 1988). There is an increase in corticotrophin-releasing hormone (CRH) in the fetal hypothalamus during the last 10 days of gestation and, in addition, it has been suggested that the placenta of the sheep can also secrete CRH (Jones et al., 1989). Endogenous opioids may also play a role in stimulating ACTH secretion via their effect upon the fetal hypothalamus rather than the pituitary. It has been shown experimentally that when exogenous opioids are infused into the fetal lamb, there is an increase in ACTH which can be abolished by the administration of the opioid antagonist naloxone (Brooks and Challis, 1988). POMC peptides and arginine vasopressin may also be involved in ACTH secretion since both increase towards the end of gestation.

The fetal adrenal becomes more responsive to ACTH stimulation with advancing age (Glickman and Challis, 1980). Maturation is induced by ACTH, particularly the pulse pattern of its secretion. Recent work has shown that insulin-like growth factors (IGFs) may have an autocrine and/or paracrine role in regulating ovine fetal adrenal function (Hann et al., 1992). Fetal growth hormone, which is elevated from 50–70 days of gestation and then falls until 100 days, before increasing to term, may also modify the response of the fetal adrenal to ACTH (Devaskar et al., 1981).

The rise in fetal cortisol stimulates the conversion of placentally-derived progesterone to oestrogen by activating the placental enzyme 17α-hydroxylase; this hydroxylates progesterone via androstenedione to oestrogen (Figure 6.1). The consequences of the rise in oestrogens in the peripheral circulation are threefold. Firstly, oestrogens have a direct effect upon the myometrium, increasing its responsiveness to oxytocin; secondly, they produce softening of the cervix by altering the structure of collagen fibres; thirdly, they act upon the cotyledon–caruncle complex to stimulate the production and release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). The latter change is induced by the activation of the enzyme phospholipase A_2 stimulated by the decline in progesterone and rise in oestrogen. This enzyme stimulates the release of arachidonic acid from phospholipids, so that under the influence of the enzyme prostaglandin synthetase, $PGF_{2\alpha}$ is formed (Figure 6.2).

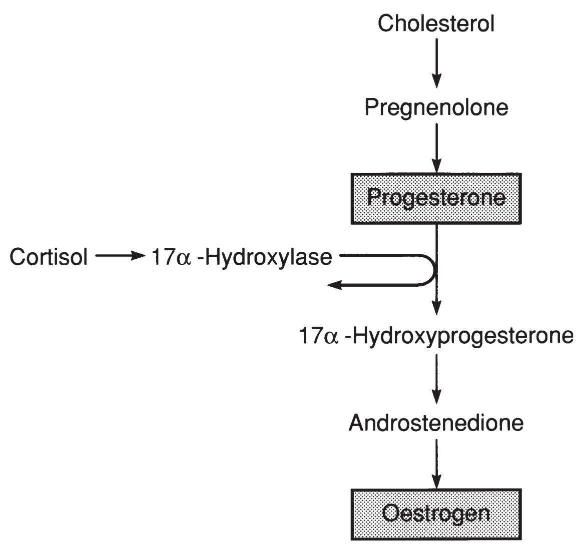


Fig. 6.1 The conversion of placentally derived progesterone to oestrogen (after Liggins, 1982).

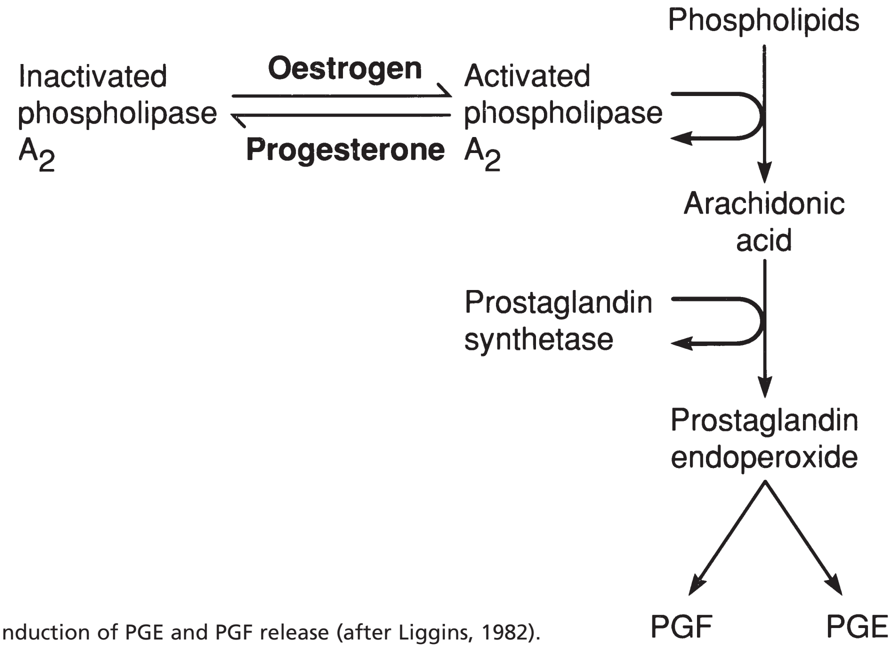


Fig. 6.2 The induction of PGE and PGF release (after Liggins, 1982).

Further stimulation of synthesis and release of the latter hormone from the myometrium can also be induced by the action of oxytocin and mechanical stimulation of the vagina.

Prostaglandins play a key role in initiating parturition; because of their molecular structure they are soluble in fat and water so that they readily pass from cell to cell via cell membranes or between cells in the extracellular fluid (Liggins, 1982). Two prostaglandins are produced by the uterus – $PGF_{2\alpha}$ in the endometrium and, during expulsion of the fetus in the myometrium, prostacyclin (PGI_2). Prostaglandins have a wide range of actions; they cause smooth muscle contraction, luteolysis, and the softening of cervical collagen as well as stimulating smooth muscle cells to develop special areas of contact called gap junctions, thereby allowing the passage of electrical pulses and ensuring coordinated contractions.

$PGF_{2\alpha}$ is considered to be the intrinsic stimulating factor of smooth muscle cells (Csapo, 1977), and thus its release is important in initiating myometrial contractions. The effect of these contractions is to force the fetal lamb towards the cervix and vagina where it will stimulate sensory receptors and initiate Ferguson's reflex, with the release of large amounts of oxytocin from the posterior pituitary. Oxytocin will stimulate further myometrial contractions and the release of $PGF_{2\alpha}$ from the myometrium. Hence both these hormones, together with uterine contraction, seem to work as a positive feedback system of increasing magnitude, thus stimulating further uterine contractions and consequent expulsion of the fetus (First, 1980).

Other important changes which are brought about by the endocrine events of parturition have been observed. For instance, maturation of the fetal lamb's lungs, especially the production of alveolar surfactant, is stimulated by cortisol, as are many other changes in fetal function and structure that enable the lamb to survive after birth. A schematic representation of the endocrine changes that are involved in the initiation of parturition in the ewe and some other species is shown in Figure 6.3, whilst overall trends in reproductive and other hormones occurring in the peripheral circulation of the ewe around the time of parturition are shown in Figure 6.4.

Cow

As stated in Chapter 3, the placenta assumes the main role of progesterone production at between 150 and 200 days of gestation, so that if the ovary

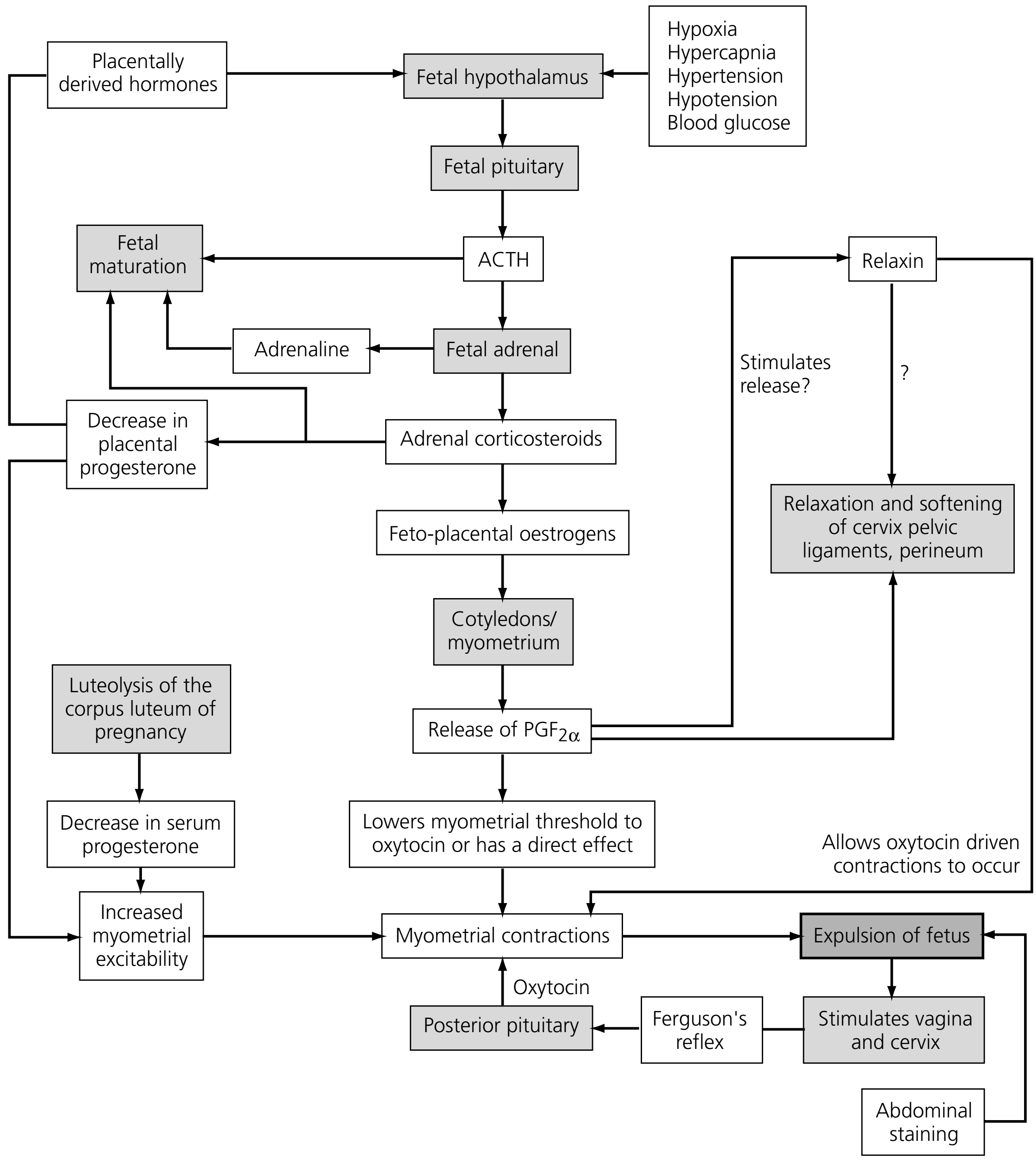


Fig. 6.3 The endocrine changes that occur before and during parturition in the sow, ewe and cow, and their effects.

containing the corpus luteum (CL), or the CL itself is removed after this stage, pregnancy will continue (McDonald et al., 1953). However, it has been observed that in cows that have been ovariectomised parturition is frequently abnormal (McDonald et al., 1953). For although the CL is not required to maintain pregnancy after this time, it has been shown that its regression plays an important role in the endocrine changes which are necessary for the initiation of parturition.

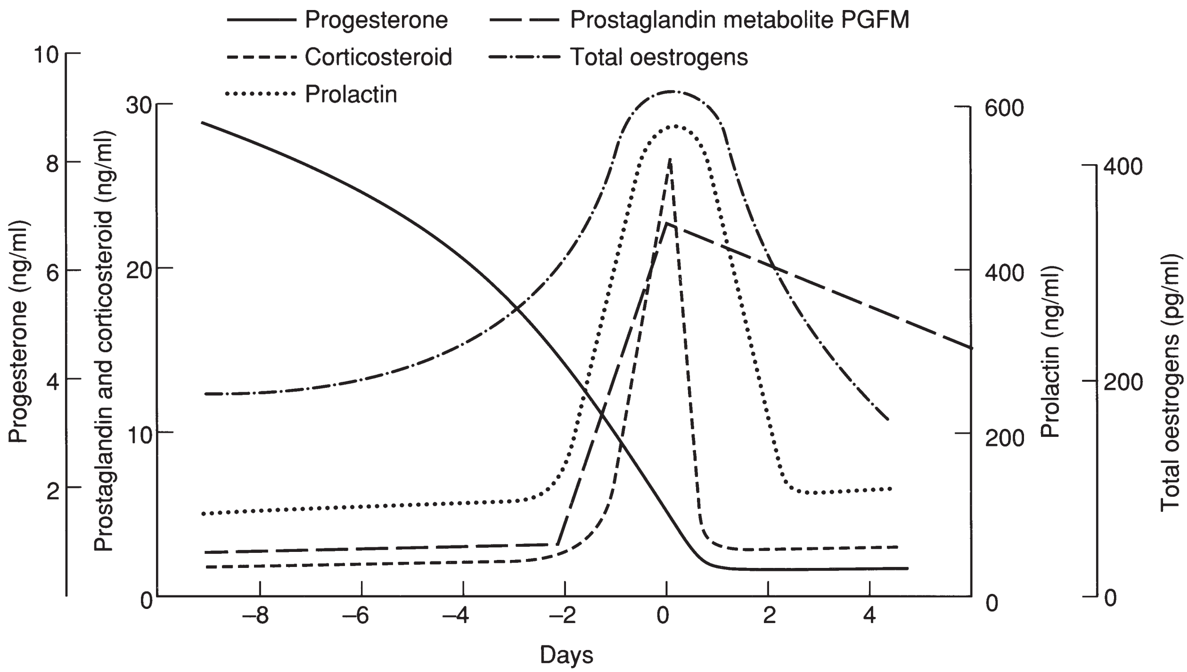


Fig. 6.4 Trends in hormone concentrations in the peripheral plasma of the ewe around the time of parturition. Day 0 at parturition.

Other mechanisms, such as the direct effect of glucocorticoids or the direct effect of placental oestrogen, may be responsible for luteal regression, but it is most likely that it is initiated by the action of $PGF_{2\alpha}$. The latter is released as a result of the effect of placental oestrogens acting upon the fetal cotyledons (see Figures 6.1 and 6.2). The endocrine changes responsible for initiating parturition are probably very similar to those described in the sheep (see Figure 6.3), on which much of the experimental work has been done. Overall trends in reproductive and other hormones occurring in the peripheral circulation of the cow around the time of parturition are shown in Figure 6.5.

Doe (nanny) goat

In this species, the CLs provide the essential source of progesterone necessary for the maintenance of gestation, since ovariectomy or extirpation of the CLs will terminate pregnancy. Placental 17α–hydroxylase, which is stimulated by the rise in fetal cortisol, diverts the synthesis of progesterone by the CLs into oestrogen. The change in the oestrogen:progesterone ratio stimulates $PGF_{2\alpha}$ synthesis as in the ewe (see Figures 6.1 and 6.2), resulting in luteolysis with a further decline in progesterone. Progesterone disappears from the circulation before parturition can occur. The endocrine changes are very similar to those in the sheep and cow.

Sow

Progesterone from the CLs is necessary for the maintenance of pregnancy throughout its entire duration. Parturition is preceded initially by increased levels of cortisol in the fetal plasma, which results in a rise in the maternal blood cortisol, oestradiol and $PGF_{2\alpha}$ metabolites and a decrease in progesterone. It is unlikely that oestrogens are responsible for stimulating the release of $PGF_{2\alpha}$ as occurs in the other species (First, 1979). Otherwise the scheme for the initiation of parturition is similar to that illustrated for the ewe. The hormonal changes in the peripheral circulation are illustrated in Figure 6.6.

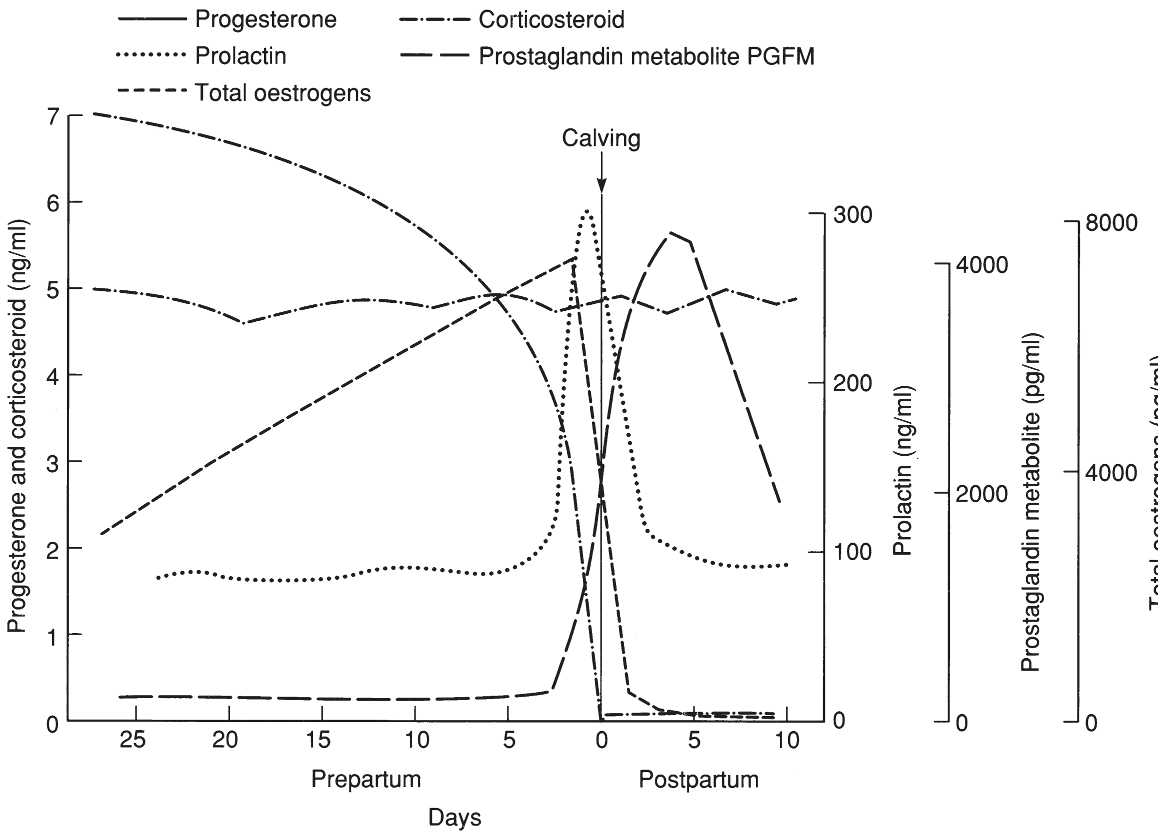


Fig. 6.5 Trends in hormone concentrations in the peripheral plasma of the cow around the time of parturition. Day 0 at parturition.

Mare

The mechanisms responsible for the initiation of parturition are not as well understood as those of the previous four species; there is less circumstantial and experimental evidence. However, it is likely that the fetal foal is responsible for the initial trigger mechanism, since the fetal adrenal undergoes rapid hypertrophy immediately before parturition (Comline and Silver, 1971) and fetal plasma cortisol concentrations have been shown to increase nearly 10-fold during the last 8 days before foaling (Card and Hillman, 1993). Since it has been shown that in the peripheral circulation of the newborn foal, β-endorphin concentrations are raised, it has been suggested that they may be involved in triggering parturition. However, it is also possible that they are produced in response to the act of parturition (Dudan et al., 1988).

The main difference from the ewe relates to the endocrine changes that occur in the maternal circulation (see Figure 3.1). Progestogens (progesterone and progestins) remain low from the middle of pregnancy until the last 2–3 months of gestation; they then increase, especially during the last 20 days, to reach a peak about 48 hours before parturition. They then decrease rapidly to low levels at the time of parturition. Plasma oestrogen concentrations decline during the last 100 days of gestation, rather than increase as in other species, reaching relatively low levels at parturition, although this is largely a reflection of the decline in oestrone and the species-specific oestrogens, equilin and equilenin, since concentrations of oestradiol–17β remain fairly constant.

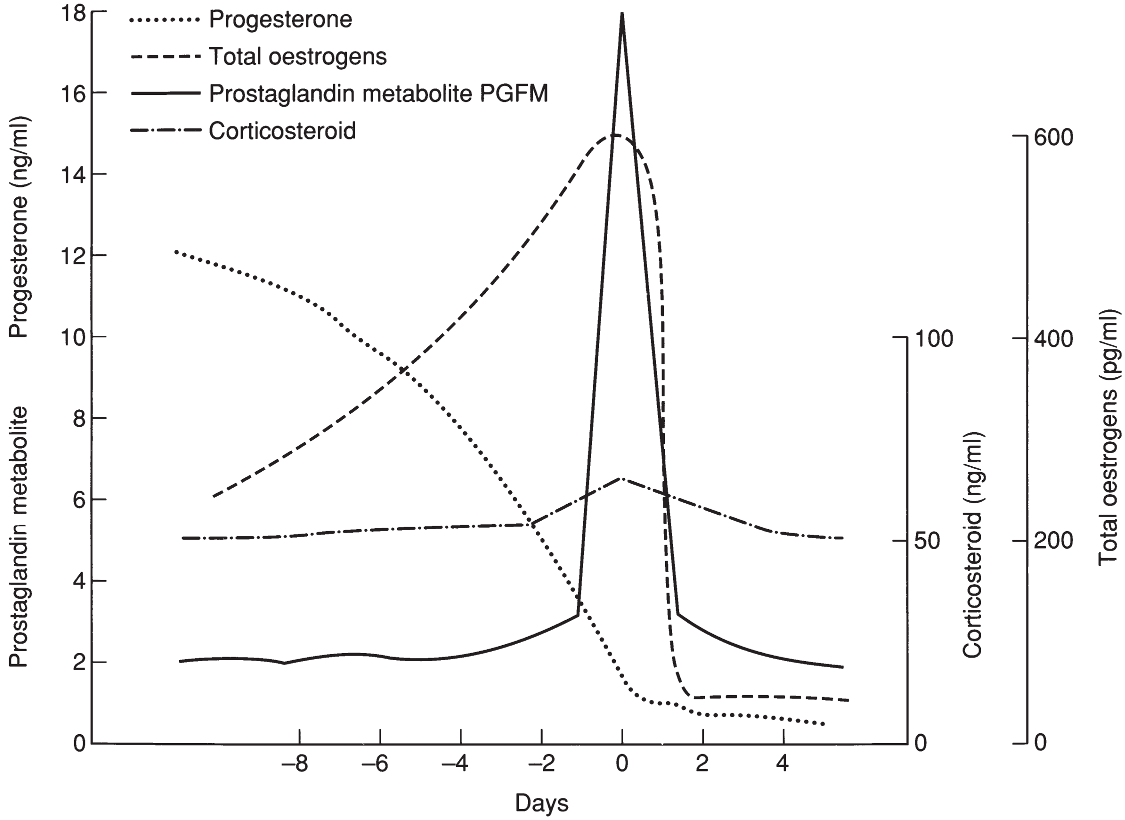


Fig. 6.6 Trends in hormone concentrations in the peripheral circulation of the sow around the time of parturition. Farrowing at day 0.

Bitch and queen cat

Far less is known about the mechanisms that are responsible for initiating parturition in either of these two species.

In the bitch, a prepartum rise of cortisol has been detected in the peripheral circulation with peaks obtained 8–24 hours prepartum (Concannon et al., 1975). Progesterone concentrations start to decline gradually from about the 30th day of gestation, and there is a precipitous fall between 12 and 40 hours before the birth of the first puppy (Concannon et al., 1975). This latter change is probably due to the release of luteolytic amounts of $PGF_{2\alpha}$ since, during the 48 hours before whelping, there is a rise in the metabolite PGFM. As the latter increased from a mean of 395 to 2100 pg/ml, progesterone decreased from a mean of 2.8 to 0.7 ng/ml (Concannon et al., 1989). Oestrogens remain at a fairly constant level throughout pregnancy and start to decline about 2 days prepartum, reaching non-pregnant values at the time of parturition (see Figure 3.22). Prolactin increases as progesterone concentrations decrease 1–2 days before whelping (Concannon et al., 1977); it is not known if this hormone plays any role in parturition.

In the queen cat, progesterone remains at between 20 and 50 ng/ml for the first two-thirds of gestation before starting to decline gradually towards term. Just before parturition it declines more rapidly to almost zero at the time of parturition (Verhage et al., 1976). Oestradiol concentrations increase slightly just before parturition.

The role of relaxin

Sources of relaxin

Relaxin, a polypeptide hormone, was shown to be responsible for causing relaxation of the pubic symphysis of guinea pigs by Hisaw in 1926. The most potent sources of this hormone are the CLs of the pregnant sow; however, it is now known to be produced by a number of other tissues such that it has a wide diversity of chemical structure and physiological effects between species.

In the pig, as well as from the CLs of the pregnant sow, it is also produced by preovulatory follicles. In the cow, the CL appears to be the main source of the hormone, with values increasing just before calving; however, because a reliable assay is not available in this species, some of the results are equivocal. There is conflicting evidence in the ewe concerning the secretion of the hormone as well as its likely source of production.

In the horse, dog and cat, the main or sole source of the hormone is the placenta. In the mare, concentrations start to rise from about 80 days of gestation, although there is considerable breed variation (Stewart et al., 1992). In the bitch, relaxin increases from about 4 weeks of gestation, and remains elevated until term, whereas in the cat there is a sudden rise from 23 days of gestation with a peak at 36 days and a dramatic decline just before parturition (Stewart and Stabenfeldt, 1985).

Actions of relaxin

Crude extracts and purified forms of the hormone have a wide range of actions on a variety of target tissues including the pubic symphysis, pelvic ligaments, cervix, myometrium and the mammary gland.

In the sow, relaxin stimulates the growth of the cervix during late pregnancy as well as causing relaxation before parturition. The latter changes, which are also influenced by the oestrogen:progesterone ratio, involve changes at a biochemical level by influencing the glycosaminoglycans:collagen ratio (O'Day-Bowman et al., 1991) and histological structure (Winn et al., 1993).

Despite the fact that there is not a reliable assay for relaxin in the cow, there is long-standing circumstantial evidence that it plays an important role in cervical relaxation at term. Studies have shown that when highly purified porcine relaxin was placed directly on the external os of the cervix at 276–278 days of gestation, cervical relaxation occurred 8–12 hours later (Musah et al., 1986). Similar results have been obtained when parturition has been induced with dexamethasone (Musah et al., 1987). The reports on the effect of porcine relaxin on the cervix of the sheep are equivocal. Recent work (Roche et al., 1993) has shown that a relaxin-like mRNA cannot encode a functional relaxin molecule, which suggests that sheep may not produce relaxin and thus, in this species, cervical relaxation may not be relaxin-dependent.

Relaxin also exerts an influence on myometrial activity, with several studies reported in domestic species, in particular the pig. In general, relaxin reduces both the frequency and amplitude of uterine contractions, particularly the former. It appears to act in concert with progesterone, oestrogens, oxytocin and prostaglandins. Thus in the sow, although the progesterone concentrations have fallen significantly 10–24 hours before farrowing, with the removal of the progesterone block (see below), myometrial activity is low. At this time, relaxin concentrations increase significantly. Furthermore, in sows there is a relationship between relaxin concentrations and the duration of farrowing (Wathes et al., 1989).

Fetal maturation

As has already been described, the fetal endocrine changes that occur in late pregnancy not only initiate parturition but also stimulate a variety of maturational changes which enable the newborn animal to survive. In the absence of these changes, sometimes referred to as 'preparation for birth' (Liggins et al., 1979), neonatal death may occur because of malfunction of immature organs. Examples of some of the changes that occur are: physical, such as closure of the ductus arteriosus and foramen ovale; and functional, such as the development of glucose homeostatic mechanisms, changes in the structure of haemoglobin from the fetal to the adult form.

If premature induction with the exogenous hormones bypasses some of the endocrine changes that normally occur, the newborn may be unprepared. This has been demonstrated when goat kids, which were born following the injection of $PGF_{2\alpha}$, were compared with those which were born after induction with ACTH (Currie and Thorburn, 1973). The link between parturition and maturation of the fetus appears to be related to the adrenal cortex and the prepartum surge of fetal cortisol (Liggins, 1978).

During its intrauterine life the fetus is in a thermally neutral environment, but at birth it has to be able to maintain its own body temperature. The mechanisms which enable this are the accumulation of brown fat and glycogen in late gestation and maturation of the thyroid gland. The latter process occurs as a result of the prepartum rise in fetal cortisol, which stimulates the monodeiodination of the thyroid hormones, thus enhancing their biological activity (Liggins et al., 1979).

The maintenance of glucose homeostasis immediately after birth, when the newborn loses its placental source of glucose, is dependent on adequate stores of liver glycogen. There is strong evidence that in sheep the stimulus for the accumulation of glycogen stores in late gestation is the rise in fetal cortisol (Jost et al., 1966). The glycogen stores are just sufficient to provide energy before sources of glucose become available from food. A similar stimulus to the production of insulin by the pancreas has also been attributed to the effect of elevated fetal cortisol, which enables the newborn to respond quickly to maintain glucose homeostasis.

The fetal adrenal medulla also shows evidence of maturational changes in that its ability to produce catecholamines, especially adrenaline, is increased in response to asphyxia (Comline and Silver, 1971). There is some evidence that adrenaline, together with fetal ACTH and cortisol, stimulates lung maturation, thus enabling normal respiratory function to occur.

Premature induction of parturition

Although it is usually possible to predict approximately when parturition will occur in domestic species there are obvious advantages in being able to predetermine when the event will occur. Many of the methods that are used have originated from studies of the endocrine changes responsible for the initiation of normal parturition. Since the first published reports of premature induction by Van Rensburg (1967) in the ewe and Adams (1969) in the cow, a large number of successful reports have been published in these two species as well as in the doe, sow and mare; little success has been achieved in the bitch and the cat.

Mare

The indications for the premature induction of foaling are few, the main one being to ensure that it occurs in the presence of skilled assistance; then if dystocia occurs it is possible quickly to correct the difficulty so as to ensure survival of the foal and reduce the danger to the mare. There are also a few occasions when, because of disease or illness in the mare, it may be advantageous for foaling to be induced.

A number of different hormone preparations have been used. Britton (1972) and Purvis (1972) described the successful induction of over 1500 foalings with an intramuscular injection of oxytocin, either with or without priming with stilboestrol dipropionate. If the cervix showed evidence of 'ripening', i.e. was soft on palpation and able to allow the insertion of one or two fingers in the external os, and the foal was in normal presentation, position and posture, oxytocin was given at a dose of 120 IU to mares between 360 and 600 kg live weight. Foaling occurred 15–60 minutes later. If the cervix was 'unripe', 30 mg of stilboestrol dipropionate in oil was given intramuscularly, followed by oxytocin 12–24 hours later, provided that the cervix had responded. Both authors recommend that a second vaginal examination is made 10–15 minutes after the oxytocin to determine the foal's position and posture so that if it is abnormal correction can be made. Purvis (1972) also recommended that the allantochorion is ruptured manually if it has not ruptured naturally by the time that the foal is well advanced into the vagina.

Purvis (1972) recorded no difficulty with placental retention although this was a problem in the cases described by Rossdale and Jeffcoat (1975). A relationship between the dose of oxytocin and placental separation has been demonstrated (Hillman, 1975), doses of less than 60 IU resulting in retention.

In the author's experience, dystocia has sometimes occurred owing to the lateral position of the foal, as a result of incomplete rotation before the onset of the second stage of parturition. Furthermore, foal viability has often been poor due to anoxia during expulsion because of premature placental separation. It is important to know the precise gestational age since induction should not be attempted before 320 days; even then because of the wide variation in the gestation length in the mare, foal viability is poor.

Dexamethasone, a quick-release synthetic corticosteroid, has been used successfully to induce foaling in ponies (First and Alm, 1977) and large 'saddle-type' mares (Alm et al., 1975). A dose rate of 100 mg every day for 4 days resulted in parturition 6–7 days after the start of treatment in the latter type, whilst the ponies responded more rapidly. The regimen was started at 321 days of gestation, with satisfactory foal survival and subsequent growth rate.

$PGF_{2\alpha}$ and its analogue fluprostenol have also been used to induce foaling. A single dose of $PGF_{2\alpha}$ is not always effective; quite often it is necessary to use repeated injections of 1.5–2.5 mg every 12 hours. In some cases these prostaglandins can cause discomfort and can result in a high incidence of dystocia due to abnormalities in the position of the foal (Allen, 1980). Fluprostenol will successfully induce foaling between 322 and 367 days of gestation when given as a single dose of 250 μg to ponies and 1000 μg to thoroughbred mares (Rossdale et al., 1976). The time from injection to the onset of second-stage labour ranged from 33 to 183 minutes; the second stage lasted 5–33 minutes, and the placenta was shed by 112 minutes. The viability of the foals was generally good, although a number suffered rib damage.

It is also possible to induce foaling by the administration of progesterone; the interval from injection to effect is very similar to that following dexamethasone (Alm et al., 1975). It is possible that, as in other species, progesterone is metabolised by the adrenal or placenta to corticosteroid (First, 1980).

Cow

The indications for the induction of calving are as follows:

- Advancing the time of calving to coincide with the availability of suitable pasture for milk production. This is used in New Zealand and parts of Ireland. In the latter country it is important that the cows calve over a period of 3 months as close to 23 March as possible (O'Farrell, 1979). O'Farrell has calculated that for every day that a cow calves after this date the yield is reduced by an average of 6.4 litres per day; he found that over 40% of the cows were in this category.
- Ensuring that cows calve at a predetermined time when skilled assistance is available so that prompt attention can be given. This should reduce calf mortality and injury to the cow.
- Reducing the birth weight of the calf by shortening the length of gestation. During the last weeks of gestation the growth rate of the calf is rapid; in some of the exotic breeds, such as the Charolais, the live weight of the calf can increase by between 0.25 and 0.5 kg per day. Thus if the dam is immature, with a small pelvis, or pregnancy is prolonged beyond 280 days, as occurs in some exotic breeds, the calf may be too large to traverse the birth canal. Premature induction can thus reduce the likelihood of dystocia due to fetomaternal disproportion (see Chapter 11).

The stage at which calving is induced must be a compromise between the birth of the calf, which is small enough to be born unaided and yet is large enough to be viable, and subsequently to have an adequate growth rate. This threshold weight will vary from breed to breed. In the pluriparous Aberdeen Angus and Hereford cow it is 40 kg, for the pluriparous Friesian 42–45 kg, for the 2-year-old Charolais 35 kg and for the 3-year-old Charolais 40–45 kg (Meniscier and Foulley, 1979).

- In diseased or injured cows where the termination of pregnancy will alleviate the condition, or where a live calf can be obtained before slaughter, premature induction may be used. Cows suffering from hydrallantois will frequently respond.

A number of different hormones have been used successfully to induce calving so that a live calf is born. Since induction before 270 days will usually result in the birth of a small, weakly calf with poor prospects of survival it is important that the date of service or insemination is accurately known.

Although ACTH has been used to induce calving, since it exerts its effect by stimulating endogenous corticosteroid production, it is best replaced by the direct administration of corticosteroids. A number of potent synthetic ones are available. There are three main categories; these are referred to as long-acting, medium-acting and short-acting, their classification being based upon the duration of the latent period (time interval from treatment to effect). Thus, when given at a normal therapeutic dose rate, the long-, medium- and short-acting corticosteroids have latent periods of 11–18, 5–11 and 1–6 days, respectively (Parkinson, 1993). It is important to give large breeds of cows an adequate dose; in the case of betamethasone up to 35 mg is necessary in the Charolais. Corticosteroids are also immunosuppressive and thus they should not be given without broad-spectrum antibiotics if infection is present; the lungs and udder should be carefully examined beforehand.

PGE_1, PGE_2 and $PGF_{2\alpha}$ and analogues of the latter have been used. In the first reported use of prostaglandins (Zerobin et al., 1973); a minority of calvings were described as being associated with 'explosive expulsions', a 42% incidence of dystocia due to poor cervical dilation was also reported by Hendricks et al. (1977). However, both $PGF_{2\alpha}$ and the analogues have been successfully used from about 275 days of gestation with a latent period of 2–3 days (Kordts and Jöchle, 1975; Day, 1977).

Good results have been obtained by using a combination of corticosteroid and prostaglandin. Beal et al. (1976) injected $PGF_{2\alpha}$ if no effect had occurred 40 hours after treatment with dexamethasone. Day (1978) obtained good results using a prostaglandin analogue, cloprostenol, administered 8 or 12 days after pretreatment with dexamethasone trimethylacetate; all the cows calved within 72 hours. In a similar trial, involving 26 adult Friesian cows ranging in gestation from 237 to 270 days, 20 mg of dexamethasone phenylpropionate was given, and induced calving in 13 cows on average 5.6 days later. Those that failed to respond received 500 μg of cloprostenol after 10 days, and all calved within 3 days; all liveborn calves survived (Murray et al., 1982). In summary, for early induction (250–275 days of gestation) a long-acting corticosteroid is administered followed by a short-acting corticosteroid or $PGF_{2\alpha}$ after 8 days if calving has not occurred; the latent period is about 48 hours. After 275 days, a medium-acting corticosteroid, with either a short-acting corticosteroid or $PGF_{2\alpha}$ after 8 days if the cow has failed to calve, is used. After 282 days, $PGF_{2\alpha}$ or short- or medium-acting corticosteroids are effective on their own.

There are, however, some disadvantages of premature induction of calving. It is not always effective. The birth weight of the calf is lower than it would have been at term, and thus the subsequent growth rate is reduced. There is also a high incidence of retained fetal membranes, up to 53% when 'short-acting' preparations are used (Wagner et al., 1971), although it is less common following the use of slow-release preparations (Welch et al., 1973; O'Farrell and Crowley, 1974). Milk yield is initially affected, with a delay in reaching peak lactation, although there appears to be very little influence on the overall yield (Bailey et al., 1973; Welch et al., 1977; O'Farrell, 1979). Subsequent fertility is fairly normal although the calving to conception interval and the number of services per conception are slightly increased in those cows that retain their fetal membranes. There is a reduction in the quality and quantity of colostral immunoglobulins, especially following the use of slow-release corticosteroid preparations, but it is unlikely that the calf will not acquire an adequate passive immunity.

Sow

On average, 5–7% of all piglets are stillborn, and it is estimated that 75% of stillbirths occur during parturition. The time interval between the birth of the first and last piglets influences the stillbirth rate so that prolonged farrowing results in an increase. Probably 80% of the stillbirths occur in the last third of the litter to be born.

It has been shown that the time interval between the birth of two live piglets is generally shorter than the time interval which precedes the birth of a stillborn piglet. The death of the piglet may have been due to a delay in expulsion, although there is good evidence that dead piglets are expelled more slowly. One of the causes of stillbirth is the premature rupture of the umbilicus; surveys have shown that 94% of stillborn piglets had ruptured umbilical cords at birth compared with 39% when all piglets were included (Randall, 1972).

The stillbirth rate could be reduced by greater care and attention during farrowing; however, the time of farrowing can be difficult to predict. For this reason, the induction of farrowing of groups of sows at predetermined times has many attractions because this can enable a skilled person to be in attendance to a group of sows during normal working hours.

Since there is also evidence that delayed or prolonged parturition can increase the stillbirth rate, then methods which accelerate the process, or at the very least prevent delays, have obvious attractions.

There are also a number of other management requirements which have stimulated a need to plan and regulate the timing of farrowing:

- Group farrowing facilitates multiple suckling and allows cross-fostering to take place. Thus the piglets from sows with large litters or agalactia have a greater chance of surviving and being reared.
- Groups of sows and litters can be managed on an 'all in, all out' principle, thus enabling disinfection and cleaning to be performed more efficiently.
- Group farrowing facilitates group weaning.
- Farrowing can take place on certain days of the week and during normal working hours; as well as reducing the stillbirth rate it can reduce the death rate due to overlaying of newborn piglets.
- It increases the reproductive efficiency by reducing the farrowing interval by a few days.

Synthetic corticosteroids have been used successfully to induce parturition in sows by injecting them on days 101–104 with a single daily dose, 75–100 mg; farrowing occurs on day 109. The procedure is expensive, and piglets born on day 109 have a poor survival rate and the use of synthetic corticasteroids therefore has no practical application.

Since the first reports of successful induction of farrowing using $PGF_{2\alpha}$ (Diehl et al., 1974; Robertson et al., 1974) or a synthetic analogue (Ash and Heap, 1973) a large number of reports have been published worldwide. It is now an accepted procedure in the pig industry. More recently, attempts have been made to improve its efficiency by combining prostaglandins with other hormones.

The basic procedure with prostaglandin treatment is that either 10 mg of $PGF_{2\alpha}$ or 175 μg cloprostenol is injected intramuscularly on days 112–113 of gestation, and farrowing will occur on average 28 hours later. Thus, if the timing of injection is between 08.00 and 10.00 hours, the majority of sows will farrow during normal working hours. Adjustments have to be made for herd variations in the average gestational length.

In general, the earlier the time of induction, the lower the birth weights; however, if attempts are made to delay the time of induction in order to obtain large birth weight the degree of synchronisation is not as good.

In an interesting study involving the use of dinoprost to induce farrowing on 229 occasions (Young and Harvey, 1984), it was found that 95% of farrowings occurred within 48 hours of injecting dinoprost, the majority (76%) within 24–36 hours, which corresponded with normal working hours on the farm. Those sows which did not farrow within 48 hours were deemed not to have responded to the injection of prostaglandin. Apart from very small litters (up to five piglets), litter size had no effect upon the response to prostaglandin; however, the duration of farrowing was directly related to litter size.

There is apparently no difference in the efficiency of $PGF_{2\alpha}$ (dinoprost) or the analogue cloprostenol, although it is generally recognised that side-effects such as biting cage bars and increased respiration rate are greater following $PGF_{2\alpha}$ (Boland et al., 1979; Einarsson et al., 1981; Widowski et al., 1990).

Improved expulsion of the piglets was observed when cloprostenol was combined with oestradiol benzoate. Bonte et al. (1981) reported that the best expulsion occurred when 10 mg of oestradiol benzoate was given 24 hours before cloprostenol although there was some improvement when 1 mg of oestradiol benzoate was given 5–6 hours after cloprostenol. Kirkwood and Thacker (1995) treated sows with 3 mg oestradiol on day 112 followed by 10 mg $PGF_{2\alpha}$ on day 113, and found that 38% of sows farrowed before 8.00 hours on day 114 compared with 5% in the control group. Oxytocin has also been used routinely in combination with $PGF_{2\alpha}$ as part of induction protocols, the most common approach being to administer it 24 hours after prostaglandin. This usually resulted in a significant reduction in the interval to farrowing, although the response may be related to the litter size (Chantaraprateep et al., 1986). Others have used repeated injections of oxytocin (Zarro et al., 1990). It is important that dose rates of oxytocin of about 10 IU should be used since higher dose rates will cause myometrial spasm and delay farrowing.

Ewe

Parturition can be induced in the ewe by means of ACTH, corticosteroids and oestrogens. The indications for induction are limited since dystocia due to fetomaternal disproportion is not as common as in cows. However, a system which can guarantee that lambing will occur only during the hours of daylight when skilled assistance is available might reduce any problems due to dystocia and increase lamb survival rates. However, it is not possible to shorten gestation length appreciably without increasing lamb mortality.

As with other species, an accurately known gestational age is important. When corticosteroids such as dexamethasone, flumethasone and betamethasone are given by a single intramuscular injection within 5 days of term, normal parturition occurs in 2–3 days. Induction is also possible with two intramuscular injections of 1–2 mg of oestradiol benzoate 5–6 days before term or with a single injection of 15 mg of oestradiol benzoate 5 days before term (Bosc et al., 1977). Cahill et al. (1976), using similar methods, had a higher than normal incidence of dystocia with poorer lamb survival.

Unfortunately, attempts to concentrate lambing during the hours of daylight by premature induction with corticosteroids have not been very successful (Bosc, 1972). Induction of lambing in groups of ewes that have been synchronised with progestogens (see Chapter 1) can ensure that ewes lamb over a relatively short period of time in a single batch.

Doe (nanny) goat

Parturition has been successfully induced with ACTH, corticosteroids, $PGF_{2\alpha}$ and analogues, and oestrogens; however, lactation sometimes occurs prematurely (Currie and Thorburn, 1977).

Bitch and queen cat

Except for the induction of abortion using $PGF_{2\alpha}$, epostane and a prolactin inhibitor cabergoline, attempts to induce parturition in these species have been unsuccessful.

Accelerating parturition

Oxytocin, administered as a bolus injection, has long been used to treat sows which appeared to be suffering from uterine inertia as shown by prolonged farrowing times.

Routine use of oxytocin 1–2 IU used repeatedly in delayed farrowing was effective in accelerating the process. Doses in excess of 10 IU induce uterine spasm and are contraindicated (Zerobin, 1981). Depot or slow-release oxytocin preparations are not effective.

Studies by Pejsak and Tereszczuk (1981), in which 10 IU of oxytocin was given to 836 primiparous and pluriparous gilts and sows by the intramuscular or intranasal route as a routine procedure, demonstrated some favourable results. In their regimen, oxytocin was given immediately after the birth of the first piglet and was repeated if there was a delay of more than 1 hour before the birth of the next piglet. If no response was observed in 10–20 minutes, a vaginal examination was performed. All the oxytocin-treated sows farrowed within 10 hours whilst some in the control group exceeded this duration.

A β-blocking agent, carazolol, has been used to shorten the duration of farrowing with encouraging results. The rationale behind its use is that during pregnancy, because β receptors in the uterus become dominant, the stress resulting in adrenaline release will cause relaxation of the uterus. If these receptors are blocked with carazolol, then in stressed animals (especially gilts) the adrenaline will have little or no effect upon the myometrium, the uterus will retain its tone and parturition will not be delayed. In a double-blind trial, involving 1000 sows using a dose rate of 0.5 mg/50 kg given at the beginning of labour, there was a significant reduction in the duration of farrowing ($P < 0.05$), a reduction in the stillbirth rate and in the incidence of obstetrical complications, especially in gilts (Bostedt and Rudolf, 1983). There may also be some effect upon milk letdown.

Delaying parturition

β-Adrenergic agents

β-Adrenergic agents which stimulate the β_2 receptors of uterine muscle cells can abolish uterine contractions and delay parturition for a short time. One such substance, clenbuterol, has been used successfully in cows, sows and ewes (Ballarini et al., 1980; Collins et al., 1980; Jotsch et al., 1981). In cows, provided that the cervix is not fully dilated and second stage has not commenced, an injection of 0.3 mg of clenbuterol hydrochloride (10 ml) followed by a second injection of 0.21 mg (7 ml) 4 hours later will inhibit calving for 8 hours after the second injection. As well as being used as a management tool it can be effective in ensuring improved relaxation of the vulva and perineum in heifers.

In sows, it causes relaxation of the myometrium and hence interrupts expulsion of the piglets. After several hours there is a return of spontaneous myometrial contractions without any adverse effects upon the viability of piglets. Zerobin (1981) obtained good responses with a dose rate of 150 μg. He also recommends the use of oxytocin 20–40 IU to reverse the effect of the clenbuterol; this is said to produce strong myometrial contractions without spasm of the smooth muscle. He recommends the combination of both drugs to manage farrowing.

Course of parturition: physiology

The essential components of the birth process are the expulsive forces, the fetus(es) and the birth canal. Normal birth will result when these forces are sufficient to expel a normal, correctly disposed fetus (and fetal membranes) through a birth canal of adequate dimensions.

The expulsive forces consist of the contractions of the myometrium and the abdominal musculature; the relative importance of these two components varies from species to species. During pregnancy the fetus occupies as small a space as possible; in order to do this it flexes its limbs and neck and, particularly in the monotocous species, assumes a position so that its dorsum is adjacent to the greatest curvature of the uterus. In order to negotiate the birth canal the fetus must be correctly disposed so that its body conformation is as ‘streamlined’ as possible to match the shape and direction of the birth canal, this being particularly important in monotocous species. Finally, the birth canal must allow the passage of the correctly disposed fetus. Changes occur in the maternal structures which allow this to occur. The cervix must dilate, the bony pelvis and its related ligaments must relax, and the vagina, vulva and perineum must soften.

Myometrial contractions

The hormones which bring about some of these changes have already been discussed. The mechanisms involved in the myometrial contractions have not. The myometrium is formed from two main types of muscle protein, namely myosin and actin. The contractions of the myofibrils occur because of the formation of covalent cross-linked bonds between the actin and myosin filaments. Contractions are initiated when the ATPase of myosin light chain (MLC), which is one of the myosin components, is phosphorylated by the action of MLC kinase (MLCK). This enzyme, MLCK, is activated by a calcium-binding protein – calmodulin. Myometrial relaxation occurs because of dephosphorylation of MLC by the action of MLC phosphatase or cAMP-dependent protein kinase which inhibits MLCK, and hence phosphorylation of MLC. It is important to consider the structural arrangement of the smooth muscle bundles. In domestic species with a bicornuate uterus the myometrium comprises two layers. In the outer layer the bundles are arranged parallel to the long axis of the uterus so that when these contract the uterus is shortened cephalocaudally. The myometrium is continuous with the cervix, which is fairly well secured within the pelvic cavity; thus when the longitudinal bundles contract, the uterine horns will be pulled caudally. When the uterus contains a full-term fetus the ability of the uterus to shorten is reduced and, as a result, the contractions may cause some dilatation of the cervix. The inner layer of the myometrium is formed by bundles of fibres arranged concentrically around the longitudinal axis; thus these contractions will constrict the uterine lumen (Porter, 1975).

During pregnancy, as a result of the stimulus of oestrogens on protein synthesis and the localised influence of distention by the developing conceptus, there is hypertrophy of the myometrium. In early pregnancy there is also some evidence of hyperplasia. As a result of this, the length of the myofibrils is increased 10-fold and the width two-fold. Since the myometrial mass is increased its work capacity is increased.

As well as the physical changes in the myometrium, there are also changes in the electrophysiology of the smooth muscle. It has been demonstrated that in many species during pregnancy there is an increase in the resting membrane potential. With the prepartum rise in oestrogens and the removal of the progesterone block there is discharge of action potentials and the initiation of myometrial contractions. The resting membrane potential of the oestrogen-dominated myometrial cell is also close to the threshold level for the spontaneous firing of action potentials. Thus, if the myometrium is stretched, there is slight depolarisation and discharge of action potentials. However, in the case of the rat myometrium there is no triggering of action potentials in response to stretch when it is under the dominance of progesterone; at present there is no report of a similar effect in domestic species.

Effects of progesterone and oestrogens on myometrial activity

Progesterone dominance during pregnancy ensures that the myometrium remains relatively quiescent, although there is some evidence in some species that relaxin and PGI_2 may also play a role (see below). Oestrogens have the reverse effect. Although the mechanisms involved are not fully understood, it has been suggested that oestrogens might exert their effect by: (1) increasing contractile protein synthesis; (2) increasing the number of agonist receptors for oxytocin and prostaglandins; (3) increasing calmodulin synthesis; (4) increasing MLCK activity; and (5) increasing the number of gap junctions, which are low-resistance pathways for the transmission of electrical and molecular information between smooth muscle cells. Oestrogens, in this way, increase the effectiveness of the myometrium as a contractile unit. Progesterone has the opposite effects. It: (1) reduces the number of gap junctions; (2) reduces the number of agonist receptors; (3) inhibits the synthesis of prostaglandins and the release of oxytocin; and (4) increases calcium binding.

Role of prostaglandins and oxytocin

Prostaglandins play a critical role in parturition, not only in the initiation of the process but in the control of myometrial contractions. These actions are facilitated because the molecular structure of prostaglandins enables them to move freely through extracellular fluids and lipid cell membranes. Whereas $PFG_{2\alpha}$ and PGE are responsible for stimulating uterine contractions, it has been shown that PGI_2 inhibits uterine contractions (Omini et al., 1979). Prostaglandins actions are mediated through specific receptors, and as a consequence they influence the number of gap junctions and also the movement of Ca^{2+} between the myofibrils; $PGF_{2\alpha}$ and PGE, enhance and PGI_2 inhibits these changes.

The pattern of oxytocin release during late pregnancy and parturition has been studied in the ewe (Fitzpatrick, 1961), goat (Chard et al., 1970), mare (Allen et al., 1973), cow (Schams and Prokopp, 1979) and sow (Forsling et al., 1979). It is interesting that in all these species the oxytocin levels during late pregnancy and the early stages of parturition remain fairly low and increase to reach peak values at the time when the fetal head emerges from the vulva, and when the fetal membranes are expelled. Therefore, it is likely that oxytocin plays only a minor role in the initiation of uterine contractions. The main release of this hormone occurs as a result of the stimulation of sensory receptors in the anterior vagina and cervix

(Ferguson's reflex). There is certainly a good correlation between electromyographic activity and oxytocin release in the sow (Ellendorff et al., 1979), which is suggestive of a local positive-feedback mechanism operating in the uterus of this species.

Oxytocin receptors increase during late gestation and with the onset of parturition; this is dependent mainly on the decline in progesterone and increase in oestrogens. Little is known in domestic species about their distribution in the circular and longitudinal muscle layers. Oxytocin stimulates uterine contractions in two ways: firstly, by increasing prostaglandin release, with which there is a synergistic effect; secondly, by increasing Ca^{2+} release which increases MLC phosphorylation (MacKenzie et al., 1990).

Stages of labour

Traditionally the process of parturition has been divided into three separate stages referred to as the stages of labour. Whilst it is convenient to consider the process in this way, it is important to remember that the stages do not start and end abruptly but pass gradually from one to the other.

First stage of labour

The changes that occur during this phase of parturition are not visible externally but are important because they prepare the birth canal and the fetus for expulsion. A number of important changes occur. Firstly, the structure of the cervix changes so that it can dilate; secondly, there is the onset of myometrial contractions; and finally, the fetus assumes the disposition for expulsion, which involves rotation about its longitudinal axis and extension of the extremities.

The change in the structure of the cervix has been studied by Fitzpatrick (1977b), who has described a loosening of the ground substance of its structure due to changes in the composition of collagen components. There is also increased incorporation of water, which permits the collagen fibres to separate from each other particularly under the extension forces, and possibly also allows ready access of previously inactive proteases to susceptible sites for the breakdown of the collagen molecules (Fitzpatrick and Dobson, 1979).

The cervix of the cow dilates, with the external os opening before the internal os (Abusineina, 1963); the same has also been reported in the ewe (Fitzpatrick, 1977b). The time taken for cervical dilatation varies. In the sow, using sequential vaginal exploration, it was found that it takes between 1 hour and 2 days, with 50% taking 6–12 hours (Schmidt, 1937). The mechanism responsible for dilatation is still not fully understood. For many years it was assumed to be a passive process brought about by the passage of the fetus and the fluid-filled fetal membranes through the ripened cervix. It has also been suggested that it is mainly an active process caused by the contraction of the longitudinal muscle bundles. However, it has been found that in some ewes only weak myometrial contractions precede dilatation (Hindson et al., 1968), and in some women the cervix can dilate in the absence of uterine contractions (Liggins, 1978). More recently, Ledger et al. (1985) demonstrated that in ewes, even when the cervix and uterus were isolated surgically, cervical softening still occurred. Thus the biochemical changes, previously described, are more important than the contributions from the smooth muscle of the cervix or myometrium. These changes are probably not just the degradation of collagen but rather a remodelling of the cervical matrix with new collagen and proteoglycan synthesis (Challis and Lye, 1994). It is likely that in normal parturition it is a combination of active and passive mechanisms.

In the cow, there is initially wide dilatation of the external os, whose perimeter is palpable as a frill at the cranial end of the vagina. The cone-shaped cervix then undergoes a simultaneous shortening before the internal os dilates, and when this has occurred the vagina and uterus form a continuous canal which becomes tightly engaged by the distended allantochorion.

The first stage of labour is also characterised by the onset of regular myometrial contractions, which frequently produce signs of discomfort and mild colic, the degree of response varying from species to species and individual to individual. However, in most cases there is restlessness with elevated pulse and respiratory rates; the body temperature usually falls a degree or so. In the sheep and goat during late gestation, in some cases as early as 2 months before parturition, uterine contractions occur once every 30–60 minutes; they are of low amplitude but of 5–10 minutes' duration. This pattern continues until at least the last 4 days prepartum when the frequency and amplitude increase. It is only in the last 12 hours, and in some ewes in the last 2 hours, that clearly coordinated contractions occur at a regular frequency (30 per hour), of short duration (1 minute) and substantial amplitude (20–25 mmHg) (Fitzpatrick and Dobson, 1979). Ward (1968) found that there is a significant increase in myometrial activity during the last 4 hours before the expulsion of the lamb.

In the cow, the myometrial contractions show a transition from isolated, uncoordinated waves during late pregnancy – 'contractures' – to a regular coordinated peristaltic type nearer to expulsion of the calf. The frequency also increases from 12 to 24 per hour in the last 2 hours, and 48 per hour just before expulsion (Gillette and Holm 1963).

Myoelectrical activity increases during the last 24 hours before birth in association with pressure changes, but strong progressive changes occur only just before expulsion (Taverne et al., 1976). One feature identified in the cow by Gillette and Holm (1963), which will also be discussed below in the sow, was the presence of cervicotubular and tubular–cervical contractions. The function of the former type is not known in this species. The myometrial contractions in the sow are more complex. However, as in other species, there is a tendency for their duration, frequency and amplitude to increase and for them to become more regular 12–72 hours before the onset of the second stage (Zerobin and Spörri, 1972; Ngiam, 1979). Contraction frequencies of 8–24 per hour, and durations of 0.5–3.5 minutes, at amplitudes up to 60 mmHg, have been recorded from about 24 hours prepartum. A change in the pattern of the contractions was observed by Zerobin and Spörri (1972) at the time that milk appeared in the teats. The same authors, using pressure recordings, and Taverne et al. (1979), using myoelectrical techniques, identified the presence of cervicotubular and tubular–cervical contractions. The latter authors observed that once one of the horns was empty, cervicotubular contractions decreased or disappeared, yet tubular–cervical contractions were present in the opposite horn. Since it has been known for some time that uterine volume is important in regulating myometrial activity by altering myoelectrical activity (Csapo et al., 1963), it has been postulated that the cervicotubular contractions prevent the premature displacement of piglets, thus ensuring orderly expulsion from the horns (Taverne et al., 1979).

The contractions of the uterine musculature cause other changes in the uterus and probably also in the fetus. In the placenta, the attachments to the endometrium become less intimate and the superficial cells undergo fatty degeneration, while in those species with a deciduate placenta, separation of the margins, with haemorrhage, is beginning. The increased resistance to blood circulation in the maternal side of the placenta causes a correspondingly greater flow of blood to the fetus. On the maternal side, this impediment to circulation may aid diversion of blood to the mammary glands.

As regards the fetus in first-stage labour, it becomes more active and disposes itself in a manner which will allow it to negotiate the birth canal. Thus, in the foal and puppy there is a progressive rotation from the ventral to the dorsal position, while the forelimbs, head and neck become extended. In the case of the calf and lamb extension only is necessary to change the fetus from its gestational posture to that of parturition. The nature of the mechanism whereby the forelimbs become straightened in front of the body is unknown. In the bovine species this is a unique attitude which is never repeated after birth. In his studies of the first stage of labour, Abusineina (1963) noticed that the flexed knees of the calf first occupied the dilating cervix; 30 minutes later the digits were in the cervix. The author suggests that at this time the fetus is practising righting reflexes and that it extends the carpal joints in its efforts to 'stand up in utero'. It is likely also that these spontaneous fetal movements occur in response to increased uterine pressure caused by the myometrial contractions of the first stage. If this view is correct, then the mother, through the medium of an indifferent myometrial function, could be partly responsible for fetal dystocia due to postural errors. With further reference to the importance of myometrial function, it is significant that when birth is premature cervical dilatation is often incomplete and fetal postural defects are then common; also retention of the afterbirth is likely. All these clinical effects are due to uterine inertia, which may result from a disordered sequence of preparturient endocrine events.

Second stage of labour

In the monotocous species this refers to the expulsion of the fetus; however, in polytocous species the fetal membranes are sometimes voided together with fetuses and hence this stage cannot be separated from the third stage.

The sign of the onset of second stage is the appearance of abdominal contractions. In the cow it has been shown that 8–10 of these are superimposed upon the onset of each myometrial contraction whose frequency at this stage is 24–48 per hour, so that one contraction is almost immediately followed by another (Gillette and Holm, 1963; Zerobin and Spörri, 1972). Similar observations were made in the ewe where the frequency of contractions increased to 40 per hour, with only very short periods of rest, and intrauterine pressure was increased to 30–40 mmHg with each contraction (Fitzpatrick and Dobson, 1979). In the cow, the disappearance of waves of cervicotubular contractions during second stage has been observed (Zerobin and Spörri, 1972).

In many species the superimposition of abdominal contractions upon myometrial contractions has been demonstrated; this is shown in an intrauterine pressure recording obtained from a sow (Figure 6.7). It should be remembered that these abdominal contractions which cause straining are not related directly to the release of oxytocin and should not be confused with Ferguson's reflex. The coordination between the two is due to the fact that the myometrial contractions force the fetus into the pelvic inlet, which activates the pelvic reflex and stimulates straining; this is a similar response to the one that stimulates defaecation. The straining forces the fetus against the cervix and anterior vagina, thus initiating Ferguson's reflex, so that the oxytocin which is released causes further contractions of the myometrium.

The allantochorionic sac ruptures, as a consequence of its backward movement being restricted by its placental attachments, and a gush of urine-like fluid escapes from the vulva. The distended amnion, together with parts of the fetus, is forced into the pelvic inlet, thus stimulating the pelvic reflex, which induces powerful contractions of the

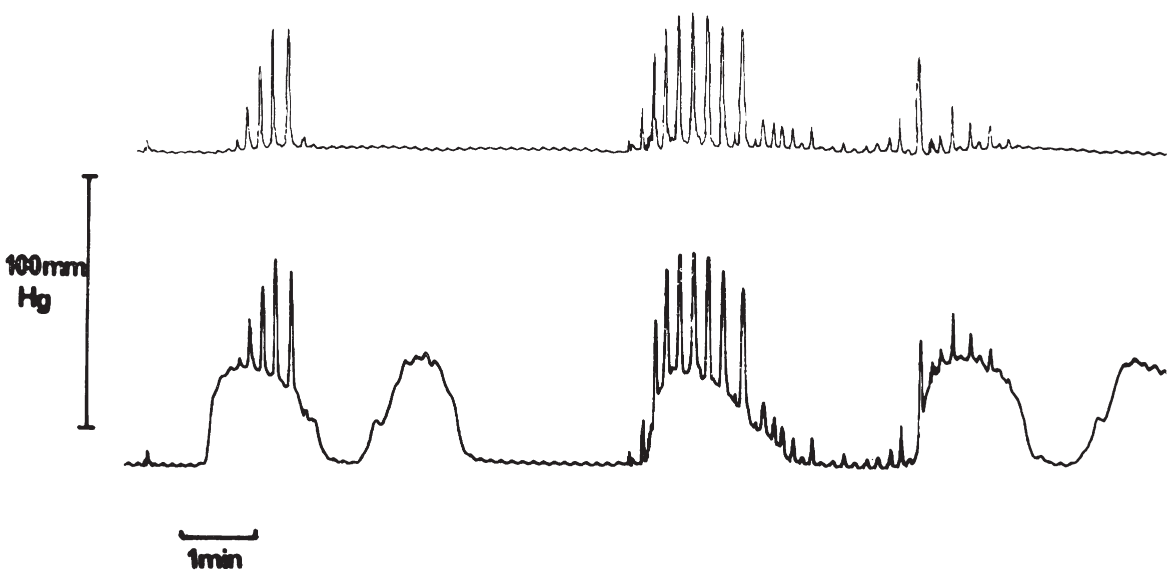


Fig. 6.7 Pressure changes at parturition in the sow. Upper trace is from a balloon-tipped catheter placed in the peritoneal cavity. Lower trace is from an intrauterine balloon-tipped catheter.

abdominal muscle. Similar, substantial straining occurs later when first the fetal shoulders and later the fetal hips engage the pelvis. These distensions of the maternal birth canal cause great increases in the release of oxytocin from the posterior pituitary and this, in turn, accentuates the myometrial contractions; thus there is a conjunction of uterine and abdominal expulsive efforts. As the intermittent straining continues, the amnion traverses the vagina and appears at the vulva as the 'water-bag'. With further straining fetal limbs appear in the water-bag; in the case of the monotocous species one limb slightly precedes the other. The amnion is progressively expelled and may, or may not, become ruptured by a fetal foot, with escape of some of the lubricant amniotic fluid. The fetal head next occupies the vulva, and at this time the contractions of the uterine and abdominal muscles reach a climax of expulsive effort, maximum effort coinciding with the birth of the fetal occiput. When the head is born the mother may rest for a while but soon a further bout of straining causes the fetal thorax to pass through the vulva. Usually, birth of the hips quickly follows and the hindlimbs may be expelled at the same time; in many cases in the monotocous species, however, no further expulsive effort occurs after the birth of the hips, the hindlimbs remaining in the vagina until they are freed either by movement of the young one or by the mother rising from the ground.

Attempts have been made to quantify the importance of the two components involved in the expulsion of the fetus. In the ewe the combined effect of the myometrial and abdominal contractions is about two-and-a-half times the effect of the uterus on its own (Ward, 1968; Hindson et al., 1965, 1968). Similar observations have been made in the cow (Gillette and Holm, 1963), although since the myometrium is involved alone during the first stage of labour it probably contributes to about 90% of the total work done in the expulsion of the calf. The abdominal effort is of high intensity at certain critical stages of delivery.

If the fetus is born in the amnion, or if the partially ruptured amnion covers the face of the offspring, fetal movement quickly causes it to be ruptured or drawn away; respirations, often accompanied by a cry, then begin. There seems to be very little risk of a healthy fetus being suffocated by an enveloping amnion. The stimulus to breathing is apparently the impact of air at the nostrils, for occasionally during the expulsive phase of equine and bovine births when the protruding face has become uncovered, breathing has begun some time before delivery was completed.

When the mother gives birth in lateral recumbency the offspring is often born with an intact umbilical cord, and some minutes may elapse before the cord is ruptured by movement of the young animal or mother. It is important to allow this to happen naturally, for artificial and premature rupture, or ligation, of the cord may deprive the newborn of a large volume of blood which would normally pass to it from the placenta. When rupture occurs, the two umbilical arteries and urachus retract towards, or into, the abdomen, and this prevents haemorrhage.

In physiological birth the fetus does not come in direct contact with the genital tract, for the surrounding amnion has served as a glistening and well-lubricated sheath through which the fetus has passed.

The second stage of labour is complete when all fetuses have been delivered; it lasts from an average of 17 minutes in the mare to an average of 4 hours in the sow. Its duration and the degree of effort associated with it are usually greater in primigravida than in multigravida.

In the mare, cow and ewe (when monotocous) the fetus is usually delivered in anterior presentation, dorsal position and extended posture (see Chapter 8), although a small proportion of normal deliveries may occur in posterior presentation, dorsal position and extended posture. In the polytocous bitch and sow up to 40–45% of fetuses may be normally delivered in posterior presentation.

During its passage from the uterus to the exterior, the fetus of the monotocous species follows an arched route. This tends to reduce the dorsoventral diameter of the fetal pelvis and also tends to keep the fetal pelvis high in the birth canal where the maternal bisiliac diameter is widest.

Third stage of labour

After birth of the young, regular abdominal contractions largely cease. Although a temporary lull has been recorded in the cow (Gillette and Holm, 1963), myometrial contractions persist; in general, they decrease in amplitude but become more frequent and less regular. These contractions are important for dehiscence and expulsion of the fetal membranes. Not only do the waves of contractions passing from uterine tube to cervix persist, but in both the cow and sow there is the reappearance of contractions in the reverse direction (Zerobin and Spörri, 1972; Ngiam, 1977); the former authors noted their return in the cow within 10 minutes of the expulsion of the calf. Taverne et al. (1976) reported in the sow the presence of regular contractions of a frequency of 15–27 per hour, which frequently progressed in a peristaltic fashion over the entire length of the uterus.

During the last 5 days of gestation, maturational changes occur in the placenta; these are likely to be related to the changes in the endocrine environment that trigger parturition (see pp. 156–161). Grunert (1984) has identified collagenisation of the placentome and flattening of the maternal crypt epithelium in the cow. In the same species he also observed significant cellular changes, such as leucocyte migration and increased activity, and a reduction in the number of binucleate cells in the trophectoderm. However, these changes may occur as a result of the maturational changes rather than causing them.

A weakening of the acellular layer of adhesive protein, the 'so-called' glue line that has been demonstrated in the cow between the cotyledonary and caruncular epithelium, is probably important in ensuring placental separation (Bjorkmann and Sollen, 1960).

The effect of the contractions is to open up the endometrial crypts, which in the case of those species with cotyledons resemble the openings of a succession of fans. The fetal villi have shrunk, owing mainly to the sudden loss of turgidity related to the escape of blood from the fetal side of the placenta when the umbilical cord ruptures. Exsanguination of the placenta is also aided by the squeezing effect of myometrial contractions. These actions, together with some of the early degenerative or maturational changes which are seen in the caruncles of the ewe and cow, cause separation of the fetal membrane. As a result, the apex of the allantochorionic sac becomes inverted and as the sac is 'rolled' down the cornua the fetal villi are drawn out of the crypts. When a large portion of the afterbirth becomes detached and inverted it forms a mass within the maternal pelvis which stimulates reflex contractions of the abdominal muscles; this straining completes the expulsion of the allantochorionic sac, which is seen to have its smooth, shining allantoic surface outermost. In the polytocous species, the dehiscence and expulsion of the fetal membranes are interspersed with the fetal births; but only the expulsion of the last afterbirth simulates the third stage of the monotocous species. The third stage lasts from an average of 1 hour in the mare to 6 hours in the cow. With the exception of the mare, domestic animals normally eat the afterbirth.

The nature of the third-stage uterine contractions can be easily appreciated with the exercise of a little patience by direct palpation of the cow's uterus per rectum. At intervals of a few minutes, profound waves of contraction are generated, during which the texture of the uterus is transformed from a flaccid state into a condition of intense tone.

With the exception of the sow, the females of the other domestic species indulge in intensive licking of the newborn offspring. Within an hour of birth it is normal for the young of all species to be suckling, and it is known that the stimulus of suckling causes release of oxytocin, which promotes the 'letdown' of milk as well as an augmentation of myometrial contractions. This has been clearly demonstrated in the sow, where suckling resulted in greater synchrony of the contractions and an increase in the number of tubocervical contractions (Ngiam, 1979). Hence suckling exerts a favourable influence on expulsion of the afterbirth. In the mare, the resumption of substantial contractions of the uterine musculature in the third stage causes abdominal pain, and it is quite common for expulsion of the membranes to be preceded by mild symptoms of colic.

CARE OF PARTURIENT ANIMALS

Mares approaching term should be put in a handy paddock during the day and brought in at night. As soon as the udder and teats become distended, or waxing occurs, the mare should be put in a foaling box at night and kept under continuous but unobtrusive observation. The majority of mares foal between 18.00 hours and midnight, so that by daybreak in natural conditions the foal has suckled and can gallop away. It is still uncertain to what extent foaling may be delayed as a result of some (to the mare) untoward environmental influence. Where continuous vigil is kept by relays of students who, being curious, may be rather obtrusive, the mare seems as likely to foal by day as during the night. The cervix does not apparently require the same degree of preparatory dilatation as in the cow. The author has seen a mare foal with ease immediately after a gynaecological examination at which the cervix seemed relaxed but not at all dilated.

If the presentation is seen to be normal, i.e. two feet and muzzle at the vulva, then the mare is almost certain to deliver the foal; an exception is 'dog-sitting position' where, with forelimbs and head showing, the presentation *looks* normal. As soon as an irregular presentation, position or posture is recognised, or if no progress occurs within 10 minutes of the onset of straining, a veterinary examination should be called for (see p. 309). In these ideal circumstances the obstetrician will have little more than an hour in which to arrive and deliver a live foal – often an impossibility because he or she cannot be located or cannot cover the distance in time. The early dehiscence of the allantochorion in equine dystocia makes stillbirth the rule rather than the exception. Even if the foal is dead on arrival, however, the veterinary surgeon's prompt attention will make a much more favourable prognosis for the mare.

As soon as a cow shows complete relaxation of the posterior border of the sacrosciatic ligament she should be put in a clean, well-bedded box and kept under frequent observation. If after 12 hours of restlessness there is no straining, a veterinary examination should be made to exclude primary uterine inertia, failure of the cervix to dilate and uterine torsion. If a cow comes into a normal second stage and there is no progress after an hour's straining she should be examined to ascertain the cause of the obstructive birth.

Heavily pregnant ewes should be kept in a handy paddock or in a lambing yard or pen whose location can be frequently changed during the lambing season.

Sows should be well washed and introduced to a farrowing crate several days before the expected farrowing. The majority of sows farrow at night, and there is a substantial loss of piglets due to overlying by the sow; in fact, more than half the piglet deaths up to weaning occur within 48 hours of farrowing. By using a $PGF_{2\alpha}$ analogue to control the time of farrowing, Hammond and Matty (1980) were able to reduce both the number of stillbirths and the mortality due to crushing by the sow and thus significantly increase the number of piglets weaned (at 3 weeks).

It is clear that the mother, by reacting to environmental influences, can exert some control over the time of parturition. For example, most mares and sows, and probably other domestic species kept in more natural conditions, produce their offspring at night in quiet, undisturbed surroundings. However, constant obtrusive interference can override this natural tendency. For example, in five beagle bitches it was observed that electromyographic activity of the uterus during late gestation was influenced significantly by external stimuli (Van der Weyden et al., 1989). Parturient bitches which are transferred for whelping to a strange environment may suffer from nervous voluntary inhibition of labour. The maternal stress occasioned by the adverse surroundings is considered to inhibit the release of oxytocin, or the resultant adrenaline secretion stimulates the β receptors of the myometrium causing relaxation.

Studies of 1151 calvings of beef cattle in Canada (Yarney et al., 1979) and of 522 parturitions in Friesian cows in Britain (Edwards, 1979) showed that the distribution of calvings was fairly uniform throughout the 24-hour period, but that the disturbances caused by farm staff at feeding and milking times exerted significant inhibiting effects, particularly in milking cows of third and later parities. There was also evidence from the beef cattle study and from data on lambing times collected by George (1969) that there is a genetic effect on the time of parturition; thus of the several beef breeds studied, 55.9–59% of cows with a Hereford grandparent calved during the day (between 07.00 and 19.00 hours), and under uniform husbandry more

Merino ewes lambed at night and more Dorset Horn ewes during the day.

Mare

The imminence of labour can be recognised by the degree of mammary hypertrophy, waxing of the teats and possibly the escape of milk from the glands (Figure 6.8).

The best indication that the first stage has begun is the onset of patchy sweating behind the elbows and about the flanks. Although it occurs in the majority of mares, it is by no means invariable. It commences about 4 hours before the birth of the foal and increases as the stage progresses.

Initially the mare yawns, there are no obvious indications of pain and food is generally taken readily. Respirations are normal and the pulse is about 60. (This increase in pulse rate is not significant of the onset of labour, for it develops during the terminal stages of pregnancy.) There is evidence that body temperature may become slightly subnormal during the first stage (36.5–37°C).

Fig. 6.8 Mammary hypertrophy, tumefaction of the teats and waxing in a thoroughbred mare 4 hours before the birth of the foal.

As the stage advances, the mare becomes restless and tends to wander aimlessly around the loose-box. The tail is frequently raised or held to one side. There may be swishing of the tail or slapping of it against the anus and kicking at the abdomen. As the end of the stage approaches the mare becomes very restless. This is indicated by crouching, straddling of the hindlimbs, going down on the knees or sternum and rising again, glancing at the flank. The stage terminates with rupture of the allantochorionic membrane and the escape of urine-like, allantoic fluid from the vulva. Its quantity is not copious. It will be noticed that there is no reference to visible straining during this period in the mare.

The onset of the second stage occurs abruptly. It is characterised by the appearance of the amnion or the commencement of forcible straining. There is never very much delay between them, and they often coincide. Very soon after straining begins, the mare goes down. She passes on to her side with limbs extended, and generally remains in this position until the foal is born. The presence of the transparent bluish-white water-bag (amnion) at the vulva is quickly followed by the appearance in it of a digit. Straining efforts recur at fairly regular intervals; each bout comprises three or four powerful expulsive efforts followed by a period of rest, generally of about 3 minutes. One forelimb precedes the other by a distance of about 7–8 cm and this position is maintained until the head is born. The point is a significant one, for it indicates that one elbow passes through the bony pelvic inlet before the other and in this way nature has provided for the foal to present the minimum obstruction at the pelvic inlet. During its delivery, the head is generally in the oblique position; it may even be transverse – the cheek lying on the limbs – but this is probably due to rotation of the cervical joints within the pelvis and should not be taken as evidence that the presentation was oblique. (The second stage is illustrated in Figure 6.9.)

The greatest and longest effort is associated with birth of the head; the chest presents less difficulty, and following this the hips slip out easily. Although equine delivery is comparatively rapid it constitutes a tremendous effort, and after expulsion of the foal the mare may remain

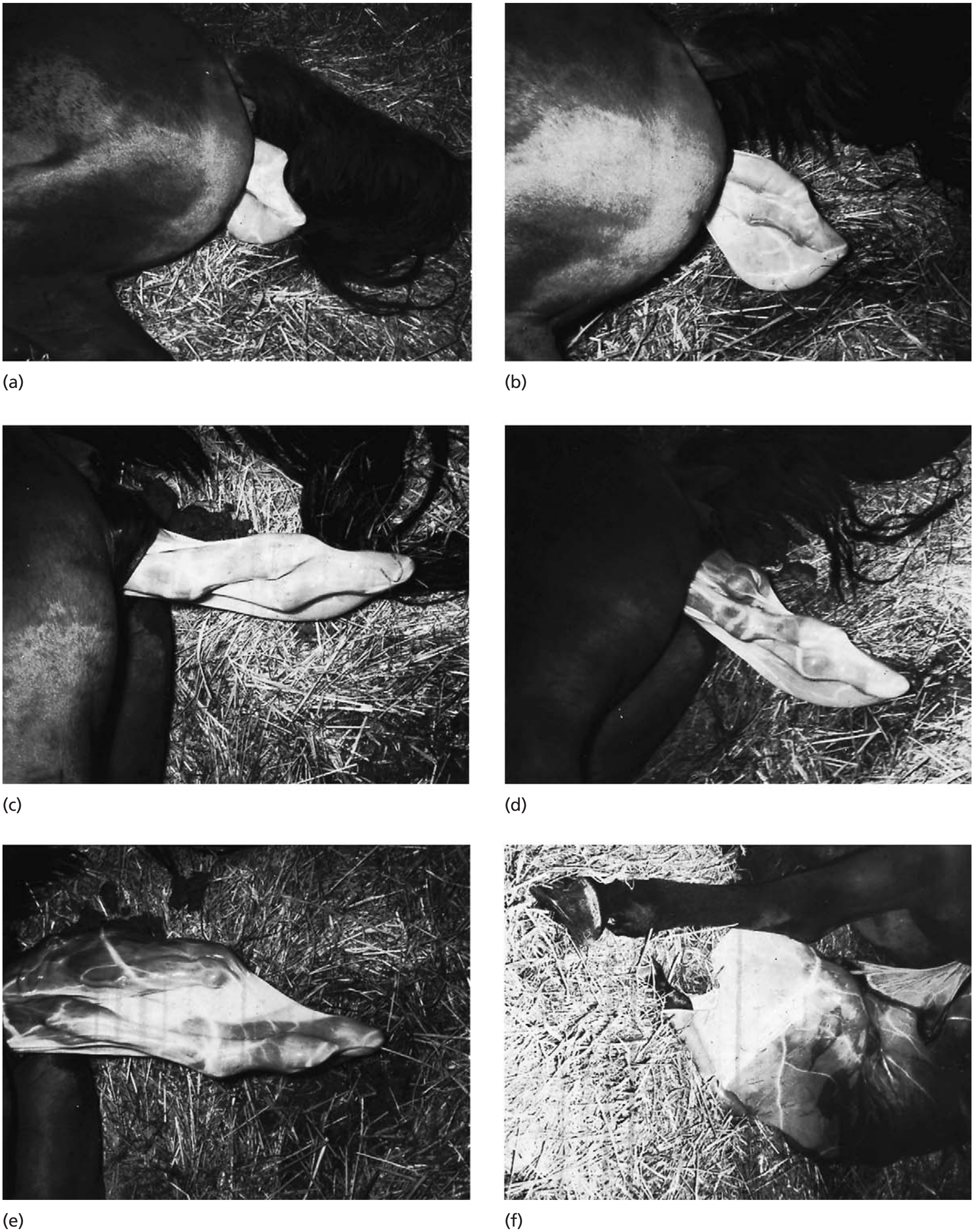


Fig. 6.9 The second stage of labour in the mare. Note the relative positions of the forelimbs and head. The foal is born in the amnion but breaks out of the sac without difficulty. In the final photograph much of the allantochorion has been passed but the umbilical cord is still intact.

Figure continued overleaf

(g)

(h)

(i)

(j)

(k)

(l)

Fig. 6.9 *continued.*

lying on her side exhausted for anything up to 30 minutes.

The umbilical cord is intact when the foal is born. It subsequently ruptures, 5–8 cm beneath the belly, as the result of movement by either the mare or the foal. Usually also the foal is born within the amnion, the membrane being ruptured by the movements of the foreparts of the fetus. Respiratory movements may be seen within the intact amnion.

The lower portions of the foal's hindlimbs often remain within the mare's vagina for some minutes after the rest of the foal is born. Their final emergence is due to movement of the foal rather than to the expulsive efforts of the mare.

The duration of the second stage in the mare is about 17 minutes; it may be as short as 10 minutes. The longest the writer has observed within normality was 70 minutes, and in this case it was seen that the greater part of the placenta came away with the fetus. It is probable that this is near the limit of the time available if parturition is to remain normal, for in the mare separation of the placenta tends to proceed rapidly once the second stage begins, and if delivery occupies too long a time it is likely the fetus will succumb from this cause.

In the majority of mares the membranes are expelled quickly after the birth of the foal, generally within 3 hours; in fact, they may fall away in about 30 minutes. The average duration of the third stage is about 1 hour. Occasionally, however, cases are met in which periods up to 24 hours elapse before the membranes fall away, yet the animal suffers no ill-effect. Straining is not a feature of the third stage, the afterbirth being expelled by the myometrial contractions. The recognition of the exact time at which a case becomes pathological and interference is necessary is a difficult problem. As has already been pointed out, the membranes are passed with the allantoic surface (smooth and shiny) of the allantochorion outermost. This statement holds for those cases in which expulsion occurs early after parturition, but it has been noticed that in those in which there is delay, the placental surface (roughened and red) is outermost, indicating that separation was complete before expulsion began.

Cow

The immediate approach of labour has been recognised by slackening of the pelvic ligaments and the change of the mammary secretion from a relatively transparent, honey-like secretion to an opaque cellular secretion – colostrum. About 54 hours before birth of the calf, Ewbank (1963) noticed a fall in the cow's body temperature of 0.6°C. He observed that a cow showing signs of imminent labour would be unlikely to calve during the succeeding 12 hours if its temperature was 39°C or more. Parturition will usually begin within 12 hours of the appreciation of complete relaxation of the posterior borders of the sacrosciatic ligaments. The first stage of labour can easily be recognised by direct palpation of the cervix.

There is great variation in the intensity of the symptoms of the first stage; in fact, many subjects, particularly multigravida, show none. Others, usually heifers, may show signs of abdominal pain for periods up to 24 hours before the cervix is completely dilated. The first stage usually lasts about 6 hours. Another feature of the cow is that occasional straining may occur during the first stage. Food is only 'picked'; rumination is irregular; there may be 'lowing' or kicking at the belly. The animal is obviously restless; she may stand with her back arched and tail raised; she may go down and rise again frequently. The line of demarcation between the first and second stages is not clear-cut, as in the mare. Body temperature is generally normal, but the pulse rate is often increased to between 80 and 90.

In 40% of normal calvings observed by the author the intact allantochorion reached the vulva as a 'first water-bag'.

The second stage is less intense but of longer duration than in the mare. Straining is less frequent and the animal often remains standing at first (Figure 6.10). During the passage of the head through the vulva, however, the cow generally goes down and remains recumbent until the calf is born. She may lie on her side, but more often adopts sternal recumbency. Taking the appearance of the water-bag as the time of its onset, the second stage may occupy from 30 minutes to 4 hours, the average duration being about 70 minutes. The second stage is longer in heifers than cows, and

(a) (b) (c) (d) (e)

Fig. 6.10 The second stage of labour in the cow. Note the relative positions of the fetal head and forelimbs. The allantoic and amniotic sacs can be clearly distinguished in (c).

male calves take longer to be born. In twin births, Owens et al. (1984) noted that intense straining for the birth of the second calf began 10 minutes after the delivery of the first calf. During the second stage, temperature may rise to 39.5 or 40°C, but this is by no means constant and is probably dependent on the degree of effort required. The pulse rate may increase to 100 or more. About 20% of calves are born almost completely enclosed in the amnion.

Placental separation occurs more slowly in the cow than in the mare and thus the stage of expulsion may occupy considerably longer without jeopardising the life of the young one. The process of expulsion is similar to that described for the mare. The umbilical cord is shorter in the calf than in the foal, and its rupture generally occurs as it falls from the vulva.

Expulsion of the fetal membranes usually takes place about 6 hours later; occasionally it may be delayed to 12 hours, but when 24 hours elapse and the membranes are still in the uterus it is probable that the cause is pathological retention. Unless prevented from doing so, it is customary for the cow to eat the fetal membranes. It will also be noticed during the first and second stages that there is a tendency to lick up vulval discharges.

Bitch

The imminence of parturition has been indicated by the animal preparing her bed. In primigravida the onset of lactation coincides with parturition, but in multigravida milk may be expressed from the teats for several days prior to its onset. There is a transient drop in body temperature of at least 1.2°C within the 24 hours before the onset of labour.

There is nothing characteristic about the first stage, but it is generally noticed that the bitch is restless, indifferent to food and inclined to pant. It is most obvious in primigravida and occupies about 12 hours.

Electromyographic (EMG) pattern during late pregnancy comprises episodes of myoelectrical activity (EMEAs) lasting 3–10 minutes and recurring at a low frequency (maximum 2.5/hour). During the last 7 days before whelping and especially during the last 48 hours, more short bursts (<3 minutes) appeared between the EMEAs; this was closely correlated with the decline in progesterone concentrations. The total duration of EMG and the burst frequency increased dramatically as the progesterone concentration and body temperature fell 12–24 hours before whelping (Van der Weyden et al., 1989).

The onset of the second stage is indicated by straining. In the majority of cases the animal remains in her bed in sternal recumbency, although sometimes she may stand and move about during straining efforts. The water-bag of the first fetus appears at the vulva, and following a series of efforts attains the size of a golf-ball. It is generally ruptured by the bitch who licks vigorously at her vulva. As with other species, delivery of the head coincides with the greatest effort; in the majority of instances, once this is born the remainder of the fetus follows easily. Expulsion of the first fetus may occupy up to an hour, but seldom longer if the process is normal. It is often quicker, a matter of a quarter of an hour or so. About 40% of puppies are born in posterior presentation.

The umbilical cord is intact at the birth of the puppy, but it is quickly torn by the mother, who bites it away.

As a rule the bitch rests for a time after the birth of her first puppy. She lies licking her young one, which soon begins to suckle. She pays frequent attention to her vulva and licks up any discharges. The fetal membranes are generally voided in 10–15 minutes and are promptly eaten by the bitch.

Straining recommences after a variable delay. This delay may be 30 minutes only; it may comprise 1–2 hours. (The writer has seen it occupy 7 hours in a bitch pregnant with two fetuses only.) The effort required and time occupied for the delivery of the second fetus is usually less than for the first. This may be followed by a further period of rest, but quite frequently a third puppy quickly follows the second.

The stage of expulsion of the fetuses is most irregular; one bitch may have her first puppy and then rest for several hours, then deliver two or three more in quick succession and then rest again before expelling several more; another may expel them at fairly regular intervals throughout the period. There is no rule. In an exceptional case, a bitch may deliver the whole of her litter in an hour or so. Unlike the sow (see later), there is a tendency for the puppies to be expelled from alternate horns. In addition, reversal of the presentation or 'leapfrogging' of puppies is very uncommon (Van der Weyden et al., 1989).

Expulsion of the fetal membranes is also irregular. They may come individually. In other instances a puppy may be born with the membranes of its predecessor around its neck.

The total time occupied by the second stage will depend chiefly on the number of fetuses, but as a general rule when the litter is within the usual limits (four to eight) it occupies about 6 hours. The question arises of what is the maximum time it may occupy, especially when the number of fetuses is very high (10–14). The writer would put it at 12 hours at the outside. It is very improbable that puppies born after this time, even without assistance, will be alive.

The fetal membranes of the last fetus are generally expelled with it or shortly afterwards. Exceptionally, however, there is a delay of up to 24 hours before parturition is finally completed.

A feature of parturition in the bitch is that much of the uterine discharge is dark green in colour. This is due to the breakdown of the marginal haematoma ('green border') and to the escape of the blood pigment, biliverdin or uteroverdin.

Cat

During the last week of pregnancy, the queen will seek out a suitable nesting area for kittening. Most cats are secretive about kittening and will select a quiet, undisturbed spot, whilst some socialised pet cats show less interest in selecting a suitable nesting position and become more demanding of human contact.

Mammary development becomes noticeable in the last week of pregnancy, and this may be particularly prominent in maiden queens. Rectal temperature may fall a day or two before parturition, but this is not a consistent feature.

During the first stage of parturition the queen may become restless, frequently visiting the site selected for kittening, whilst other queens hide away quietly in the chosen nesting area, occasionally lying down and straining unproductively. The second stage begins with straining in lateral recumbency. Expulsion of the kittens is usually rapid, with only a short interval between each birth, and parturition is usually completed within a few hours. However, in other cases, the pattern of fetal expulsion may be much more variable and, on some occasions, part of the litter may be born one day and the remainder 24 hours or more later. If the queen is alarmed, this may disrupt the pattern of births and she may move the kittens already born to a new nesting area before resuming parturition. The placentae are usually expelled still attached to the fetuses or shortly afterwards and are quickly consumed by the queen. Breakdown of the marginal haematoma releases the pigment which gives a brown coloration to the parturient discharge. Soon after birth, the kittens will seek out the nipples and begin to suckle.

Ewe

In the ewe the course of parturition is very similar to that described for the cow, except that the incidence of twinning and even triplets is high in those ewes which have 'been done well' previous to mating time or in breeds with high fecundity. Wallace (1949) found that 72% of ewes complete second-stage labour in 1 hour and that the majority of ewes pass the afterbirth within 2 or 3 hours of the expulsion of the lamb. Ninety-five per cent of lambs were presented anteriorly. Spontaneous birth may occur despite retention of a forelimb. In their observations of ovine parturition. Hindson and Schofield (1969) noted that in twin births where one fetus occupied each horn, one horn developed contractility before the other. This observation supports the author's contention that dystocia in cattle and sheep due to simultaneous presentation of twins is more likely when both fetuses occupy the same horn.

Sow

From 60 to 75% of sows farrow at night (Bichard et al., 1976; Kovenic and Avakumovic, 1978). The fetal membranes of adjacent piglets are usually fused and because individual or aggregated afterbirths may be expelled during the phase of fetal expulsion as well as after the birth of the last fetus, it is unrealistic to speak of separate second and third stages of labour in porcine species. Good accounts of farrowing have been given by Jones (1966) and by Randall (1972).

Sows in late pregnancy are mostly asleep in lateral recumbency but within 24 hours before the birth of the first piglet a marked restlessness develops – apparently caused by the discomfort of the first-stage labour pains – accompanied by bed-making activity. The intensely active period is followed by recumbency and rest but, after a variable pause, clawing and champing of the bedding is resumed. There are several alternating periods of rest and bed-making and then, in the hour preceding the birth of the first piglet, the sow settles quietly into lateral recumbency.

Conspicuous mammary growth is a feature of late gestation; 1–2 days before farrowing the individual glands are clearly demarcated, turgid, tense and warm, and milk can be expressed from the prominent teats during the final 24 hours.

There is progressive swelling of the vulval labia from about 4 days before parturition and the mucosa becomes reddened.

Prepartum temperature variations of between 37.5 and 38.5°C, but with no constant temperature change, have previously been reported, but Elmore et al. (1979) recorded a 1°C rise at 12–15 hours before the birth of the first piglet.

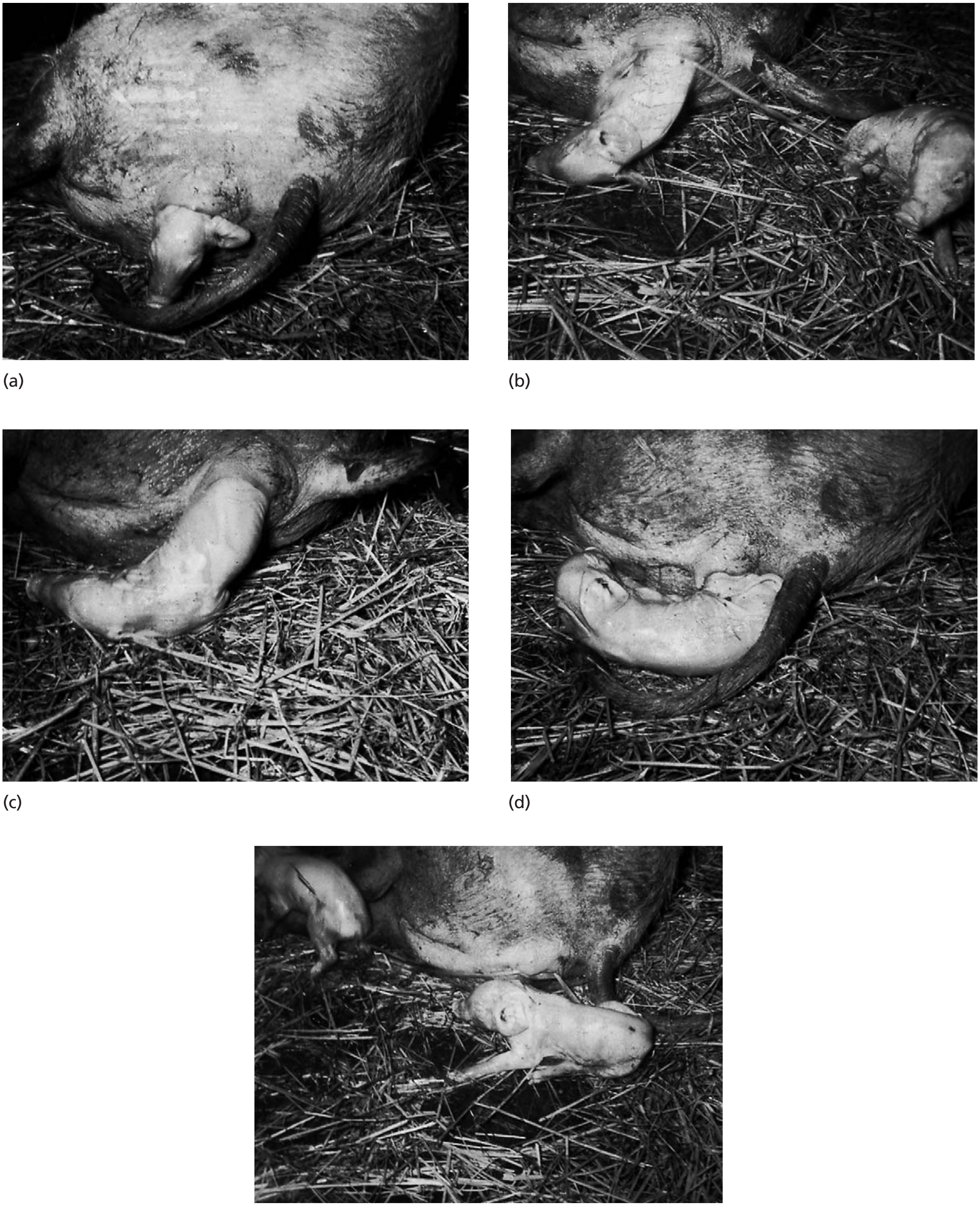
Parturient sows usually remain in lateral recumbency but gilts more particularly may get up after the birth of the first or second piglet, or change from one side to the other, or from lateral to ventral recumbency. After the prepartum quiet period there is intermittent straining accompanied by paddling leg movements. The birth of the first pig (and subsequent ones) is heralded by the passing of a small quantity of fetal fluid and by marked tail-switching. The greatest parturient effort is expended over the first piglet, succeeding fetuses being expelled with surprising ease and sometimes with projectile force. The allantochorion and amnion usually rupture as the conceptus traverses the birth canal but occasionally piglets are born within the amnion, and not uncommonly, a fetus becomes surrounded by the membranes of another fetus. Only small amounts of fetal fluid are voided.

The expulsive phase of parturition is illustrated in sequence by Figure 6.11. By means of laparotomy and transuterine marking of the uterine location of fetuses of the miniature pig between 80 and 105 days of gestation and then observing their birth sequence, Taverne et al. (1977) found that the offspring were delivered randomly from both uterine horns. As had previously been observed by Perry (1954), there were occasional instances of a piglet apparently overtaking its neighbour in the uterus. In 18 of 95 piglets the observed presentation at birth differed from that previously detected at laparotomy but it was not determined whether these changes of polarity occurred in the uterus during gestation or within the uterine body during delivery. Both explanations would seem valid, although Taverne (1981) has postulated that the polarity change is most likely to occur because a piglet passes down from one horn into the base of the other and is then expelled into the uterine body and then the outside.

Observing the birth of 1078 piglets in 103 litters Randall (1972) recorded 55.4% anterior and 44.6% posterior presentations. In both presentations the fetus was usually in dorsal position with the presenting limbs flexed alongside the fetal body; thus the fetal snout or tail was the first part to protrude. The mean interval between consecutive births was 16 minutes and the mean duration of the expulsive stage was 2 hours 36 minutes. Between 60 and 70% of piglets were born with intact umbilical cords; in the case of the early-born fetuses the elasticity of the cord allowed the newborn piglet to reach the middle of the sow's abdomen without the cord breaking. The later-born piglets were more likely to be expelled with broken cords. Newborn piglets were remarkably active and within 2 minutes had reached a teat and attempted to suck.

The porcine fetal membranes tend to be expelled as two or three masses of joined allantochorions with the placental stalks of the umbilical cords indicating the number of separate conceptuses in each mass; single afterbirths may also be voided. One or more of the coalesced masses are commonly passed before all the fetuses are born, but the largest mass is usually passed about 4 hours after the last piglet.

When all the piglets have been expelled, the sow usually stands up and micturates profusely. She then lies down again, sometimes very clumsily – with consequent risk of crushing the surrounding piglets – and the feature of the next phase is that the sow lies quietly for a long time and allows the piglets to suck.



(a) (b) (c) (d) (e)

Fig. 6.11 The second stage of labour in the sow. Note the lack of extension of the forelimbs. The intact umbilical cord can be seen in (e).

A notable economic feature of porcine parturition is the frequency of stillbirths. They occur in about 30% of apparently normal farrowings and the overall stillbirth percentage in unassisted deliveries is 3–6% (Randall and Penny, 1970).

Because piglets are nearly always found alive when premeditated hysterectomies or hysterotomies are performed just before the onset of parturition, it is concluded that stillbirths occur during farrowing. Early-born piglets are more likely to survive than either the middle-born ones, which are of smaller size, or the late-born ones from the tips of the uterine horns (Dzuik and Harmon, 1969; Sprecher et al., 1974; Leman et al., 1979). The stillbirth rate is also influenced by litter size, being greatest in litters of four or less or in those of 14 or more, and by the polarity of the expelled fetus (Sovjanski et al., 1972). Piglets at the ovarian ends of the uterine horns have to traverse the entire length of the respective horn and in the case of small litters they may have to negotiate a previously unoccupied and non-dilated length of horn. Piglets born posteriorly are nearly four times as likely to be born dead as those presented anteriorly. Also, extension of the intervals between successive births up to 20 minutes or more predisposes to stillbirth.

High progesterone and/or low oestrogen levels, produced experimentally in the preparturient sow's blood, can delay farrowing and increase the stillbirth rate from 10 to 97% (Wilson et al., 1979); the authors suggest that such hormonal derangements may occur naturally.

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7 The puerperium and the care of the newborn

THE PUERPERIUM

The puerperium is that period after the completion of parturition, including the third stage of labour, when the genital system is returning to its normal non-pregnant state. In the polyoestrous species (the cow, mare and sow) it is important that there should be a normal puerperium since it is the practice under most systems of husbandry to breed from individuals of these species fairly soon after they have given birth. Thus any extension of the puerperium may have a detrimental effect on the reproductive performance of the individual animal concerned. The genital system does not completely return to the original pregravid state since, particularly after the first gestation, certain changes are not completely reversible.

There are four main areas of activity:

- The tubular genital tract, especially the uterus, is shrinking and atrophying due to tissue loss, thus reversing the hypertrophy that occurs in response to the stimulus of pregnancy. Myometrial contractions, which continue for several days after parturition, aid this process and help in the voiding of fluids and tissue debris; this is normally referred to as involution.
- The structure of the endometrium and deeper layers of the uterine wall is restored.
- There is a resumption of ovarian function in polyoestrous species and a return to cyclical activity.
- Bacterial contamination of the uterine lumen is eliminated.

Cow

Although the stimulus for the changes that occur during the puerperium is primarily due to the removal of the fetus, hormones such as oxytocin and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) are also probably involved. In the case of the latter, however, there is an increase after the end of parturition, in which peak values occur 3 days postpartum and do not return to basal levels until 15 days postpartum (Edquist et al., 1978, 1980). The puerperium has been studied in detail by Rasbech (1950), Gier and Marion (1968) and Morrow et al. (1969).

Involution

The reduction in the size of the genital tract is called involution; it occurs in a decreasing logarithmic scale, the greatest change occurring during the first few days after calving. Uterine contractions continue for several days, although decreasing in regularity, frequency, amplitude and duration. The atrophy of the myofibrils is shown by their reduction in size from 750 to 400 μm on the first day to less than 200 μm over the next few days.

Gier and Marion (1968) found that the diameter of the previously gravid horn was halved by 5 days and its length halved by 15 days. The results of their study are summarised in Figure 7.1 and show that after the initial rapid phase of involution the subsequent changes proceed more slowly. Others (Morrow et al., 1969) recorded a reduction in the rate of involution between 4 and 9 days postpartum, with a period of accelerated change from days 10 to 14 and a gradual decrease thereafter. Associated with this phase of rapid involution is uterine discharge. The whole of the uterus is usually palpable per rectum by 8 and 10 days postpartum in primipara and pluripara, respectively. The speed of involution of the non-gravid horn is more variable than that of the previously gravid horn, which depends upon its degree of involvement in placentation.

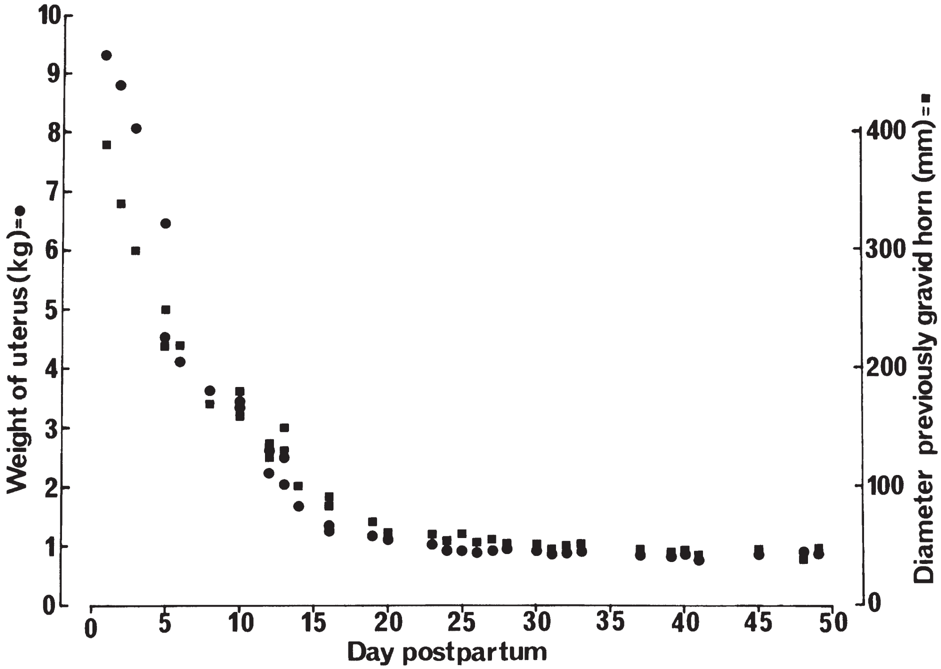

Fig. 7.1 Gross changes in the uterus of the cow during the puerperium. (Drawn from the data of Gier and Marion, 1968).

There is some dispute about when uterine involution is complete; the differences are probably only subjective. In six studies reported in dairy cattle the time taken for complete involution ranged from 26.0 to 52.0 days, whilst in three studies in beef cattle it was 37.7–56.0 days. The changes after 20–25 days are generally almost imperceptible.

The cervix constricts rapidly postpartum; within 10–12 hours of a normal calving it becomes almost impossible to insert a hand through it into the uterus, and by 96 hours it will admit just two fingers. The cervix also undergoes atrophy and shrinkage due to the elimination of fluid and the reduction in collagen and smooth muscle. Gier and Marion (1968) found that the mean external diameter was 15 cm at 2 days postpartum, 9–11 cm at 10 days, 7–8 cm at 30 days and 5–6 cm at 60 days. A useful guide that involution is occurring normally is to compare the diameter of the previously gravid horn with that of the cervix, since at about 25 days postpartum the latter starts to exceed the former.

Prostaglandins may have a role in controlling uterine involution, although the postpartum rise in the metabolite of $PGF_{2\alpha}$ may be a reflection of the process of involution rather than the cause. Eley et al. (1981) have shown a positive correlation between PGFM concentrations in the peripheral circulation and the diameter of the uterine horn. Using exogenous $PGF_{2\alpha}$ twice daily for 10 days starting from 3 days postpartum, uterine involution has been accelerated by 6–13 days; however, the number of animals was small and the frequency and duration of the treatment regimen were very atypical of the normal situation (Kindahl et al., 1982). Since the increase in the uterine mass during pregnancy is due to a combination of increases in both collagen and smooth muscle, then involution must be associated with a reduction of these tissues. This has been shown very clearly in the study of Kaidi et al (1991) in relation to collagen degradation, but in relation to the loss of smooth muscle the results were equivocal (Tian and Noakes, 1991a). The studies of the latter authors also showed that exogenous hormones such as oestrogens, $PGF_{2\alpha}$, and long-acting oxytocin analogues do not influence the rate of involution (Tian and Noakes, 1991b).

Restoration of the endometrium

Although placentation in the cow is considered to be of a non-deciduous type it is well recognised that during the first 7–10 days after calving there is usually a noticeable loss of fluid and tissue debris. This is sometimes referred to by the herdsperson as the 'second cleansing' or 'secundus'. In human gynaecology the postpartum vaginal discharge is referred to as lochia. The presence of such a discharge in cows is normal, although sometimes individuals will mistake it for an abnormal discharge due to uterine infection and request treatment.

The lochial discharge is usually yellowish brown or reddish brown; the volume voided varies greatly from individual to individual. Pluripara can void up to a total of 2000 ml, although it is more usually about 1000 ml. In primipara it is rarely more than 500 ml and in some animals it is occasionally nil, owing to the complete absorption of the lochia. The greatest flow of lochia occurs during the first 2–3 days; by 8 days it is reduced, and by 14–18 days postpartum it has virtually disappeared. At about 9 days it is frequently blood-stained, whilst before it ceases it becomes lighter in colour and almost 'lymph-like'. Normal lochial discharge does not have an unpleasant odour.

The lochia are derived from the remains of fetal fluids, blood from the ruptured umbilical vessels and shreds of fetal membranes, but mainly from the sloughed surfaces of the uterine caruncles. The slough occurs following degenerative changes and necrosis of the superficial layers, first described by Rasbech (1950). The changes that occur are illustrated diagrammatically in Figure 7.2.

After the shedding of the allantochorion the caruncle is about 70 mm long, 35 mm wide and

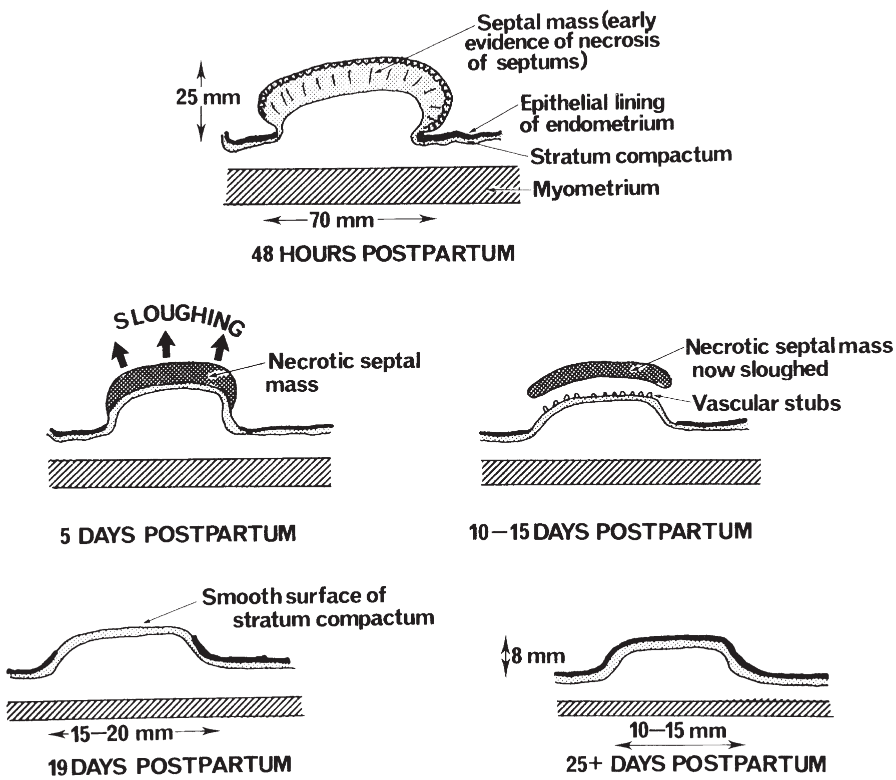

Fig. 7.2 The changes that occur in the caruncles of the cow during the puerperium. (Drawn from the data of Gier and Marion, 1968).

25 mm thick. The endometrial crypts frequently contain remnants of the chorionic villi which were detached from the rest of the allantochorion at the time of placental separation. Within the first 48 hours postpartum there is evidence of early necrotic changes in the septal mass of the caruncle; the caruncular blood vessels become rapidly constricted and are nearly occluded. At 5 days the necrosis has proceeded rapidly, so that the *stratum compactum* is now covered by a leucocyte-laden necrotic layer. Some of this necrotic material starts to slough and contributes to the lochia. Small blood vessels, mainly arterioles, then protrude from the surface of the caruncle, from which there is oozing of blood, causing a red coloration of the lochia. By 10 days, most of the necrotic caruncular tissue has sloughed and undergone some degree of liquefaction and by 15 days postpartum sloughing is complete, leaving only stubs of blood vessels protruding from the exposed *stratum compactum*. This eventually becomes smooth by 19 days, owing to the disappearance of the vessels.

Regeneration of the epithelium of the endometrium occurs immediately after parturition in those areas which were not seriously damaged and is complete in the intercaruncular areas by 8 days. Complete re-epithelialisation of the caruncle, which is largely derived from centripetal growth of cells from the surrounding uterine glands, is complete from 25 days onwards, although the stage at which complete healing occurs is variable.

Whilst these changes are taking place the caruncles are becoming smaller (Figure 7.2), so that at 40–60 days they consist of small protrusions 4–8 mm in diameter and 4–6 mm high. They also differ from those of nullipara because they are larger and have melanin pigmentation and a more vascular base.

Return of cyclical activity (ovarian rebound)

Anovulatory follicular waves occur periodically during pregnancy, with the emergence of follicles of up to a maximum of 6 mm in diameter. However, because of the prolonged period of inhibition during pregnancy, due to the continuous negative-feedback effect of progesterone secreted by the corpus luteum and placenta, the pituitary is refractory postpartum, as demonstrated by a lack of response to the administration of gonadotrophin-releasing hormone (GnRH) (Lamming et al., 1979). This eventually recovers with time. As a result of the absence or low output of gonadotrophins the ovary is relatively quiescent and the cow is in the anoestrous phase, which may be prolonged in suckler and high-yielding cows. However, during this postpartum phase the ovaries frequently contain numerous large anovulatory follicles which quickly become atretic; these are sometimes incorrectly diagnosed as cysts (see Chapter 22).

In the immediate postpartum period both oestradiol and progesterone are low. The anterior pituitary is capable of releasing FSH during the first few days postpartum (Schallenberger et al., 1982), so that with the sporadic release of endogenous GnRH there is a gradual and sustained rise in plasma FSH. After about 7–10 days, this is sufficient to result in the emergence of the first follicular wave; this occurs at about 4 days in dairy cattle, and 10 days in beef cattle. The ability of the pituitary to release luteinising hormone (LH) is much slower, for although the early release of GnRH causes some rise in LH, it quickly returns to basal levels. If a very large dose of endogenous GnRH is given within 10 days of calving there is no release of LH (Foster et al., 1980). If standard doses of GnRH are given at 10 and 16 days postpartum in milked cows, then LH rises; however, in autumn-calved suckler beef cows 20 days had to elapse and in spring-calved suckler beef cows 30 days had to elapse (Lamming et al., 1982). Further evidence of the refractory state of the hypothalamus and pituitary gland has been demonstrated by the failure of a 1 mg dose of oestradiol benzoate to elicit a surge of LH at 0–5 days postpartum; a response was obtained by 10 days which was increased by 25 days. A dominant follicle may emerge from the first follicular wave, but ovulation will occur only if the dominant follicle produces enough oestradiol to stimulate adequate LH secretion in the form of one pulse per hour; if this occurs, then there is a first ovulation at 21 days in dairy and 31 days in beef cattle (Adams, 1999). Insulin growth factor (IGF-1) is also involved in the early onset of folliculogenesis and ovulation, by stimulating follicular granulosa cell aromatase activity and oestradiol synthesis. After ovulation, there is a luteal phase which may be of normal length with a return to oestrus after 18–24 days, or it may be much shorter, 14 days or less; the latter occurred in 25% of dairy but in 78% of beef cattle (Adams, 1999). These short luteal phases probably arise because of inadequate preovulatory development of the follicle so that it either becomes luteinised in the absence of ovulation, or more likely luteinisation of the CL is inadequate. These short luteal phases are more prevalent the earlier the return of normal ovarian activity, i.e. 100% at 0–5 days, 60% at 10–15 days and 10% at 25–30 days postpartum (Terqui, et al., 1982).

However, it is now accepted that the first sign of oestrus is not always a true reflection of the onset of cyclical activity (Moller, 1970; King et al., 1976). This is because the CNS requires prior exposure to progesterone to elicit behavioural signs; a similar phenomenon occurs in ewes at the beginning of the breeding season (see Chapter 1). Using continuous time-lapse video recording of herds, 50, 94 and 100% of cows were identified in oestrus at the first, second and third postpartum ovulations (King et al., 1976); however, with daily observations the frequencies of detected oestrus were 16, 43 and 57%, respectively.

The availability of the milk progesterone assay has enabled the onset of cyclical activity to be determined by the presence of elevated progesterone concentrations. In a survey of 533 dairy cows in four herds (Bulman and Wood, 1980) nearly half (47.8%) of the cows had resumed normal cyclical ovarian activity within 20 days of calving and by 40 days this had increased to 92.4%. In this study only 4.9% appeared to have a delayed return to cyclical activity, i.e. had not returned by 50 days postpartum, and 5.1% of the cows subsequently ceased normal cyclical activity having initially returned. A small number, 1.9%, had prolonged luteal activity, presumably due to a persistent corpus luteum or luteal cyst (see pp. 431–445). These ovarian abnormalities depressed fertility, as measured by the calving to conception interval, which was 98 days for those with a delayed start to ovarian activity, 102 days for those with persistent luteal function, and 124 days for those cows where there was cessation of cyclical activity, compared with 85 days for normal cows.

Other endocrine organs are involved in controlling ovarian function postpartum (Peters and Lamming, 1990). The uterus exerts an influence since it has been known for some time that the majority of ovulations postpartum occur in the ovary contralateral to the previously gravid horn (Gier and Marion, 1968); the effect is less, the later ovulation occurs. It has also been shown that prostaglandin metabolite (PGFM) usually returns to normal levels before the first postpartum ovulation (Thatcher, 1986). Similarly, the ovario-uterine axis exerts an inhibitory effect on pituitary LH secretion during the early postpartum period; experimental hysterectomy results in a rapid increase in plasma gonadotrophin concentrations (Schallenberger et al., 1982).

The adrenal cortex plays an important role in influencing the return to oestrus postpartum. Adrenocorticotrophic hormone (ACTH) (Liptrap and McNally, 1976) and corticosteroid administration (da Rosa and Wagner, 1981) suppress the secretion of LH. Stimulation of the teat and milk removal cause a rise in glucocorticoids (Wagner and Oxenreider, 1972; Schams, 1976). Suckling, which is known to delay the return of cyclical ovarian activity, may exert its effect by modifying the tonic release of GnRH and LH by the release of opioid peptides. The role of prolactin is equivocal, for although bromocriptine treatment during lactation had little or no effect on LH release in cows, there appears to be a reciprocal relationship between the hypothalamic control of LH release and prolactin release. Opioid antagonists increase LH and decrease prolactin secretion; the effects of the agonists are the reverse. The mammary gland has also been shown to have an endocrine role (Peters and Lamming, 1990).

Elimination of bacterial contamination

At calving, and immediately postpartum, the vulva is relaxed and the cervix is dilated thus allowing bacteria to gain entry into the vagina, and thereafter the uterus. A wide range of bacteria may be isolated from the uterine lumen; Elliott et al. (1968) identified 33 different species, those most frequently isolated being Arcanobacterium (*Actinomyces Corynebacterium) pyogenes, Escherichia coli*, streptococci and staphylococci (Johanns et al., 1967; Elliott et al., 1968; Griffin et al., 1974). The last authors stressed that the flora fluctuated as a result of spontaneous contamination, clearance and recontamination during the first 7 weeks postpartum. In all studies there is a decrease with time in the percentage of uteri from which bacteria are isolated. This is exemplified in the study of Elliott et al. (1968) in which 93% of uteri examined within 15 days of calving were contaminated, compared with 78% between 16 and 30 days, 50% between 31 and 45 days, and only 9% between 46 and 60 days.

Blood, cell debris and sloughed caruncular tissue provide an ideal medium for bacterial growth; however, in most cases the bacteria do not colonise the uterus to produce a metritis/endometritis (see Chapter 22). The main mechanism involved in the elimination of the bacteria is phagocytosis by migrating leucocytes; however, persistence of uterine contractions, sloughing of caruncular tissue and uterine secretions all assist in the physical expulsion of the bacteria. Early return to cyclical activity is probably important since the oestrogen-dominated uterus is more resistant to infection. However, there is evidence that in some cases early return to oestrus may be disadvantageous (Olson et al., 1984; and see Chapter 22), in that if the bacteria are not eliminated at the first oestrus then the cow enters the first luteal phase where progesterone is the dominant hormone.

Factors influencing the puerperium

Uterine involution. Many of the methods used to measure the rate of involution have been largely subjective and thus inaccurate; however, with the advent of transrectal ultrasound imaging, accurate measurements of uterine and cervical dimensions are now possible (Tian and Noakes, 1991a). Some of the factors are:

- *Age*. Most observers have found that involution is more rapid in primipara than pluripara.
- *Season of year*. If there is any influence, involution is probably most rapid in spring and summer.
- *Suckling vs. milking*. Results are contradictory; it may be a breed influence on the effect of time to return of cyclical ovarian activity.
- *Climate*. There is evidence that heat stress can accelerate and inhibit the speed of involution.
- *Periparturient abnormalities*. Dystocia, retained placenta, hypocalcaemia, ketosis, twin calves and metritis delay involution. Periparturient problems cause an overall delay in the completion of this process of 5–8 days (Buch et al., 1955; Tennant and Peddicord, 1968).
- *Delayed return to cyclical ovarian activity*. This inhibits involution.

Restoration of the endometrium. There is little related documented evidence; however, retained fetal membranes and metritis inhibit healing, whilst ovarian rebound to cyclical activity may have an influence.

Return of cyclical activity (ovarian rebound). Factors include:

- *Periparturient abnormalities*. A number of authors have shown that a whole range of periparturient problems delay ovarian rebound.
- *Milk yield*. There is much contradictory evidence on the influence of current milk yield; some authors have demonstrated an effect of the lactation preceding calving. It is frequently difficult to differentiate the influence of nutrition and milk yield.
- *Nutrition*. In both beef suckler and dairy cows, inadequate feeding, especially of energy, during the dry period and after calving inhibits ovarian rebound. This will usually be shown as poor body condition score. Ovulation of the dominant follicle will occur after 3.2 ± 0.2, or 10.6 ± 1.2, follicular waves in beef cows in good and poor body condition score, respectively (Crowe, 2000). The effect of nutrition on ovarian function is likely to be mediated by insulin and IGFs.
- *Breed*. Whilst it has been known for some time that there is a longer delay in beef compared with dairy cows there is evidence of a breed effect within the two groups especially in the former.
- *Parity*. Most observers have recorded a delay in primipara compared with pluripara – up to the fourth lactation. Conflicting opinions have probably arisen because of the problems of separating the influences of nutritional status, milk yield and weight loss.
- *Season of the year*. There is good evidence that photoperiod has an effect. This has been shown

by experimentally subjecting heifers to continuous darkness, which inhibited the return of cyclical activity (Terqui et al., 1982). Peters and Riley (1982) showed that suckler cows that calved between February and April were acyclic significantly longer than those that calved between August and December. By stimulating the effects of short day length using exogenous melatonin it has been possible to delay the return to oestrus and ovulation in postpartum beef cows (Sharpe et al., 1986).
- *Climate*. Cows in tropical climates show a delay compared with those in temperate zones.
- *Suckling intensity and milking frequency*. The greater the frequency of milking and the intensity of suckling (number of calves), as well as calf presence, the longer the period of acyclicity. This can be reversed in beef suckler cows by restricting the access of the calf to suckle from 30 days postpartum.

Elimination of bacterial contamination. The consequence is the development of metritis and endometritis (see Chapter 22):

- *Magnitude of bacterial contamination*. A massive bacterial flora may overwhelm natural defence mechanisms.
- *Nature of bacterial flora*. Many obligate Gram-negative anaerobes, such as *Fusobacterium necrophorum* and *Bacteroides* spp., exhibit synergy with Gram-positive aerobic contaminants.
- *Delayed uterine involution*.
- *Retained placenta*.
- *Calving trauma to the uterus*.
- *Return of cyclical ovarian activity*. There is contradictory evidence since, with an early return to oestrus, there is an early oestrogen peak which should assist in the elimination of the bacteria. However, if the level of contamination is such that a significant bacterial flora persists after the first oestrus the subsequent luteal phase may allow the bacteria to proliferate (Olson et al., 1984).

Mare

The puerperium is shorter in the mare than in the cow, with rapid involution and relatively good conception rates at the first postpartum oestrus. For the reader who wishes to study the subject in greater detail than outlined below there are two excellent papers (Andrews and McKenzie, 1941; Gygax et al., 1979).

In pony mares it is usually possible to identify the outline of the uterine body and horns by rectal palpation at about 12 hours postpartum; in thoroughbreds it is longer. Lochial discharge is relatively slight in most mares and usually ceases by 24–48 hours after foaling, although in a few cases it can persist for up to a week. The uterine horns shrink rapidly, reaching their pregravid size by day 32. Although the previously non-gravid horn was initially smaller, it shrinks at a slower rate. The cervix remains slightly dilated until after the first oestrus.

Ovarian rebound is rapid, the foal heat occurring 5–12 days postpartum. Evidence of follicular activity can be determined as early as the second day. Although conception rates at this first oestrus are lower than at other times, a large number of mares are fertile, which proves that the endometrium is capable of sustaining a pregnancy. Andrews and McKenzie (1941) found that the endometrium was fully restored by 13–25 days postpartum.

There is nothing comparable with the degeneration and sloughing of the endometrium that occurs in the cow; small amounts of villous debris are frequently attached to the maternal crypts but are removed by autolysis. The maternal crypts disappear as a result of lysis and shrinkage of the epithelial cells of the endometrium, condensation of their contents and collapse of the lumen of the crypt. By 14 days the endometrium is usually quite normal, apart from some pleomorphism of the luminal epithelium, but in some mares inflammatory changes may persist for several weeks (Gygax et al., 1979).

As in the cow, bacterial contamination of the uterus from the environment is a frequent occurrence, the species most frequently isolated being β-haemolytic streptococci and coliforms. These organisms are usually eliminated at the foal heat; if not, although they may increase during the subsequent dioestrus, they usually disappear at the second postpartum oestrus.

Retained fetal membranes delays involution, whilst exercise is said to hasten it. The process is more rapid in primipara than in pluripara.

Ewe and doe (nanny) goat

The puerperium in both these species is very similar to that in the cow, being typical of ruminants in general. The main difference is that, since they are both seasonal breeders, parturition is followed by a period of anoestrus. There is little information available for the doe so that the changes that are described relate only to the ewe, although it is unlikely that there will be major differences.

Involution

There is rapid shrinkage and contraction of the uterus, particularly during the third to 10th days postpartum, as determined by measurements of uterine weight and length, diameter of uterine body and previously gravid horn. According to these measurements, involution is complete by 20–25 days (Uren, 1935; Hunter et al., 1968; Foote and Call, 1969). Using sequential radiography and radio-opaque markers, uterine involution has been shown to be complete by about 28 days in suckling ewes, although an unexplained increase in uterine dimensions has been reported at 42 days (Tian and Noakes, 1991b; Regassa and Noakes, 1999). Involution in the ewe is also due to collagen breakdown, since although tissue collagen concentrations remain fairly constant with advancing pregnancy, there is a 7- to 8-fold increase in uterine mass; the reduction in size can only be a reversal of this process.

Restoration of the endometrium

As in the cow, there are profound changes in the structure of the caruncles with degeneration of the surface, necrosis, sloughing and subsequent regeneration of the superficial layers of the endometrium. There is evidence, determined by the slaughter of animals 3 days before the expected date of lambing, of prepartum hyaline degenerative changes. This occurs in the connective tissue at the base of, and adjacent to, the endometrial crypts and also involves both directly and indirectly the walls of the arteries and veins, thus reducing their lumens; the fetal villi are unaffected (Van Wyk et al., 1972).

After dehiscence and separation of the placenta there is further hyaline degeneration of caruncular tissue, which results in constriction of the blood vessels at the base of the maternal crypts. There is necrosis of the surface layer of the caruncle so that at about 4 days postpartum the most superficial layers are undergoing autolysis and liquefaction, which are responsible for the dark reddish brown or black coloration of the lochial discharge at this time. By 16 days postpartum, necrosis of the whole superficial part of the caruncle has occurred with, in most cases, separation of the brown necrotic plaque so that it is lying free in the uterine lumen. The caruncles now have a clean, glistening surface, and the process of regeneration is completed by the re-epithelialisation of the caruncles by about 28 days.

The quantity of lochia voided is variable. Initially it arises from blood, fetal fluids and placental debris but as the puerperium proceeds the liquefied, sloughed caruncular tissue contributes to it.

Return of cyclical activity (ovarian rebound)

Although in temperate climates ewes normally become anoestrus after lambing there are numerous reports of ovarian activity occurring within a few days to 2 weeks postpartum. Follicular growth is common but ovulation is unusual and when it does occur it is usually associated with a silent heat. Failure of follicular maturation and ovulation is probably due to inadequate release of LH as a result of a deficiency in GnRH synthesis and secretion. As a result, basal LH levels and the pulse frequency of episodic LH secretion are inadequate to stimulate normal ovarian function (Wright et al., 1981). It is possible that the time of the year when the ewes lamb has a profound effect, with those that lamb early and within the normal breeding season being more likely to have normal ovarian rebound. Hafez (1952) has suggested that it is most likely to occur in those breeds which have a longer-than-average breeding season.

Elimination of bacterial contamination

Although it would have been expected that similar events to those previously described for the cow and mare would occur, the author was unable to isolate bacteria from uterine swabs obtained from 10 ewes, 1–14 days postpartum, at surgical hysterotomy. More recently, using sequential transcervical swabbing of 13 ewes during the first week postpartum, bacteria were isolated from four ewes; thus in the other nine ewes the uterus was sterile.

Sow

There are a number of studies which describe the changes that take place during the puerperium of the sow (Palmer et al., 1965; Graves et al., 1967; Svajgr et al., 1974). It is important that the changes should occur rapidly, with a return to a normal pregravid state, so that pregnancy can be established as quickly as possible after weaning.

Involution

Apart from the rapid initial uterine weight loss which occurs in the first 5 days postpartum, involution is fairly uniform and is complete by 28 days. After day 6, most of the loss of weight is due to changes in the myometrium, notably a reduction in cell numbers, cell size and amounts of connective tissue. The decrease in the thickness of the endometrium and myometrium is completed by 28 days.

Restoration of the endometrium

The uterine epithelium 1 day after farrowing is of a low columnar or cuboidal type and there is evidence of the extensive folding that is present during pregnancy. The epithelial cells at 7 days are very low and flattened and show signs of degenerative changes; however, there are also signs of active cell division which is subsequently responsible for regeneration of the epithelium. This latter process is complete by 21 days and is capable of sustaining pregnancy.

Return of cyclical activity (ovarian rebound)

Suckling and subsequent weaning have a profound effect upon ovarian rebound and indirectly on other puerperal changes in the genital tract. In most cases there will be no return to oestrus and ovulation until the piglets are removed. In the study by Palmer et al. (1965) there was no evidence of ovulation during suckling periods of up to 62 days. In general, the later the time of weaning, the shorter the time interval to the first oestrus; for example, if the litter is weaned at 2, 13, 24 and 35 days postpartum the mean times to first oestrus were 10.1, 8.2, 7.1 and 6.8 days, respectively (Svajgr et al., 1974). The time to the first ovulation can also be shortened by the temporary removal of the whole litter for varying periods during the day (partial weaning), or the permanent removal of part of the litter (split weaning) (Britt et al., 1985).

There is rapid regression of the corpora lutea of pregnancy, with signs of cellular degeneration by 3 days postpartum, so that by day 7 they consist mainly of connective tissue. There is considerable follicular activity during suckling, with follicles sometimes reaching a diameter of 6–7 mm. This is sometimes associated with behavioural oestrus shortly after farrowing but in no cases is there ovulation; the follicles become atretic.

In a study of the endocrine changes of the postpartum sow, Edwards and Foxcroft (1983) showed that, irrespective of whether weaning occurred at 3 or 5 weeks, the great majority of sows showed a preovulatory LH surge within 7 days of weaning. At the time of weaning there was a transient rise in basal LH of about 2 days' duration but, unlike the cow, there was no consistent change in the episodic release of LH. Prolactin concentrations are high during lactation but decline rapidly to basal levels a few hours after weaning; mean FSH concentrations rise 2–3 days after weaning. Follicular growth and ovulation are inhibited during lactation because of suppressed LH secretion; this probably occurs as a result of direct neural inhibition of GnRH synthesis and release. Inadequate nutrition, particularly severe weight loss, can delay the onset of cyclical ovarian activity, as can the season of the year (Britt et al., 1985). It is generally accepted that exposure to the boar has the reverse effect.

The time of weaning, and thus the time of first oestrus, also has other effects on reproductive function, owing to the time taken for the completion of the puerperium. Fertilisation rates and pregnancy rates are improved the later the time of weaning, and hence the later sows are served after farrowing.

Bitch

Since the bitch is monocyclic, parturition is followed by anoestrus, the onset of the next heat being unpredictable. Regression of the corpora lutea of pregnancy is initially rapid, so that by 1 or 2 weeks postpartum they have been reduced in size. However, thereafter it is much slower, so that even after 3 months the corpora lutea measure 2.5 mm in diameter.

The rate of involution is similar to that of other species and the uterine horns are restored to their pregravid size by 4 weeks. The lochial discharge immediately postpartum is very noticeable because of its green colour due to the presence of uteroverdin; unless there are complications this should change to a bloodstained, mucoid discharge within 12 hours.

In the non-pregnant bitch, the surface of the endometrium undergoes desquamation followed by regeneration, with repair completed by 120 days after the onset of oestrus (see Chapter 1). After pregnancy and normal parturition, the time taken for regeneration of the endometrium is about 2 weeks longer. The areas of placental attachment are not readily identifiable immediately postpartum but by 4 weeks they are easy to identify. Desquamation of the epithelial lining of the endometrium starts at 6 weeks postpartum and is complete by 7 weeks; the whole process of regeneration has ended by 12 weeks.

Cat

Lactation will usually suppress oestrus effectively (Schmidt et al., 1983) but if the queen has no kittens to suckle or only one or two, she may show a postpartum oestrus 7–10 days after parturition.

THE NEWBORN AND ITS CARE

The sudden change at birth from the constant, controlled, cosseted environment of the uterus to the variable and frequently stressful free-living environment demands great adaptability from the newborn. In domestic species, however, provided that parturition is normal, most survive this transition without assistance. It should also be remembered that during the latter part of gestation the fetus is already undergoing a number of maturational changes, probably stimulated by the hormonal changes that occur in the initiation of parturition, in preparation for the free-living state (see Chapter 6). However, there is often a high mortality rate more often associated with dystocia; in cattle, for example, it has been estimated that 64% of calf losses occur within 96 hours of birth (Patterson et al., 1987), and those born following dystocia are 2.4 times more likely to develop infectious diseases within the first 45 days of life than those born as a result of a normal birth (Toombs et al., 1994). At birth, and for a variable period of time afterwards, a number of important events must occur, and it is the responsibility of the person involved in supervising or assisting at the parturition to assist the newborn so that the likelihood of survival is enhanced.

Onset of spontaneous respiration

During fetal life episodes of muscular movements similar to those of respiration have been observed in a number of species; whether these are truly the precursors of the continuous respiratory movements of the newborn is debatable. However, if parturition occurs normally then spontaneous respiratory movements will occur within 60 seconds of expulsion; if there is a delay then respiratory movements can sometimes occur before the offspring has been completely expelled.

There are probably a number of factors that are responsible for the initiation of spontaneous respiration. During the birth process, PO_2 and blood pH are falling and PCO_2 is rising due to the start of placental separation and occlusion of the umbilicus, thus restricting gaseous exchange. These changes have been shown in the lamb to stimulate chemoreceptors in the carotid sinus (Chernick et al., 1969). Tactile and thermal stimuli are also important, for it has been shown that if the face of the fetal lamb is cooled there is stimulation of respiratory movements (Dawes, 1968), whilst the licking and nuzzling of the dam probably provides some stimulus.

The first respiratory movement is usually a deep, forceful inspiration which is necessary to force air into the lungs. It has been demonstrated in the fetal lamb that a pressure of 18 cm of water is necessary to force air into the lungs at the first inflation; once this has occurred only a small pressure is needed to cause full inflation thereafter (Reynolds and Strang, 1966). Pulmonary surfactant, produced by type 2 pneumocytes during the maturation of the fetus at the end of gestation, assists in the initial lung expansion and alveolar stabilisation. Although the initial work done in the first breath is greater in mature than in immature fetal lungs, for the second and subsequent breaths it is much less; this is because the alveoli remain partially inflated after exhalation. As a result of lung inflation, the pulmonary vascular bed opens up followed by a sudden increase in pulmonary blood flow. Such changes in the vascular dynamics result in the rapid closure of the ductus arteriosus and foramen ovale followed by the ductus venosus several hours later; the consequence of these changes are that instead of gaseous exchange occurring via the placenta, it now occurs via the lungs (Grove-White, 2000). Survival of the newborn is dependent on the rapid onset of normal, spontaneous respiration. In a normal calf, respiration usually commences after 30 seconds of birth, being irregular at first before settling down to 45–60 breaths per minute (Grove-White, 2000).

Once birth is complete, it is important first to ensure that the upper respiratory tract is cleared of fluid, mucus and attached fetal membrane. This can be done with the aid of fingers or, preferably, with a simple suction device. Elevation of the rear of the calf, particularly by suspension from the hindlimbs, results in the escape of copious quantities of fluid. Some of this comes from the stomach and it may not necessarily be beneficial since it has been shown that one-third of this fluid can be absorbed from the lungs of the newborn via the lymphatic system (Humphreys et al., 1967). In addition, pressure on the diaphragm by the abdominal viscera can itself interfere with normal respiratory movements. Brisk rubbing of the chest with straw or towels frequently provides the necessary tactile stimulus to stimulate respiration, whilst a portable oxygen cylinder and resuscitator are useful pieces of equipment to have available. They comprise a small portable cylinder of oxygen, a reducing valve, a rebreathing bag and either a face mask, or an intranasal or endotracheal tube; the latter is preferable since, unless the oesophagus is occluded by pinching, the abomasum will also be inflated. If spontaneous respiration does not commence then it is necessary to provide positive pressure ventilation. This should be done with the calf in sternal recumbency; the person involved blows into the tube, and compresses a rebreathing bag attached to an oxygen source or a resuscitator such as the Richie. Respiratory stimulants such as coramine and adrenaline are not particularly useful; however, a mixture of solutions of crotethamide and corpropamide placed on the tongue can stimulate respiratory activity in some cases. Over-enthusiastic compression of the chest can sometimes cause injury to the ribs and the thoracic organs.

In most cases, if resuscitation does not result in spontaneous respiration in 2 or 3 minutes it is unlikely that the newborn will survive, even though there is a good strong pulse and heart beat.

Acidosis

The fetus at the time of a normal birth will usually have a mild metabolic and respiratory acidosis; in the case of the former, this is corrected within a few hours, whereas the latter may last up to 48 hours (Szcenci, 1985). Dystocia is likely to cause a severe respiratory and metabolic acidosis. Severe acidosis will have an adverse effect on both respiratory and cardiac function, and in the case of the calf will reduce vigour, the suck reflex resulting in reduced colostrum intake and impaired passive immunity (Grove-White, 2000). One of the simplest methods of assessing the degree of acidosis is to determine the time to the calf assuming sternal recumbency. Following a normal calving this was 4.0 +/– 2.2 minutes, where as following traction it was 9.0 +/– 3.3 minutes; a time >15 minutes was found to have a high predictive value for death of the calf (Schuijt and Taverne, 1994). The presence of good muscle tone and a pedal reflex are indicators of a well-oxygenated calf with fairly normal acid–base status. The presence of scleral and conjunctival haemorrhages is indicative of hypoxia and acidosis and carries a poor prognosis; similar lesions are present extensively at necropsy in calves that die at birth (Grove-White, 2000).

A calf requiring resuscitation is likely to be suffering from both a metabolic (low plasma bicarbonate concentration) and a respiratory (high PCO_2) acidosis. The PCO_2 will be reduced with improved alveolar gas exchange and tissue perfusion; however, the metabolic acidosis may be treated with sodium bicarbonate (Grove-White, 2000). The origin of the metabolic acidosis is due primarily to the production of lactic acid by tissues. When sodium bicarbonate is used to neutralise the acid, CO_2 and H_2O are produced; the former will exacerbate any respiratory acidosis. Thus it is important that the calf is breathing normally so that it can expire this additional CO_2.

Preferably, treatment of a metabolic acidosis should be carried out after its degree has been assessed following blood gas analysis or the use of the Harleco apparatus. However, under field conditions this is seldom possible, in which case the dose rate can be computed. Grove-White (2000) recommends that, for a newborn calf whose history and clinical signs suggest that it is acidotic, sodium bicarbonate at a dose rate of 1–2 mmol/kg as a bolus intravenous injection of 50–100 ml (35 gm in 400 ml of lukewarm water) can be used quite safely.

Injuries at parturition

Manipulative obstetrical procedures, particularly traction (see Chapter 12), can result in injury to the newborn. In a study involving the post-mortem examination of 327 calves that died during the perinatal period (within 48 hours of birth), 13.2% had fractured ribs, 4.3% diaphragmatic tears and 2.8% fracture of the spine in the thoracolumbar region (Mee, 1993).

Thermoregulation

In the period immediately following birth, the newborn has to adjust to an environment whose temperature may fluctuate widely and which is also usually below that of the uterus.

Following birth, the body temperature of the newborn falls quickly from that of the dam before it eventually recovers; the degree of decline and speed of recovery vary from species to species and with the environmental temperature. In the foal and calf, the fall is transient; in the lamb recovery occurs within a few hours; the piglet takes up to 24 hours or even longer in cold conditions; whilst in the kitten and puppy the period before the temperature recovers to approximately that of birth is 7–9 days.

In the newborn, thermoregulation is controlled in two ways. Firstly, the metabolic rate is increased to three times the fetal rate soon after birth. The increased rate is dependent upon adequate substrate, and since glycogen and adipose tissue reserves are low in the newborn it is very important that immediate and adequate food is available. However, the metabolic rate can increase only to a certain level, known as summit metabolism; if this is insufficient to maintain body temperature then hypothermia occurs (Alexander, 1970). The second method of thermoregulation is to reduce heat loss. The newborn has little subcutaneous fat and hence insulation is poor. The body surface is wet and thus heat is lost due to evaporation, whilst in species such as the pig the coat provides little protection. Heat loss is greatest in smaller individuals because they have a greater surface area per unit of body weight.

Thermoregulation in the newborn can be improved in a number of ways:

- Ensure that there is adequate food intake.
- Arrange for birth to occur in at least a thermally neutral environment and in those species where thermoregulation is delayed this environment should be maintained. The newborn puppy should be placed in an environmental temperature of 30–33°C for the first 24 hours, which can be reduced to 26–30°C by 3 days. Puppies born at a normal room temperature of 18–22°C can suffer a fall in rectal temperature of 5°C.
- Reduce heat loss by ensuring that the coat is adequately and quickly dried. A proper nest area should be provided with good insulation and supplementary heating in polytocous species, which will also encourage the huddling together of the litter, thus reducing the overall surface area. In the case of lambs,

simple plastic jackets can be an effective way of reducing heat loss.

Umbilicus

At birth the umbilicus usually ruptures passively, or in some species, such as the dog, the dam bites through the structure; there are few indications for ligation. Premature severance, especially in the foal, should be prevented, since it has been shown that in the foal the pulse can persist for up to 9 minutes after expulsion, thereby ensuring an adequate blood volume (Rossdale, 1967).

Provided that birth occurs in a clean environment with adequate hygiene it should not be necessary to handle the umbilicus. However, if there is an outbreak of 'navel ill' it may be necessary to introduce some prophylactic measures. The navel should be carefully cleansed with an antiseptic solution, dried and treated with an antibiotic spray or dressing.

Nutritional deficiencies and infectious agents

In a study involving the perinatal death of 22 calves born after an unassisted calving, there was some evidence that deficiencies of selenium, iodine and other trace elements were involved (Mee, 1991). When there is a high neonatal mortality rate in the absence of dystocia, the possibility of such deficiencies should be investigated, as well as the possibility of the presence of infectious agents, since these may cause not only pregnancy failure but also stillbirth and weakly offspring (see Chapters 23, 25, 26, 27 and 28).

Protection from an excitable or vicious dam

Occasionally the dam will attack or savage the newborn, in which case it may be necessary to provide some physical protection and resort to the use of tranquilliser drugs.

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8 General considerations

INTRODUCTION

Dystocia means difficult birth; the corresponding Greek word for normal birth is eutocia. The diagnosis of dystocia is frequently based on a high degree of subjectivity, since there are situations that one person will consider to be normal, but another will consider difficult. For this reason, some of the data on the incidence, causes or efficacy of treatment of dystocia are not very reliable, although there are many circumstances when distinguishing between the two will present no difficulty. The diagnosis and treatment of dystocia constitute a large and important part of the science of obstetrics, and require a good understanding of normal parturition, sensitivity to the welfare of both dam and offspring, and good and sensitive practical competences. In addition, veterinarians must always try to prevent dystocia where possible, by the application of sensible sire and dam selection, and good husbandry and health care.

CONSEQUENCES AND COST OF DYSTOCIA

The consequences of dystocia are numerous, and will depend upon the severity. Firstly, there are the financially unquantifiable effects on the welfare of dam and offspring. Secondly, there are the quantifiable financial consequences. Dystocia results in:

- increased stillbirth rate and mortality of the offspring
- increased neonatal morbidity
- increased mortality rate for the dam
- reduced productivity of the dam
- reduced subsequent fertility and increased chance of sterility
- increased likelihood of puerperal disease in the dam
- increased likelihood of culling.

The economic importance of bovine dystocia is emphasised in a number of different published reports. The biggest single economic loss is due to stillbirth and early calf mortality. Sloss and Dufty (1980) showed that about a third of the total of 17% of fetal and calf losses occur at the time of parturition, and that most of these arise from calving difficulties. Calves born to cows which suffered from dystocia were five times more likely to die neonatally than those following normal calvings, and accounted for 43.6% of all neonatal deaths (Azzam et al., 1993). Collery et al. (1996), in a study involving both dairy and beef suckler herds in Ireland, found that of those calves that were dead at birth, 68% were born following corrected dystocia. In a study in Colorado, USA, involving 73 units, Wittum et al. (1994) reported that 17.5% and 12.4% of calf mortality were associated with dystocia and stillbirth, respectively; the mean cost per calf death was $216. Kossaibati and Esslemont (1995) estimated that at that time in the UK, the direct and total costs were £141 and £310, respectively; these figures will be influenced by the current commercial value of calves.

To this loss of offspring must be added maternal deaths and subsequent infertility which derive from dystocia in all species, as well as the cost of treatment and the diminished productive capacity of the dam. In a study in Bavaria, Sauerer et al. (1988) reported that the culling rate of cows which suffered from dystocia was increased by 18%, and the calving index extended by 11–14 days. Milk yield was not affected unless the calf was stillborn (and therefore probably associated with a more severe dystocia), in which case the yields were reduced by 50, 126 and 148 kg for the first, second and third lactations, respectively. In a large study involving 71 618 Holstein cows in the USA, it was shown that differences between cows that had a normal calving, and those that had an extremely difficult calving for milk yield, fat yield, protein yield, number of days open, services/conception and percentage cow deaths were 703.6 kg, 24.1 kg, 20.8 kg, 33 days, 0.2 services and 4.1%, respectively.

Salman et al. (1991) indentified dystocia as the disease with the largest veterinary treatment cost in the Colorado National Animal Health Monitoring System. Similarly Miller and Dorn (1990) found that, in Ohio, 5% of the total cost of disease prevention and occurrence per cow per year was due to dystocia. Kossaibati and Esslemont (1995) calculated that the average farm labour cost of a single dystocia in the UK was £5.

Data on the effects of dystocia on the profitability of sheep enterprises are not extensive compared to those for cattle. The losses will be largely restricted to the increased incidence of lamb mortality which, as can be seen from Table 8.1, shows that the percentage of perinatal mortality ranged from 17–49%, and that dystocia was responsible for between 10 and 50% of this mortality. The effects on fertility will be less apparent, because in most production systems ewes have at least 6 months after they have lambed before they are served again. Severe trauma at parturition will result in lesions to the genital tract which may cause subfertility or sterility. It is much more difficult to quantify other production losses, but it is likely that severe dystocia will reduce milk production, and as a consequence, will reduce lamb growth rates and extend the times to weaning and reaching slaughter weight.

It is well recognised that in the sow most stillbirths occur intrapartum as a result of dystocia and the delayed expulsion of the litter. There are substantial data on stillbirth rates in sows. It is generally accepted that it should not exceed 7% of total piglets born, and if it exceeds 10% then there is a need for an investigation (Carr, 1998). In the mare, because of the relatively early separation of the placenta, foal survival is very short; thus if there is dystocia there will be stillbirth. In a survey involving 3527 cases of abortion, stillbirth and perinatal foal mortality in horses, 196 could be directly attributed to dystocia, and dystocia was probably also involved in a substantial number of the other causes of neonatal death. Birth-related trauma was present in 50% of dystocia cases. Dystocia was found to be responsible for 20% of foal mortality within 48 hours of birth (Haas et al., 1996). Mares that suffer from severe dystocia should not be served at the foal heat; if they are, then pregnancy rates will be lower than normal for this stage, and a small number will suffer from traumatic injuries which may result in sterility.

CAUSES OF DYSTOCIA

Obstetricians have usually regarded dystocia as being either maternal or fetal in origin; however,

Table 8.1 Incidence of perinatal mortality and its relationship to dystocia

Author(s)	*Year*	*Country*	*Breed(s)*	*Total no. lambs*	*% Perinatal mortality*	*Mortality due to dystocia*
Moule	1954	Australia	Merino	2467	18	23
McFarlane	1961	Australia	NS	15	49	NS
Hight and Jury	1969	NZ	Romney	7727	18	32
Dennis and Nairn	1970	Australia	Merino	3301	25	10
Welmer et al.	1983	UK	Cheviot, Blackface, Welsh	2453	26	22–53
Wilsmore	1986	UK	Polled Dorset	227	17	50

NS = not stated

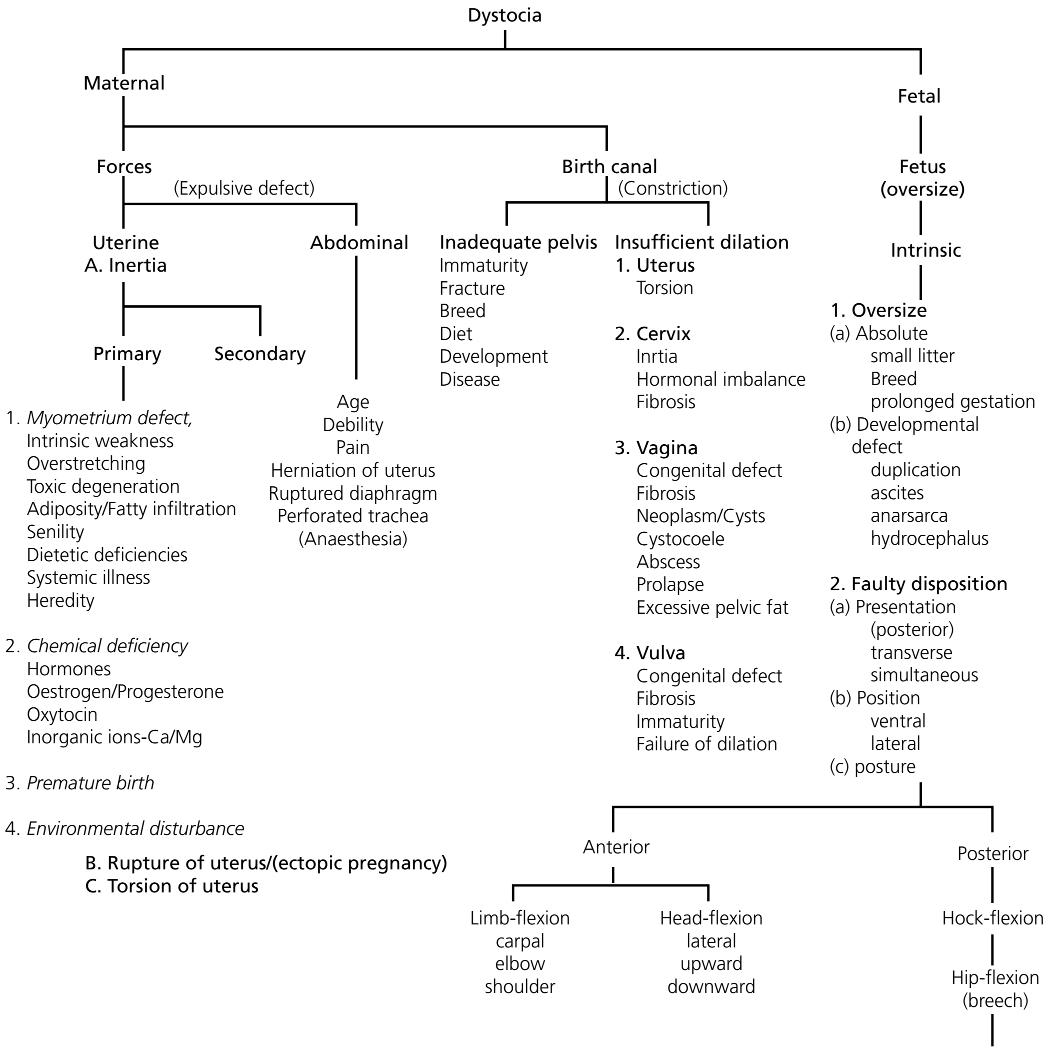

Fig. 8.1 The causes of dystocia.

there are sometimes occasions when it can be difficult to identify the primary cause, and also others when there will be a change in the dominant cause during the course of the dystocia. More realistically, dystocia should be considered in relation to defects in the three components of the birth process – the expulsive forces, the adequacy of the birth canal, and the size and disposition of the fetus. Difficult birth will occur when the expulsive forces are insufficient, when the birth canal is of inadequate size and shape, or when the presenting diameter of the fetus is unable to pass through the normal birth canal because it is too large or its disposition prevents it from doing so. The types and causes of dystocia are set out in Figure 8.1, and the specific diagnosis and the treatment of these will be described in the following chapters.

INCIDENCE OF DYSTOCIA

As was mentioned earlier in this chapter, whether the birth process is considered normal or abnormal can often be very subjective. In addition, consideration must always be given to the effect of breed, age and parity in the interpretation of results. The overall incidence thus varies from species to species, between breeds within species, and between ages and parities within breeds.

Cattle

The incidence of dystocia in cattle has been widely studied because of its effects on productivity. In addition, as will be discussed later, there has been substantial effort to prevent dystocia due to feto-maternal disproportion. It is impossible to provide a single figure for the incidence of dystocia in cattle because there are a large number of variables such as breed, age of dam, body weight of dam, sex of calf, singleton or twins, breed of sire, body condition of dam. It is less common in dairy than in beef cattle. The situation is well summarised in the study of Edwards (1979) in a Friesian herd. He found that dystocia was more common in primipara than in pluripara, and more common with the birth of male than female calves, and also with twins. In relation to parity, there were 66.5%, 23.1% and 14.3% assisted deliveries in first, second and third calvings, respectively.

More recent large studies confirm these original findings. In a large survey in Ontario (123 herds) involving a wide range of breeds (Angus, Hereford, shorthorn, Limousin, Saler, Simmental, Charolais), the overall mean dystocia incidence was 8.7% (median 5.8%), of which 5.3% were classified by the farm as being easy (traction by one person), and 3.4% as being difficult (McDermott et al., 1992). Further breakdown shows that parity, twins and sex of calf, as well as breed of sire and dam, influence the frequency of dystocia (Table 8.2).

This same study also provided some interesting data on culling rates. For the study as a whole the overall culling rate was 9.8%; however, when the data on culling related to the difficulty of calving is examined, then 7.9% of cows/heifers which had a difficult calving were culled, compared with 2.9% which were retained (McDermott et al., 1992).

The influence of age at calving, and also the influence of calf sex and birth weight, are illustrated in a large study involving purebred Angus heifers, a breed which traditionally is considered to be relatively easy calving (Berger et al., 1992). In this study, which involved 53 herds and 83 467 calvings in the USA, dystocia was recorded according to whether it was necessary for some assistance to be provided, or whether it was a difficult calving requiring substantial help. The data are summarised in Tables 8.3 and 8.4.

Certain breeds of cattle have a high frequency of muscular hypertrophy (double muscling), which is of genetic origin and for which certain

Table 8.2 Influence of parity, calf sex, twins and breed of sire and dam on the frequency of dystocia in cattle. These data were obtained from 123 cow-calf herds in Ontario (McDermott et al., 1992)

Factor	*Total number of calvings*	*% Easy calvings*	*% Difficult calvings*
Twins	73	13.7	4.1
Singles	4296	5.2	3.4
Primiparous	667	14.1	12.3
Pluriparous	3702	3.8	1.8
Heifer calf	2083	4.4	2.2
Bull calf	2065	6.1	4.5
Breed of dam			
Hereford	1186	4.0	2.4
Limousin	264	4.2	1.1
Charolais	284	6.0	3.2
Simmental	354	11.6	6.5
Breed of sire			
Hereford	1056	4.3	2.9
Limousin	1236	4.9	2.4
Charolais	896	5.6	3.3
Simmental	729	8.8	6.2

Table 8.3 The relative frequencies of dystocia severity in Angus heifers at different ages (Berger et al., 1992)

Age of dam in months	*Sex of calf*	*Total number of calvings*	*% Some assistance*	*% Difficult*
< 23	M	7543	21.0	6.2
< 23	F	7909	13.0	2.6
24–25	M	48 859	7.2	4.0
24–25	F	49 557	8.1	2.1
26–27	M	16 892	6.0	3.0
26–27	F	16 716	6.5	1.3
28–29	M	6448	8.7	2.6
28–29	F	6473	5.5	1.4
> 29	M	4018	8.0	2.0
> 29	F	4027	4.4	0.7

Table 8.4 Relative frequency of severity of dystocia and birth weight and sex of calf (Berger et al 1992)

Birth weight (kg)	*Sex of calf*	*Total number of calvings*	*% Some assistance*	*% Difficult*
20	M	23 949	11.3	2.2
20	F	25 069	6.4	1.1
21–25	M	3085	5.9	1.0
21–25	F	5588	3.9	0.4
26–30	M	13 023	9.3	1.3
26–30	F	19 118	6.6	0.8
31–35	M	21 165	16.4	3.5
31–35	F	19 368	11.5	2.2
36–40	M	10 372	29.0	10.1
36–40	F	5007	23.7	7.0
40	M	2164	33.6	27.8
40	F	542	30.0	20.6

breeds in particular have been bred in the last two decades. The highest frequency occurs in the Belgian Blue and Piedmont breeds, in which there is a higher proportion of the expensive cuts of meat of high lean content and of high quality. However, the frequency of dystocia is very high, and with it the undesirable consequences of high calf mortality rates and reduced fertility. For this reason, the author's view is that it is unethical to breed from such animals when it is known that there will be a high probability of severe dystocia and the need for an elective caesarean operation.

Sheep and goats

The incidence of dystocia is influenced by breed (Table 8.5), ranging from 1% in Scottish Blackface (Whitelaw and Watchorn, 1975) to 77% in the Texel (Grommers, 1977). In the goat, the frequency of dystocia is generally low, being comparable with that of the Scottish Blackface: between 2–3%. Faulty fetal disposition can cause dystocia. In a study by Wallace (1949), it was found that 94.5% of presentations were anterior longitudinal and only 3.6% were posterior. The commonest faulty disposition was unilateral flexion of one forelimb; if the lamb is small, this may not result in dystocia (Table 8.6).

Horses

There are relatively few studies which provide reliable information on the incidence and causes of dystocia in the horse. In general, it can be stated that despite being a monotocous species, where

Table 8.5 Incidence of dystocia (assisted births) in sheep

Author(s)	*Year*	*Country*	*Breed*	*Total no. of parturitions*	*% Dystocia*
Laing	1949	NZ	Suffolk	NS	70
Gunn	1968	UK	Blackface	15 584	2.5
			Cheviot		4.2
George	1975	NZ	Merino	1510	4.2
Whitelaw and Watchorn	1975	UK	SC Cheviots	1009	12
			NC Cheviots	569	2
			Blackface	433	1
George	1976	NZ	Dorset Horn	1509	34
Grommers	1977	Netherlands	Texel	NS	77
Wooliams et al.	1983	UK	Blackface, Cheviot, Welsh	2000+	5.3

NS = not stated

Table 8.6 Classification of ovine births according to the type of presentation. (Data from Wallace, 1949)

Presentation	*Number*
Anterior, with head and both forefeet extended	191 (69.5%)
Anterior, with head and one foreleg normal, other leg retained	49 (17.8%)
Anterior, with head presented and both forelegs retained	18 (6.5%)
Anterior, with forefeet presented and head retained	2 (0.7%)
Breech presentation – both hind legs retained	7 (2.5%)
Posterior – lamb being right way up and both hind legs presented	2 (0.7%)
Posterior – lamb upside down, i.e. ventrosacral position	1 (0.4%)
Other miscellaneous types	5 (1.8%)
Total	275 (100%)

the fetus is relatively large in comparison with the dam (unlike the situation in polytocous species), the incidence of dystocia is low. There are large breed variations. For example, Vandeplassche (1993) quotes 4% for thoroughbreds and trotters; 10% in Belgian draft horses, this relatively high level being due to fetal muscular hypertrophy; and 8% in Shetland ponies because of a large skull. However, in many breeds of pony the incidence is 2%. In an interesting on-farm study, involving 517 spontaneous foalings and including a wide variety of breeds (quarter horses, standardbreds, thoroughbreds and miniature horses), the total number of dystocias was 517 (11.2%), ranging from 8–19% on different farms. When the details for the different breeds are examined, then the incidences were 16%, 10.5%, 8.9% and 19% for the quarter horses, standardbreds, thoroughbreds and miniature horses, respectively (Ginther and Williams, 1996). All studies have shown that dystocia occurs more frequently in primipara than in pluripara.

Pigs

In the pig, dystocia is generally considered to be less common than in the monotocous species. In addition, many large intensive breeding units attempt to reduce dystocia, or certainly reduce its consequences, by the induction of farrowing (see Chapter 6). Figures of 2.9% in 103 farrowings (Randall, 1972), 0.25% in 772 farrowings (Jones, 1966) and 0.25–1% (Jackson, 1995) have been reported in the literature.

Dogs and cats

Details concerning the frequency of dystocia in the dog are few; this is because of the wide between-breed variations and the tendency for breeders to intervene, in some cases prematurely and unnecessarily. In addition, there are some breeds which are achondroplastic and brachycephalic, where normal birth rarely if ever occurs, and elective caesarean operations are the routine. A retrospective study by Walett-Darvelid and Linde-Forsberg (1994) of 182 cases of dystocia found that 42% of bitches that had whelped before had previously suffered from dystocia.

In one of the few studies of the frequency of dystocia in the cat (Gunn-Moore and Thrusfield, 1995), dystocia was reported to have occurred in 5.8% of 2928 litters involving 735 queens. There were some interesting breed differences; for example, in a large colony of cats of mixed breeding the frequency was 0.4%, whereas in litters of Devon Rex it was 18.2%. Pedigree litters were at a significantly higher risk than cats of mixed breeding (odds ratio being 22.6). Dolichocephalic and brachycephalic types were found to have a significantly higher level of dystocia than mesocephalic types.

PREVENTION OF DYSTOCIA

As with all diseases and disorders, veterinarians should be endeavouring to prevent and reduce the incidence of dystocia. For certain categories, such as faulty fetal disposition, our knowledge of the mechanisms that occur during the first stage of parturition, that are responsible for ensuring that the fetus assumes the correct disposition for its normal expulsion, is very incomplete. However, there are some types of dystocia which can be reduced significantly; these are invariably based on good husbandry. The principal one is fetomaternal disproportion. It has been known for some time, largely based on anecdotal evidence, that the pelvic canal size varies between breeds. For example, in cows of the Channel Island breeds the pelvic canal is relatively much larger than in other breeds, and cows of these breeds will readily give birth unaided when they are pregnant as embryo transfer recipients with calves of breeds with muscular hypertrophy. There are two approaches to reducing the frequency and the severity of dystocia. Firstly, the size of the birth canal should be adequate, secondly, the size and conformation of the calf should be such that it can pass through the birth canal.

For some years, since the early attempts to measure the size of the pelvic canal, there has been considerable interest in using this measurement as a method of predicting ease of calving. There are differences of opinion as to its value, largely based on the accuracy of measuring the pelvic area using pelvimeters inserted in the rectum. Deutscher (1995) is of the opinion that pelvic area is moderately to highly heritable, and can be increased in a herd by the selection of breeding heifers and bulls. He found that yearling heifers should have a pelvic area of at least 120 sq cm to deliver a 27 kg calf at 2 years of age. The pelvic area:birth weight (in lb) ratio should be 2:1. Similarly, Gaines et al. (1993) found that the ratio of the pelvic area at calving and calf birth weight significantly affected ($P < 0.01$) the incidence of dystocia, but the pelvic area *before* calving was not an accurate predictor. Others have doubted its true value (van Donkersgoed et al., 1993). Although excess body condition score has been considered to increase the incidence of dystocia, because of the presence of large amounts of retroperitoneal fat in the pelvic canal, not all studies have confirmed this. It is likely that only very fat cows will have problems, and it is good husbandry practice to ensure that this does not occur.

The selection of sires which result in a low dystocia frequency due to fetomaternal disproportion has been recognised for many years, as illustrated by the use of breeds such as the Angus and Hereford as sires for dairy heifers. Other aspects of good husbandry can prevent dystocia due to fetomaternal disproportion or reduce the adverse consequences if it occurs; this is discussed in detail in Chapter 11.

Little attention has been paid to the study of the basic causes of the other large category of dystocia – namely, faulty disposition of the fetus. It is unlikely that its aetiology will be clarified until the normal birth mechanism involved in parturient extension of the limbs from the flexed gestational position is understood. It seems likely to the author that the uterus, through its myometrial activity, plays a part in this limb extension; postural defects are more common with twins and with premature births, and in both of these instances a degree of uterine inertia is commonly present. Hormone changes, ratios and concentrations (particularly that of progesterone), which occur as a result of the cascade which stimulates the onset of parturition (see Chapter 6), are probably important in determining limb posture.

For example, Jöchle et al. (1972) have found that when progesterone was given to cows in which labour had been induced by flumethasone, there was a high incidence of dystocia due to postural deviation. This may be related to the influence of the endocrine changes on myometrial activity (see Chapter 6).

OBSTETRICAL TERMINOLOGY

We have used the term faulty or abnormal fetal disposition to describe the situation where the fetus has failed to assume the disposition which enables it to be expelled unaided per vaginam. In order to be able to provide a description of the disposition which any veterinarian will understand, there is an agreed terminology first defined by Benesch. This involves the use of the terms presentation, position and posture, each of which has a specific meaning in relation to veterinary obstetrics.

Presentation signifies the relation between the longitudinal axis of the fetus and the maternal birth canal. It includes longitudinal presentation, which can be anterior or posterior depending on which fetal extremity is entering the pelvis; transverse presentation, ventral or dorsal according to whether the dorsal or ventral aspect of the trunk is presented; and vertical presentation, ventral or dorsal. Vertical presentation is very rare, and only the obliquely vertical 'dog-sitting' presentation in the horse needs to be considered.

Position indicates the surface of the maternal birth canal to which the fetal vertebral column is applied. It can be dorsal, ventral and left and right lateral.

Posture refers to the disposition of the movable appendages of the fetus and involves flexion or extension of the cervical or limb joints: for example, lateral flexion of the neck or hock flexion posture.

TYPES OF DYSTOCIA WITHIN SPECIES

Cattle

For many years it has been customary to classify fetal oversize into absolute and relative; the former describes an abnormally large fetus, whilst the latter refers to a normal-sized fetus but where the maternal pelvis is smaller than normal. A more appropriate terminology is fetomaternal or feto-pelvic disproportion; the former of these two terms will be used in this book. Fetomaternal disproportion is the commonest cause of dystocia in cattle (Table 8.7). The incidence of which is dependent on such factors as:

- breed, being especially common in those with a high incidence of muscular hypertrophy; this can be compounded in a breed such as the Belgian Blue where there is also a small pelvic inlet
- immaturity of the dam at the time of breeding, and hence calving
- the use of an inappropriate sire either without or within the breed
- the use of embryos derived from in vitro fertilisation (IVF).

Table 8.7 Causes of dystocia in 635 beef cattle (after Sloss and Johnston, 1967)

Cause	*% All dystocias*
Fetomaternal disproportion	46
Faulty fetal disposition	26
Incomplete cervical and vaginal dilatation	9
Uterine inertia	5
Uterine torsion	3
Cervical prolapse	3
Pelvic fracture	2
Uterine rupture	2
Cervical neoplasia	0.5
Fetal abnormalities	5

Surprisingly, cows in high-condition score at calving have been shown to produce heavier calves, but without an increase in dystocia (Spitzer et al. 1995).

Dystocia due to faulty fetal disposition at the time of calving is lower, i.e. 26% (Sloss and Johnston, 1967 – Table 8.7). A survey of 3873 calvings over a 21-year period in Colorado, USA, showed that in 96% of the calvings the fetus was in normal disposition; in the remaining 4% the disposition was abnormal. In these 4% (155 in total), 72.8% of the fetuses were in posterior presentation and dorsal posture, 11.4% had unilateral carpal or shoulder flexion, in 8.2% the calf was a breech, in 2.5% there was lateral deviation of the head, 1.9% had incomplete extension of the elbow, in 1.35% the calf was in posterior longitudinal presentation and ventral position, in 1.35% it was in transverse presentation, and in 0.6% it was in oblique ventro-vertical presentation/position.

The incidence of fetal monsters is relatively high in the cow; they are generally of the distorted and celosomian types, *schistosoma reflexus* and *perosomus elumbis* being commonest (see Chapter 4). In a survey by 21 veterinarians from 1966–85 in the state of Victoria, Australia, 1.3% of the dystocias attended were due to *schistosoma reflexus* (Knight, 1996). In a survey in Poland from 1970–74, 115 or 12.9% of 891 fetuses or newborn calves with developmental congenital abnormalities were also due to this abnormality; all resulted in dystocia (Cawlikowski, 1993). Achondroplastic calves, typified by the ‘bulldog’ calf of the Dexter–Kerry breed, are also encountered.

Departures from longitudinal presentation are uncommon, because the anatomical arrangement of the uterine cornua and the absence of a distinct uterine body do not favour transverse presentation. Postural irregularities of the head and limbs are common, particularly carpal flexion, lateral deviation of the head and ‘breech presentation’. Simultaneous presentation of twins is a well-recognised cause of bovine dystocia, and one of the first duties of the obstetrician when proceeding to manipulative delivery is to ensure that the presenting limbs belong to the same fetus. Uterine inertia, often associated with hypocalcaemia, is well known, particularly in pluriparous Jersey cows; uterine torsion has its highest incidence in cattle, while instances of incomplete dilatation of the cervix are occasionally seen.

Mare

According to Vandeplassche (1972), only about 5% of the more serious equine dystocias are of maternal origin, and they are mainly uterine torsions. Most cases result from irregularity of presentation, position and posture of the fetus, of which the commonest single cause is lateral deviation of the head. Fetomaternal disproportion and uterine inertia are rare, except in some draught breeds. Transverse presentation of the foal across the uterine body (either dorsotransverse or ventrotransverse) is well known, and another form of transverse disposition in which the extremities of the fetus occupy the uterine horns is notorious and peculiar to the equine species. In respect of the influence of presentation of the fetus on dystocia, Vandeplassche (1993) summarises the presentations in 170 000 normal equine births in Belgium, compared with the presentations diagnosed in 601 dystocia cases brought to his clinic in Ghent (Table 8.8). Whereas posterior and transverse presentations occurred in only 1.0% and 0.1%, respectively, of normal births, they were present in 16% and 16% of dystocia cases. An obliquely vertical or 'dog-sitting' position of the fetus is another well-known dystocia peculiar to horses. In a more recent study, Leidl et al. (1993), from the Munich Veterinary School, examined the causes of 100 dystocia cases referred to their clinic. They found that 61 were due to faulty fetal disposition, 17 due to uterine torsion, 10 due to fetomaternal disproportion, 4 associated with twins, 4 due to incomplete dilatation of the birth canal and 3 due to uterine ventral deflection. These detailed studies involve cases referred to clinics. In a study by Ginther and Williams (1996), details were collected from eight stud farms involving four different breeds of horse; again the study shows that faulty disposition of the fetus was responsible for causing 69% of the dystocias. Of these, flexion and retention of one forelimb accounted for 13 of the 40 cases. Examination of the causes of dystocia when fetal disposition was normal shows that fetomaternal disproportion occurred in 5, weak contraction in 5, a small or previously broken pelvis in 2 and hiplock in 2 of the 18 cases.

Table 8.8 Influence of fetal presentation on dystocia in the mare (Vandeplassche, 1993)

Presentation	*Normal foalings*	*Dystocia cases*
Anterior	168 130 (98.9%)	408 (68%)
Posterior	1700 (1.0%)	95 (16%)
Transverse	170 (0.1%)	98 (16%)

Failure of the fetus to rotate into the dorsal position, and its consequent engagement at the maternal pelvis in the ventral or lateral position, are often encountered. They may be complicated by laceration of the dorsal wall of the vagina and even of the rectum and anus.

All forms of postural irregularity occur in the mare. The head and neck may be displaced laterally or downwards between the forelegs. Such displacements may be further complicated by rotation of the cervical joints. The limbs are frequently presented abnormally; one, several or all of the joints of the limbs may be flexed, and the irregularities are classified according to their clinical significance as carpal flexion, shoulder flexion, hock flexion and hip flexion. Bilateral hip flexion is known as breech presentation. An exceptional equine postural abnormality, which occurs in anterior presentation, is displacement of one or both extended forelimbs above the fetal neck (foot-nape posture).

Gross fetal monstrosities are rare, but occasional developmental anomalies which cause dystocia are wryneck (fixed lateral deviation) and hydrocephalus. Wryneck is likely to occur with transverse bicornual pregnancy.

Sheep and goat

Wallace (1949) provided a useful basis for understanding the causes of sheep dystocia by observing all parturitions (275) in a single flock (Table 8.6). He found 94.5% anterior presentations and 3.6% posterior, strikingly similar figures to those for bovine parturitions. Gunn (1968) collected data from 15 584 births in Scottish hill flocks, and reported a dystocia incidence of 3.1% (3.5% with singles and 1.3% with twins), but McSporran et al. (1977) recorded 20–31% of difficult lambings in a particular flock of Romney sheep in which fetopelvic disproportion was prevalent.

It is uniformly believed that in sheep populations, irrespective of breed and age, fetopelvic disproportion is the commonest cause of dystocia, that its incidence varies with breed and that it frequently occurs where there is crossing of disparate breeds for commercial lamb production. Also, assistance at lambing for this type of dystocia is more frequently required in primipara; male lambs, which are larger, predispose to it. Where pelvic size of the ewe is the major factor in the disproportion, there is likely to be repeated dystocia. McSporran et al. (1977) have demonstrated that its incidence can be markedly reduced (from 31% to 3.3% in a period of 4 years) – the level in Gunn's (1968) survey for Scottish hill sheep – by culling ewes that had required assistance at consecutive parturitions, and by breeding to rams that had sired lambs of lower birth weight.

In certain breeds and flocks, the incidence of dystocia due to maldisposition exceeds that due to fetopelvic disproportion; for example, in Gunn's survey it was more than 60% (Table 8.9). It is more common in pluripara than primipara, and is more frequent with twins than with single births. Among maldisposition dystocias, shoulder flexion is commonest, followed by carpal flexion, breech presentation, lateral deviation of the head and transverse presentation. Ewes with unilateral shoulder flexion often lamb spontaneously.

Only the more difficult dystocias are referred for treatment to veterinary surgeons, and in veterinary lists of assisted lambings the incidence of particular types of dystocia varies with the prevalent breed and with flock management. In Ellis's (1958) series of 1200 cases of sheep dystocia attended in a North Wales practice over a 10-year period, lateral deviation of the head was the commonest type, whereas in Wallace's (1949) and Blackmore's (1960) reports it was cervical non-dilatation (32 and 15%, respectively). Next after these two types in the veterinary surveys came shoulder flexion, carpal flexion, simultaneous presentation of twins, breech presentation and fetal oversize. Other occasional causes of severe sheep dystocia are uterine torsion, monstrosities (including *schistosoma reflexus*), fetal duplication, fetal oedema and *perosomus elumbis*. Similarly, in Thomas's (1990) survey the number of dystocias due to fetomaternal disproportion was small (3%) because, unless it is very severe, such forms of dystocia can be dealt with by the shepherd. Similarly in this same survey, the large number of other causes were due to those disorders such as incomplete dilatation of the cervix which may require veterinary intervention such as a caesarean operation (see Table 8.9).

It appeared from Gunn's (1968) data, and from other reports, that twinning does not significantly increase sheep dystocia overall. The explanation for this is that whereas twins increase maldisposition dystocia, there is a reduced incidence of fetopelvic disproportion dystocia because of their smaller individual size.

Table 8.9 Frequency of the main causes of dystocia in sheep

Author(s)	*Year*	*Country*	*Breed(s)*	*Total no. of dystocias*	*% Disproportion*	*% Disposition*	*% Other*
Wallace	1949	NZ	Romney	100	32	53	15
Gunn	1968	UK	Cheviot, Blackface	477	35	65	0
George	1975	Australia	Merino	63	77	23	0
Whitelaw and Watchorn	1975	UK	Cheviot, Blackface	50	76	24	0
George	1976	Australia	Dorset	513	57	43	0
Thomas*	1990	UK	Mixed	328	3	42	55

* Veterinary practice-based survey

There is no doubt from all published work that posterior presentation markedly predisposes to difficult births.

Pig

The types of dystocia encountered in the sow resemble more closely those of the bitch than those of the monotocous species, maternal forms being almost twice as common as fetal forms. In Jackson's (1972) series of 202 dystocias, 37% were caused by uterine inertia, 13% by obstruction of the birth canal and 9% by downward deviation of the uterus, whereas 14.5% were caused by breech presentation, 10% by simultaneous presentation, 3.5% by downward deviation of head and 4% by fetal oversize. The incidence of fetal dystocia increases when the litter is small, for in these the size of the individual tends to be large and obstruction may result. Irregularities of limb posture, and even uncomplicated posterior presentation, often cause dystocia when the litter is small, whereas had the litter been large and its individuals small, these irregularities would not have interfered with normal expulsion. Monstrosities are not uncommon; they are generally of the double type but schistosomes, perosomes and hydrocephalic specimens also occur. Together, bladder flexion and vaginal prolapse were reported to be the third most common causes of dystocia in a study in Germany (Schulz and Bostedt, 1995), whilst hypocalcaemia should also be considered as a cause of uterine inertia (Framstad et al., 1989).

Among litters of sows attended for dystocia, there is a collective stillbirth rate of about 20%, as compared with 6% in sows which farrow unaided.

Dog and cat

It is difficult to collect meaningful data on causes of dystocia in the bitch and queen, firstly, because some experienced dog and cat breeders will be capable of treating all but the most severe causes. Secondly, many breeds of dog, and to a lesser extent cat, suffer from severe congenital deformities such as achondroplasia and brachycephalicism which can exert a major influence on the birth process. The data will be influenced greatly by the population of animals in the study. Achondroplasia results in a reduction in the dimension from the sacrum to the pubic bone, and thus reduces the size of the pelvic canal. In brachycephalic breeds the head is very broad.

In a study involving 155 cases of dystocia in bitches, which included 65 different breeds ranging in age from 1 to 11 years, 75.3% of the causes of dystocia were maternal in origin, and 24.7 fetal in origin (Walett-Darvelid and Linde-Forsberg, 1994). A further breakdown of the various subcauses is shown in Table 8.10. This shows that uterine inertia was responsible for 72% of all dystocias. The authors of this paper used the term 'primary complete uterine inertia' to indicate when the bitch failed to expel any pups, comparable with the classical definition of primary uterine inertia, and 'primary partial uterine inertia' to indicate where the bitch gave birth to at least one pup and then stopped before whelping was complete, more comparable with secondary uterine inertia.

The dachshund and Aberdeen terrier are particularly prone to primary uterine inertia. The corgi shows extreme variation in the size of its puppies and hence fetomaternal disproportion may occur. Brachycephalic breeds, together with the Sealyham and Scottish terrier, are prone to obstructive dystocia due to the fetuses having comparatively large heads and the dams having

Table 8.10 Frequency of the cause in 182 cases of dystocia in bitches (Walett-Darvelid and Linde-Forsberg, 1994)

Cause	*Number of cases*	*%*
Maternal		
Primary complete uterine inertia	89	48.9
Primary partial uterine inertia	42	23.1
Narrow birth canal	2	1.1
Uterine torsion	2	1.1
Hydallantois	1	0.5
Vaginal septum formation	1	0.5
Total	137	75.3
Fetal		
Faulty disposition	28	15.4
Fetomaternal disproportion	12	6.6
Fetal monsters	3	1.6
Fetal death	2	1.1
Total	45	24.7

narrow pelves. Large fetuses, causing fetomaternal disproportion, are commonly encountered in bitches gravid with only one or two young; disproportion may also result from a fetal monster. A primigravid bitch of the small breeds often has trouble expelling her first puppy, but provided timely assistance is forthcoming she usually expels the remainder of her litter normally. If, however, assistance is delayed, the onset of secondary inertia may make the outcome serious. Irregularities of limb posture are generally of little importance provided the puppy is of normal size. In fact, many puppies are born with their fore- or hindlimbs flexed. However, when the fetus is relatively large, these postural deviations are often the factor that causes dystocia. Not infrequently a bitch or cat, in attempting to expel a fetus with its forelimbs retained, partially succeeds in that the head is born but the thorax with the limbs becomes obstructed in the maternal pelvic inlet. Similarly a puppy or kitten may have its hindparts born while its distended thorax is obstructed.

Irregularities of head posture are common, and vertex ('butt') presentation and lateral deviation of the head are frequently encountered. An interesting feature of the latter abnormality is that it often involves the last puppy to be born.

Fetal hydrocephalus and anasarca occasionally occur, but other forms of monster are rare. In the achondroplastic types and in the kitten, gross umbilical hernia (schistocormus) is seen, but it is seldom a cause of dystocia.

Abnormalities of position are common in both anterior and posterior presentation and are themselves a cause of obstruction. Failure of the fetus to rotate prior to presentation results in its engaging in the pelvic inlet in the ventral or lateral position.

Traverse presentation is rare. When it occurs the bitch is generally gravid with a single fetus only and gestation is of the bicornual type. It is generally accompanied by uterine inertia.

In the cat, maternal causes of dystocia are more common, particularly uterine inertia. Fetomaternal disproportion and faulty disposition are the most common fetal causes. These are illustrated in Table 8.11, from a paper by Ekstrand and Linde-Forsberg (1994); the authors used the same classification for uterine inertia as described above (Walett-Darvelid and Linde-Forsberg, 1994). This study also shows the influence of breed on dystocia (Table 8.12).

Table 8.11 Causes of dystocia in queens (Ekstrand and Linde-Forsberg, 1994)

Cause	*Number of cases*	*%*
Maternal		
Uterine prolapse	1	0.6
Uterine strangulation	1	0.6
Narrow birth canal (fetomaternal disproportion)	8	5.2
Uterine inertia	94	60.6
Subtotal	104	67.1
Fetal		
Faulty disposition	24	15.5
Fetal congenital defects	12	7.7
Fetomaternal disproportion	3	1.9
Fetal death	7	4.5
Subtotal	46	29.7
Other causes	5	3.2
Total	155	100

Table 8.12 Relative frequency of cat breeds with dystocia (Ekstrand and Linde-Forsberg, 1994)

Breed	*Number*	*% Queens*
Short-haired		
British short-hair	2	1.3
Devon rex	2	1.3
Russian blue	2	1.3
Burmese	7	4.5
Foreign short-hair	1	0.6
Siamese	10	6.5
Semi-long-haired		
Balinese	3	1.9
Norwegian forest cat	2	1.3
Birman	6	3.9
Long-haired		
Persian	58	37.4
Others		
Household cat	62	40.0
Total	155	100

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9 The approach to an obstetric case

Each case of dystocia is a clinical problem which may be solved if a correct procedure is followed. The veterinary surgeon arrives with a knowledge of the various types of abnormality that may occur in that particular species, and then, by a careful consideration of the facts elicited from the owner or attendant and the information obtained from the methodical examination of the patient, the nature of the abnormality can be ascertained. A correct diagnosis is the basis of sound obstetric practice.

HISTORY OF THE CASE

Therefore, before proceeding to examine the animal, a brief history of the case should, whenever possible, be obtained. Much of it will be the outcome of questioning the owner or attendant, but many points will also be elicited from personal observation of the animal.

- Has full term arrived or is delivery premature?
- Is the animal a primigravida or multigravida?
- What is her previous breeding history?
- What has been the general management during pregnancy?
- When did straining begin? What was its nature – slight and intermittent or frequent and forceful?
- Has straining ceased?
- Has a water-bag appeared and, if so, when was it first seen?
- Has there been any escape of fluid?
- Have any parts of the fetus appeared at the vulva?
- Has an examination been made and has assistance been attempted? If so, what was its nature?
- In the case of the multiparous species, have any young been born, naturally or otherwise, and if so, when? Were they alive at birth?
- Is the animal still taking food?
- In the case of the bitch and cat, has there been vomiting?

By a consideration of the answers to these and similar questions, it is possible to form a fairly accurate idea of the case to be dealt with. The inference to be drawn from many of them is obvious, but there are several points associated with them which merit discussion.

The greatest attention will be paid to the duration of labour. Calculating the time of onset of first stage is often difficult because, as you will know from reading Chapter 6, the signs are sometimes very vague and indistinct. However, the onset of vigorous and frequent straining, together with the appearance of the amnion, the expulsion of fetal fluids, or the appearance of a fetal extremity, indicates the onset of the second stage of labour, and parturition should proceed normally. If several hours have elapsed since its onset, it is reasonably certain that obstructive dystocia exists. Nevertheless, it is probable in all species except the mare that the fetus or fetuses are still living, unless the signs have not been observed and their significance understood. In the primigravida, particularly the heifer and the bitch, it is often found that the cause of the dystocia is relatively simple, such as slight fetomaternal disproportion, and the application of a little assistance is all that is required. In the mare, the normal course of delivery is so rapid, and separation of the placenta occurs so quickly once the second stage has commenced, that any delay generally results in the death of the foal due to anoxia.

However, when the call for assistance has been delayed 24 or more hours and it is noticed that straining efforts have ceased, it may be assumed that the fetus is dead, much of the fluid has been lost, the uterus is exhausted and putrefaction of the fetus has begun. These facts in themselves, quite apart from the more detailed features of the case, indicate that the prognosis must be guarded. This is especially the case in the polytocous species, for it is probable that there are several fetuses in utero.

If the history is that efforts to deliver the animal have already been made, or when such evidence is absent but one suspects it to be the case, a search for injury of the genital canal will be the first feature of the detailed examination of the animal. If any injury is identified then the owner or attendant must be informed immediately, and the likely consequences for the health of the dam explained. Sometimes, attempts at assistance will be denied; however, it is generally accepted that, with the exception of the mare where the expulsive forces during second stage are very powerful, spontaneous injury does not occur. In this case, the honesty or accuracy of the information should be queried.

GENERAL EXAMINATION OF THE ANIMAL

The animal's physical and general condition should be noted. If recumbent, is she merely resting or is she exhausted or suffering from a metabolic disease? Body temperature and pulse rate should be noted and the significance of abnormalities considered. Particular attention should be paid to the vulva. Parts of a fetus may be protruding and it may be possible to assess the nature of the dystocia from these. Are exposed fetal parts moist or dry? Such evidence serves not only as a guide to the duration of the condition, but also to the effort that will be necessary to correct it. Should parts of the amnion protrude, what is their condition? Are they moist and glistening and is fluid caught up in their folds? If so, their exposure is recent and the case is an early one. If, however, the membranes are dry and dark in colour, it may be taken that the case is protracted.

Maybe nothing protrudes from the vulva, in which case particular attention should be paid to the nature of the discharge. Fresh blood, especially if profuse, generally indicates recent injury to the birth canal. A dark brown fetid discharge indicates a grossly delayed case. Where it is clear from the evidence already obtained that the fetus is dead and the uterus grossly infected, the desirability of inducing epidural anaesthesia before proceeding to a vaginal examination should be considered. In this way the risk of infecting the neural canal should spinal anaesthesia later be found to be necessary is reduced.

When dealing with the bitch and cat, the degree of abdominal distension should be observed, for it may be possible to make an estimate of the number of fetuses which occupy the uterus. The onset of vomiting, together with a great increase in thirst, should be regarded as grave signs in the bitch.

DETAILED EXAMINATION OF THE ANIMAL

Large animals

The animal should be effectively restrained for the safety of both the veterinarian, any assistants and the animal concerned, in a clean environment. In the case of the mare, cow, ewe and doe goat it is easier if they remain standing; in the sow the examination is best performed with her in lateral recumbency. Very rarely it may be necessary to sedate the dam if she is very fractious. Plentiful supplies of clean hot water with soap or surgical scrub should be available, as well as a table, bench or truss of straw covered with a sterile cloth, on which the instruments may be placed. Whilst it is impossible to perform obstetrical procedures aseptically in any species, the amount of contamination of the genital tract should be kept as low as possible. A plentiful supply of clean straw should be placed under and behind the animal; also, since the floor is often wet and slippery, a prior application of sand or grit is a worthwhile precaution.

With an assistant holding the tail to one side, the external genitalia and surrounding parts are thoroughly washed from one bucket, and in the mare a clean tail bandage applied since the tail hairs are frequently introduced into the vulva and vagina and can cause quite severe lacerations. The operator, having washed his or her hands and arms from another bucket and after donning a clean disposable plastic sleeve, proceeds to make a vaginal examination. The introduction of the hand through the vulval labiae almost invariably provokes defaecation in the cow and it becomes necessary to wash the vulva and the operator's arms again.

Without the previous induction of epidural anaesthesia and the resultant paralysis of the rectum, it is almost impossible to make a vaginal examination in the cow without introducing some faecal contamination. This statement certainly holds true for animals which have been fed on grass and in which faeces are semi-fluid. Usually, no serious consequence will result from this contamination of the vaginal mucous membrane, provided the latter is intact.

If on examination the vagina is found to be empty, attention should be directed to the cervix. Is it completely effaced? If it is not, is it partially dilated and is it still occupied by some sticky mucus? If so, then it may be concluded that the first stage of labour is not complete and the second stage of labour has not yet begun, and the animal should be given more time. Maybe the case is one of uterine torsion. Does the vagina end abruptly at the pelvic brim and is the mucosa drawn into tight, spirally arranged folds? In the event of the vagina being occupied by amnion only, the nature of the fetal parts presented at the pelvic inlet must be ascertained. Can a fetal tail and anus be identified? If so, it is highly probable that the case is one of breech presentation. Is it the flexed neck which is being palpated? Can the mane be detected? A search on one or other side may reveal the ears and occiput, the case being one of lateral deviation of the head. But what of the forelimbs? Can the flexed carpi be felt beneath the neck or is there complete retention of the forelimbs in addition to the head abnormality? In the mare, complete emptiness of the vagina apart from the membranes may be due to postural defects, as previously outlined, but more often indicates a dorsotransverse presentation. If it is impossible or almost impossible to reach any parts of the fetus in this species, the case is probably one of bicornual gestation. The protrusion of the allantochorion into the vagina and from the vulva – 'red bag' – indicates placental separation.

However, in the majority of cases some part of the fetus occupies the vagina – the head, a limb or limbs. Recognition of the head is not difficult; the mouth and tongue, the orbits and the ears are generally obvious. In the case of a limb, the first requirement is to ascertain whether it is a fore-limb or hind-limb. If the plantar aspect of the digit is downwards, it is highly probable that it is a fore-limb; the converse is equally true. This statement applies with greater force to the cow than the mare, for in the latter, presentation of the fetus in the ventral position is relatively common. Proof is obtained by noting the direction of flexion of the limb joints. If the joint immediately above the fetlock flexes in the same direction as the latter, the limb is a fore one, and the converse holds true. The beginner may experience some difficulty in recognising the fetal parts being palpated if they are covered by amnion. To overcome this, the torn edges of the amniotic sac should be identified and opened, and the hand inserted so that the fingers come into direct contact with the fetus. If two limbs are present, it must be established that they are both fore or hind, and if they are from the same fetus.

Not infrequently, it is necessary to repel the fetus in the uterus to ascertain the nature and direction of displaced parts. If continued straining makes this difficult, the induction of epidural anaesthesia should be considered at once, but it should be remembered that the dam's expulsive effort may be required after any corrective procedure has been performed.

In the protracted case, assessment of the exact nature of the dystocia and methods of correction may be more difficult. Often, particularly in heifers, mares and sows the vaginal wall becomes grossly swollen and oedematous so that even the insertion of a hand and arm becomes difficult and there is no room in which to carry out manipulations. Loss of fluid has resulted in the mucous membrane and the fetal parts becoming dry. Contraction of the uterus directly on the irregular contour of the fetus makes retropulsion difficult or even impossible, in which case a spasmolytic such as clenbuterol may be used, while in many cases the fetus has become impacted in the pelvis. Plenty of obstetrical lubricant is required.

The assessment of the viability of the presented fetus is necessary at an early stage in the examination because this will influence the options for treatment. This can be done by attempting to elicit reflexes such as corneal/palpebral, suck, anal if they are in posterior presentation, and limb withdrawal. Unfortunately, there is no simple method of determining if other non-presented fetuses in polytocous species are alive or dead. If the fetus is dead, then it may be important to be able to estimate the time interval since death. When there is fetal emphysema and detachment of hair, then the fetus has been dead for at least 24–48 hours. If after the fetus has been removed there is no emphysema and the cornea is cloudy and grey, then it will have been dead for 6–12 hours.

Bitch and queen cat

The bitch, unless an exceptionally large one, should be placed standing on a table. It is preferable that a person with whom the animal is familiar should hold its head and be warned that even some quiet stoical bitches may resent a vaginal examination. Fetal numbers may be assessed in some bitches by gentle abdominal palpation. However, if a B-mode realtime ultrasound scanner is available then the use of this transabdominally will enable a fairly accurate assessment of fetal numbers, and has the added advantage of being able to determine if the pups are alive by identifying the beating fetal heart. At a later stage in the examination, it might also be necessary to take radiographs.

As a general rule, the operator will proceed to make a digital examination per vaginam, especially in early cases in which it is likely that obstruction is the cause of the delay, and also in protracted ones in which it is estimated that a single fetus only remains unborn. Nevertheless, cases will be met in which it is obvious that inertia has supervened and there are several fetuses to be delivered, in which case an immediate caesarian operation or hysterectomy is indicated.

Whether or not the hair is clipped from the area around the vulva before making a vaginal examination will depend on the length of the coat. In long-coated animals it is a great convenience to do so; although it is impossible to render the area sterile, it should be thoroughly cleansed beforehand.

Sometimes on raising the tail it is seen that part of a fetus, a head or hind parts, is protruding from the vulva. Such a finding is more common in the cat than the bitch. The case is a simple one; traction on the exposed parts effects delivery without difficulty and, provided this assistance has been forthcoming early, it is probable that parturition will proceed normally. Occasionally it is found that the vagina is occupied by a fetal head or buttocks which have become impacted. In the majority, however, the pelvic canal is unoccupied and obstruction occurs at the inlet.

What is the presentation? If a head, can one detect the mouth? Or is it the occiput with the ears? If the latter, the case is one of vertex presentation. Maybe a single limb is felt, but there is no sign of the head; the case is probably one of lateral deviation of the head. Is the presentation posterior? Recognition of the tail is generally simple, although it may be directed forwards over the fetal back. Have the hindlimbs entered the pelvis or are they retained? Has the fetus rotated into the dorsal position or is the case one of ventral or lateral position? Is the uterine body unoccupied? Determination of fetal viability by attempting to elicit reflexes is unreliable.

CONSIDERATION OF TREATMENT TO BE ADOPTED

General

The great majority of dystocia cases in the monotocous species are fetal in origin, and are the outcome of either faulty disposition or oversize. In the former, the first aim of treatment is to convert it to normal, and having done this, hasten delivery by relatively gentle traction. Such correction must, if possible, be performed by manipulation, assisted perhaps by the use of simple instruments such as snares and repellers. In cases of oversize of the fetus a decision must be made promptly on whether to attempt delivery by traction or by a caesarian operation. Various studies in cattle have shown that one of the major factors which determines the outcome for the cow and calf in cases of a caesarean operation is the degree of traction to which the cow was subjected before the decision to operate was made. The rationale for the obstetrician should always be that, if presented with live and viable young at term inside a viable dam, then the only measure of success is the delivery of live and viable young, without compromising the health or future fertility of the dam. However, the decision as to whether delivery should be accomplished by traction or a caesarean operation is one of the most difficult facing the obstetrician. In addition, veterinarians will sometimes be pressurised by owners into performing a caesarean operation when it is not necessary, particularly in cows with muscular hypertrophic calves or brachycephalic/achondroplastic bitches, purely because owners want to ensure the birth of live offspring. Conversely, owners will sometimes request the use of severe and prolonged traction rather than pay for the cost of a caesarean operation. In both situations the veterinarian must remember that the welfare of both dam and offspring are paramount, and act accordingly.

With the advent of new and safer anaesthetic agents the caesarean operation should not be considered as 'the last resort', but an effective method of treatment when used appropriately. Fetotomy as a method of treating dystocia in large animals still has its place if the fetus is dead. With the greater ease and increased effectiveness of the caesarean operation, however, in many parts of the world veterinarians have lost the skills that are necessary to perform fetotomy effectively.

Uncontrolled forcible traction may lead to laceration and contusion of maternal soft tissues, pelvic nerve damage and occasionally sacral displacement. If the mother survives, a third-degree perineal laceration, deformity of the perineum, fistula of the vagina and rectum, or paralysis may ensue. The obstetrician should seek to avoid these complications at all costs.

Species-specific

Mare

The first consideration is whether attempts at correction should be made with the animal standing or recumbent, or restrained and sedated, or under caudal epidural, or general anaesthesia. The decision will be influenced in part by the size and temperament of the mare, but more especially by the type of dystocia. Not infrequently the operator begins manipulative correction with the mare unsedated and standing, but soon realises that, for success, the other states described above are preferable. It is important in such cases that this decision be made early, so that the obstetrician shall not have become exhausted as the result of prolonged but futile efforts.

Relatively simple abnormalities, such as carpal flexion or lateral or downwards deviation of the head, can often be corrected using the hand alone, particularly when the mare is comparatively small and straining has been eliminated. However, it must be remembered that the limbs of the thoroughbred newborn foal are very long (70% of their adult length), which requires a substantial amount of space to facilitate flexion and extension. When, however, one of the more difficult forms is present, such as transverse presentation, ventral position or impaction of the fetus in the pelvis, or when there is laceration of the vagina or vulva, it is generally best to anaesthetise the animal at the outset, particularly in a hospital environment. One of the advantages of general anaesthesia is that by changing the position of the mare – for example, so that she is in dorsal or lateral recumbency, or even suspended by her hindlimbs (the anaesthetists will not be very enthusiastic about this approach because of pressure on the diaphragm) – the change in the pressures on the fetus within the uterus can be utilised to facilitate correction. Whenever fetotomy is required, both sedation and caudal epidural anaesthesia should be used. In veterinary hospitals, general anaesthesia is preferable since the foal is already dead and will not be affected by transplacental transfer of anaesthetic agents.

In all severe cases, the operator should consider the advisability of seeking the assistance of a colleague, for it is always possible that the combined efforts of two will succeed where those of one alone fail.

The value of *partial* fetotomy as a treatment of equine dystocia where the fetus is dead or deformed has been emphasised by Vandeplassche (1972, 1980), but *total* fetotomy was not recommended because it usually causes severe damage, particularly to the uterus. He pointed out that in the mare, fetotomy was difficult because of powerful straining, long birth canal and early dehiscence of the placenta. A long Thygesen fetotome was the best instrument. The indications for, and results of, partial fetotomy are shown in Table 9.1.

Table 9.1 Results of fetotomy in mares suffering from dystocia (Vandeplassche, 1972 and 1980)

Cause of dystocia	*No. of mares*	*No. recovered*
Reflexion of head and neck	72	67 (93%)
Hydrocephalus of two heads	6	6 (100%)
Breech presentation with ankylosis	17	14 (82%)
Partial transverse presentation	25	21 (84%)
Deformity, ankylosis or reflexion of forelegs	12	11 (92%)
Total	132	119 (90%)

Vandeplassche found that 25% of mares retained the afterbirth after fetotomy compared with 5% after a normal birth, and that fertility after fetotomy was 42%. With improved methods of general anaesthesia and aseptic surgery, the caesarean operation has a definite place in equine obstetrics, particular indications being maternal dystocia due to bicornual gestation, uterine torsion and narrow or deformed pelvis, as well as those cases of fetal dystocia where there is oversize or faulty fetal disposition combined with maternal injury or where the uterus has contracted on to a dead emphysematous foal. Vandeplassche's maternal recovery rates for fetotomy (132 cases) and caesarean operation (77 cases) were, respectively, 90 and 81%. Because of early dehiscence of the allantochorion in mare dystocia, only 30% of foals survived the caesarean operation (as compared with 85% of calves in the cow).

Cow

In the cow, delivery per vaginam, is the foremost consideration. The delay before professional aid is sought varies greatly, and this is a factor which influences the course to be adopted. In protracted cases there is often severe impaction of parts of the fetus in the pelvis; the greater part of the fetal fluids has often been lost and there is insufficient space to repel the fetus; the fetal skin and the vaginal mucosa have lost their natural lubrication, while the vagina and vulva are often swollen and manipulation is rendered difficult. Correction of the faulty disposition in such cases may prove very difficult and may prompt an early decision to undertake fetotomy or a caesarean operation. If, however, fetal disposition is normal and the case is one of simple fetomaternal disproportion, controlled traction will be first attempted, but before this is done it is important that the vagina and those parts of the fetus occupying it shall be lubricated as well as possible. For this purpose, one of the proprietary brands of cellulose-based obstetric lubricant should be used. Failing this, the copious application of soap (often in the form of soap flakes) and water is indicated, or mucilage of linseed or acacia. Traction, however, must be employed with consideration and discretion, for if it is impossible to extract the fetus by this means, its continued application makes for more severe impaction and this renders subsequent fetotomy very difficult or even impossible. In all cases such as these, epidural anaesthesia should be induced at the outset, together with the use of a spasmolytic such as clenbuterol. As a result of these treatments, it is generally possible to repel the fetus sufficiently for the performance of intrauterine fetotomy. When applying epidural anaesthesia subsequent to handling a putrid fetus, great care must be taken to ensure that infection is not introduced into the neural canal through the medium of either the needle or the anaesthetic solution.

However, more often the case will be an early one; the calf is alive and the uterus healthy. In the heifer, it is often found that fetal disposition is normal and that obstruction is due to slight fetomaternal disposition. In these cases, it is a comparatively simple matter to apply snares to the fetal extremities and, following the principles which are described in detail in later chapters, to effect delivery by traction. As a rule, the animal remains standing during the application of snares but often goes down during the passage of the calf's head through the vulva. In the multigravid cow, while fetomaternal disproportion is sometimes encountered, it is more likely that the cause of obstruction is faulty fetal disposition. If it is found that the space required for correction is continually lost due to the effects of straining or the contracted uterus, then caudal epidural anaesthesia and clenbuterol should be given without further waste of effort. A further advantage of epidural anaesthesia is that an animal which has become recumbent often rises after its induction, which invariably makes any manipulative procedures easier because the veterinarian can stand and the intra-abdominal pressure is reduced.

If the calf is a monster, e.g. *schistosoma reflexus* presented viscerally, it is almost certain that fetotomy will be necessary before it can be delivered via the vagina. In many, especially *schistosoma reflexus* in which the head and limbs are directed towards the pelvic inlet, fetotomy may be extremely difficult, and a better means of removing the fetus is by a caesarian operation.

In cases of fetomaternal disproportion of an otherwise normal calf in normal disposition, the inclination of the operator will be to resort to traction. In many cases, this attitude is a proper one, for by this means delivery is often effected without the mother sustaining irreparable injury. However, the amount of traction must be limited to that of three persons or a calving aid (this will be discussed later) and the progress of the operation must be very closely scrutinised by the veterinary surgeon, who will pay due regard to lubrication and to the method and direction of traction. If no progress is made after 5 minutes, or if the fetus becomes lodged and fails to yield to 5 minutes of further traction, then a caesarian operation should be performed.

Here again, the operator should always consider the advisability of seeking the aid of a colleague.

Ewe

In this species, the facility with which faulty fetal disposition can be corrected will depend in large measure on the operator's ability to pass a hand through the pelvis into the uterus. In the majority of ewes this is possible, but occasionally, especially in primigravid animals of the smaller breeds, it is impossible, and delivery per vaginam may fail. The same difficulty arises in cases of incomplete dilatation of the cervix or 'ringwomb'. In this troublesome condition, unless patient digital and manual efforts to dilate the cervix soon succeed, a caesarean operation must be resorted to.

In cases of fetomaternal disproportion with normal fetal disposition, the application of snares after retropulsion of the head or hips from the pelvic inlet is not difficult, and gentle traction effects delivery. Unless the amount of vaginal manipulation is minimal, the use of caudal epidural anaesthesia should be used on welfare grounds; because the uterus of the ewe is particularly easily torn or ruptured it allows a more gentle approach to any manipulative procedures and reduces the likelihood of trauma. In addition, where faulty disposition involves the limbs or head, reposition after retropulsion is, as a rule, relatively easy. Retropulsion, replacement of lost fetal fluids and correction of a faulty disposition are made much easier by elevating the hindquarters of the ewe. This can be done by rolling her on to her back and getting an assistant to pull both hind legs upwards and forwards. In cases of lateral deviation of the head and breech presentation in which manipulative reposition fails, fetotomy using the guarded wire-saw is indicated. Owing to the smallness of the lamb, the operation is easier than in the calf.

In the ewe, it is especially important to ensure that the presented parts belong to a single fetus. The young, in cases of twins and triplets, are small and retropulsion and reposition are seldom difficult.

In ewes in which it is impossible to pass the hand into the uterus, delivery by forceps traction may be possible. The manner of the application of the forceps is similar to that later described for the bitch. Forceps of the Hobday type, of appropriate size and fitted with a ratchet to maintain a secure hold when applied, are best for the purpose. Snare forceps of the Roberts' type or various proprietary fixed snares (see Figure 12.4) are also useful in head presentations.

Great care must be taken during intravaginal manipulations that the mucous membrane at the pelvic inlet is not lacerated. It is an accident which may occur quite simply, particularly when a finger is being used to lever a head or limb upwards. Such lacerations are usually followed by infection and possibly death.

Sow

In the sow, the ease with which obstructive dystocia can be relieved depends almost entirely on the operator's ability to pass a hand through the pelvic inlet. Provided this is possible, it is usually a relatively easy matter to grip the head or hind parts and withdraw the fetus. In small gilts and in sows of breeds such as the Vietnamese pot-bellied breed, the use of a lambing snare (Figure 12.4) may be useful to apply traction to the head. The disposition of the limbs is seldom of much consequence. When such assistance has been forthcoming early, i.e. within an hour or two of the onset of second-stage labour, removal of a fetus is often followed by the normal expulsion of the remainder. Assistance in the sow is frequently delayed, however, and in these cases the obstetrician will be well advised to remove as many piglets from the uterine body and cornua as are within reach. The subsequent course will depend chiefly on the measure of delay and thus the degree of inertia which has supervened. It may be found in an hour or so that normal expulsion has recommenced, or that on further examination more fetuses are accessible to manual extraction, and by continued attention to the sow in this manner the whole litter can be removed. It is worth remembering that intravaginal and intrauterine manipulations will stimulate the release of endogenous oxytocin, and thus stimulate myometrial contractions. Quite often, however, complete inertia has developed and no further progress follows the removal of the accessible fetuses. In these, a caesarean operation is the only means of saving the sow.

The strategic use of oxytocin to induce myometrial contractions can be used to treat overt cases of dystocia, and also to hasten the expulsion of piglets if there is an extended time interval before the arrival of the next, thus preventing stillbirth. It is important to give low doses of oxytocin initially since it is a potent ecbolic and large dose rates will cause spasm of the myometrium rather than rhythmical peristaltic-like contractions. In addition, the myometrium will become refractory to its effect and it may be necessary to increase the dose rate in order to obtain a response.

In a series of 200 porcine dystocias, Jackson (1996) found that an injection of 1 ml of a solution containing 0.5 mg of ergometrine maleate and 5 units of oxytocin gave a better and more prolonged ecbolic effect. The same author observed that the greatest problem in porcine obstetrics was to know when a parturient sow had expelled all her piglets. Good, but not infallible, indications of the end of labour are that the sow rises, passes a large volume of urine and then resumes recumbency in an attitude of contentment. When it is suspected that parturition is incomplete, the clinician should pass a hand as far as possible into the uterus and sweep it gently about the abdomen in the hope of balloting indirectly a piglet in an adjacent segment of the long uterine horn. Transabdominal B-mode realtime ultrasonography can be used to locate a retained fetus (see Chapter 3). The presence of retained fetal membranes is even more difficult to determine. Where the clinical manifestations suggest that a fetus (or fetuses) is still retained and there has been no response to the administration of ecbolics, the only approach would be an exploratory laparotomy. Sows and gilts will often survive the presence of retained piglets which sometimes become mummified (see Chapter 4). Since they are occasionally seen in the uteri of culled sows and gilts at the abattoir, it is likely that although they survived they were infertile.

Bitch and queen cat

The primary consideration in the management of a case of dystocia in the bitch or queen is – shall one proceed with delivery per vaginam or shall one immediately resort to laparotomy? Factors which will influence the decision are:

- the cause of dystocia, whether obstruction or primary inertia
- the duration of second-stage labour and hence the condition of the fetuses and the uterine muscle
- the number of fetuses retained.

When the case is recent, a matter of a few hours only, one will proceed to assist the bitch or queen per vaginam. If the cause is a modest degree of fetomaternal disproportion with the fetus in anterior or posterior presentation, it is probable that traction, using the finger and vectis or forceps (these should be used with great care to prevent trauma to both dam and offspring), will succeed in effecting delivery and parturition will then proceed normally. Similarly, in cases of faulty fetal disposition, such as vertex posture or breech, traction may succeed after correction of the abnormal posture. If, however, there is gross fetomaternal disproportion, and this should be suspected in litters of one or two only, an early caesarian operation is indicated.

In protracted cases of 24 hours or more, a caesarian operation is the primary consideration, for it is probable that secondary inertia has supervened and removal of the obstructed fetus will not alter the ultimate outcome. The question sometimes arises of whether one should first attempt to remove the presented fetus per vaginam before performing surgery. It is highly likely that this fetus is infected, and interference with it through an abdominal wound will favour the development of peritonitis. There is also, of course, the possibility that forceps interference will subject the bitch to even graver risk. The author's attitude is that when the presented puppy is impacted in the pelvis, it is best to attempt its removal with forceps prior to commencing abdominal operation, but in other situations the presented fetus is best removed through laparotomy.

A further question which arises in laparotomy cases – and this has special reference to the influence of the anaesthetic agents to be employed – is how long after the onset of second-stage labour puppies are likely to remain alive. It is very improbable that the presented fetus will live longer than 6–8 hours, for by that time its placenta will have completely separated. The remaining fetuses, however, may be alive for much longer periods; it is possible that after 36 hours' delay the presented fetus may be dead with early signs of emphysema yet those occupying the anterior parts of the cornua may be alive. After a delay of 48 hours this is highly unlikely to occur.

The respective indications for the two operations, hysterotomy and hysterectomy, will be discussed in Chapter 21. However, a recent study involving 37 bitches and 26 queens which were subjected to ovarohysterectomy found it to be safe, with newborn survival rates of 75% for dogs and 42% for cats; these are comparable to those published following caesarean operations to treat dystocia (Robbins and Mullen, 1994).

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10 Maternal dystocia: causes and treatment

Dystocias, which arise in the mother due to maternal factors, are caused either by constriction of the birth canal or by a deficiency of expulsive force; they are set out in Figure 8.1. The constrictive forms, of which the most important are pelvic inadequacies, incomplete dilatation of the cervix and uterine torsion, will be considered first.

CONSTRICTION OF THE BIRTH CANAL

Pelvic constriction

Developmental abnormalities of the pelvis are generally rare in animals, but in the achondroplastic breeds of dog the pelvic inlet is flattened in the sacropubic dimension, and this, together with the large head of the fetus in brachycephalic breeds, is a common cause of dystocia. An inadequate pelvis is a very frequent cause of dystocia in bovine primipara (heifers). The pelvis is late maturing compared with some other aspects of skeletal development; however, between 2 and 6 years of age it keeps pace with, or even exceeds, overall body weight. For this reason dystocia is far less frequent in cows than heifers. All aspects of fetomaternal disproportion are discussed in Chapter 14.

Pelvic constriction following fractures, where there has been poor alignment of the pelvic bones, can be an important cause of dystocia in any species. It is in those that are particularly prone to road traffic accidents, such as dogs and cats, that the frequency is highest and for this reason it is good preventive medical practice to ensure that any bitch or queen cat that has suffered from such an injury is radiographed before breeding, to ensure that the pelvic canal is capable of allowing a normal fetus to pass through at parturition without obstruction.

Incomplete dilatation of the cervix

The cervix provides an important protective physical barrier for the uterus during pregnancy. Several days before, and during, the first stage of parturition the cervix undergoes considerable changes in its structure so that it can dilate, becoming completely effaced and thus allowing the fetus(es) to pass from the uterus into the vagina, and thence to the exterior. The changes in the cervix are described in Chapter 6. Incomplete dilatation occurs in cattle, goats and sheep; in the latter species it is one of the commoner causes of dystocia. The degree of incompleteness of dilatation varies from virtually complete closure, to the situation where there is just a small frill of cervical tissue present which is sufficient to reduce the size of the birth canal thereby causing obstruction. The fact that it is a disorder of the ruminant cervix perhaps suggests a common aetiology, since in all three species the cervix is a tough fibrous structure with substantial amounts of collagen.

Cattle

In cattle, incomplete dilatation may occur in both the heifer and the multiparous cow. In the latter, the condition has generally been ascribed to fibrosis of the cervix resulting from injury at previous parturitions, but it is doubtful if this explanation is correct. It is more likely to be due to hormonal dysfunction which normally causes the cervix 'to ripen', or it is a failure of the cervical tissue (most likely collagen) to respond. The characteristic signs of discomfort, associated with the first stage of parturition are often mild and transient only, so that often it is difficult to ascertain accurately for how long labour has been in progress. For this reason, it is possible that weak uterine contractions which would be relatively ineffectual in causing dilatation of the ripened cervix, may be involved in the pathogenesis.

In the multiparous cow hypocalcaemia, perhaps subclinical, would impair uterine contractions and perhaps impair cervical dilatation.

On vaginal examination, the cervix is normally found to comprise a frill about 5 cm broad, separating the vagina from the uterus, and it is clear that delivery by traction must inevitably cause severe tearing. Often the amniotic sac has passed through the cervix and may be present at the vulva; it may have ruptured with escape of amniotic fluid. Sometimes fetal limbs have passed into the anterior vagina. At this stage it is advisable to determine if the cow is showing signs of hypocalcaemia, but even if not it is advisable to administer calcium borogluconate subcutaneously and wait several hours. It is possible that, if dilatation occurs after this time interval, the cow had not completed the first stage of parturition at the time of the first examination. The danger in deciding to wait several hours before interfering in the hope that the case is simply one of delay and that normal dilatation will later occur, is that the calf may die. The author has on occasion waited for a further 12 hours, by which time the calf had died, without any change in the cervix, in which case a caesarian operation should have been performed. It is probably sensible to leave the cow for a maximum of 2 hours, and then if there is no progress in parturition the alternative option should be followed.

Also, in some cases of abortion, the cervix fails to dilate properly and the fetus is retained, subsequently to undergo putrefactive maceration in the uterus. Incomplete dilatation of the cervix frequently accompanies uterine torsion. In addition, the disorder may also be diagnosed incorrectly when an earlier cause of dystocia at term has resulted in failure of the calf to be expelled after the cervix has dilated normally, allowing bacteria to enter the uterus followed by maceration.

Sheep and goats

Incomplete dilatation of the cervix of the ewe and doe goat is descriptively named ‘ringwomb’. It accounts for a substantial number of the ovine dystocia cases referred to veterinary surgeons; for example, Blackmore (1960) reported 28%, and Thomas (1990) reported 27%. The condition is suspected when, after protracted restlessness, the ewe does not progress to the second stage of labour. Manual exploration of the birth canal reveals that the cervix is in the form of a tight, unyielding ring which will admit only one or two fingers. Usually the intact allantochorion can be felt beyond the cervix, but occasionally this membrane has ruptured and a portion of it may have passed into the vagina; the latter observation distinguishes the condition from a protracted first stage, with which it may easily be confused and thus wrongly diagnosed. If there is a fetid vaginal discharge and necrotic fetal membrane in the vagina, in the presence of a non-dilated cervix, there is no doubt that the condition is abnormal, which may be due to retention after a failed abortion, or dystocia due to some other reason in which the lamb(s) was/were not expelled after the cervix had dilated normally.

When there is doubt over the diagnosis, the ewe should be left for 2 hours and then re-examined to ascertain if any further cervical dilatation has occurred, as in normal first-stage labour. Caufield (1960) found that only about 20% of cases of cervical failure recognised by him opened naturally, but even these required some assistance to lamb. Others have found that patient effort to dilate the cervix by digital manipulation is rewarding, and Blackmore (1960) was successful by this means in the treatment of 28 of 32 cases of ringwomb. Many experienced shepherds will attempt digital dilatation. Some veterinarians regularly use a spasmolytic such as vetrabutine hydrochloride (Monzaldon, Boehringer Ingelheim Ltd); however, the author cannot see the logic of such a preparation since it does not affect the composition of the cervical tissue which is such an important part of the ripening and subsequent dilatation process. If effective, then it may be because it delays parturition by virtue of it inhibiting uterine contractions, thereby giving the cervix a longer time to ripen and relax. The method of vaginal hysterotomy, whereby the cervix is retracted with vulsellum forceps and then incised by shallow cuts ‘at the points of the compass’, has its advocates, particularly in New Zealand, but such a brutal approach cannot be condoned on welfare grounds. Furthermore, such trauma must affect cervical function subsequently and probably requires culling of the ewe.

Many cases of ringwomb in ewes follow preparturient prolapse of the vagina and both conditions occur in similar circumstances of breed and environment. Hindson (1961) has drawn attention to an apparent connection between the incidence of 'ringwomb' at parturition and the prevailing weather conditions during pregnancy. Thus, in two summers and early autumns when there was plentiful, good-quality grazing preceding the tupping seasons there were 158 and 123 cases of ringwomb in his Devon practice, whereas following a very dry summer when grazing was poor, only 62 cases were seen. In the latter season, there was a high incidence of single lambs (probably due to a lack of flushing) and the ewes had to range widely to get sufficient keep. Far less is known about the causes of the disorder in doe goats where it occurs sporadically. Treatment is the same as for the ewe.

Hindson et al. (1967) were able to produce ringwomb experimentally by the injection of 20 mg of stilboestrol into pregnant ewes as early as 85–105 days of gestation. During this type of dystocia the myometrial contractions were normal, and the authors therefore concluded that natural ringwomb was a cervical rather than a myometrial disorder. Hindson and Turner (1972) suggested that ringwomb might be caused by ingestion of oestrogenic substances by pregnant sheep – as, for example, by grazing on red clover pasture or by feeding on herbage or grain contaminated with a fungus like *Fusarium graminaerum*. Mitchell and Flint (1978) demonstrated that when synthesis of prostaglandin was experimentally reduced, cervical ripening did not occur. More recent studies on cervical ripening in the ewe have shown that not only is there degradation of cervical collagen, but rather a remodelling of the cervical matrix with new collagen and proteoglycan synthesis (Challis and Lye, 1994). These changes are endocrine-mediated and obviously do not occur when there is ringwomb. As yet, we are uncertain of the deficiency and thus until such time as we know why and how it occurs, it will be necessary to treat cases as has already been described.

Incomplete relaxation of the posterior vagina and vulva

This is a relatively common finding in dairy heifers. It seems to be associated with heifers which are in overfat body condition, or in herds where the animals have been moved just before calving, or where the process of calving has been interrupted by too frequent observations or interventions.

Treatment requires the patient application of slow and gentle traction. If excessive force is used because of impatience then there will be perineal damage which might be so severe that a third-degree perineal laceration will occur (see Chapter 18). If continuous progress is made then delivery can be affected. If the vulva will not dilate properly then an episiotomy should be carried out (see Chapter 12). If there is any doubt about the likelihood of success with continuing attempts at vaginal delivery, a caesarean operation should be performed. There are occasions when large numbers of heifers in a group will be affected. The reason for this is not known; however, if a substantial number are affected then treatment with clenbuterol at the first signs of the onset of the first stage of parturition will delay calving and give the heifer extra time for the vulva, vagina and perineum to soften and relax, thus reducing the chances of dystocia.

Leidl et al. (1993) reported that 3% of the dystocias in mares referred to the Munich Veterinary School's obstetrics clinic were due to incomplete dilatation of the birth canal; these were all associated with what is described as premature delivery (abortion).

Vaginal cystocele

This is the name given to a condition occasionally encountered in the parturient mare and cow in which the urinary bladder lies in the vagina or vulva. It is of two types:

- Prolapse of the bladder through the urethra. This is more likely to occur in the mare consequent on the great dilatability of the urethra and the force of straining efforts in this species. The everted organ will occupy the vulva and will be visible between the labiae.
- Protrusion of the bladder through a rupture of the vaginal floor. In this condition the bladder will lie in the vagina and it will further differ from the previous one in that the serous coat of the organ will be outermost.

It is important to differentiate both the above from protrusion of fetal membranes; this is particularly the case in the mare, where the prolapsed bladder and the velvety (villous) surface of the allantochorion are very similar ('red bag'). In both conditions, the first aim of treatment is to overcome straining; this is best effected by the induction of epidural anaesthesia with or without sedation. This must be followed by retropulsion of those parts of the fetus which already occupy the vagina. In the case of the prolapsed bladder, it is then necessary to invert the organ again by manipulation. Where there is a protruded bladder, it must be replaced through the tear in the vaginal wall and the latter sutured. In the mare, if the tear is large the procedure is best done under general anaesthesia. The fetus should be delivered by traction after the correction of any faulty disposition.

Neoplasms

Neoplasms of the vulva and vagina may occur in all species and thus serve as potential causes of dystocia, because of physical obstruction, although in fact it is seldom that they do so. In the cow, papillomata, sarcomata and submucous fibromata of the vagina and vulva occur, while in the bitch the vaginal submucous myxofibroma is common.

Neoplasms of the cervix are so rare in animals as to be of no consequence in a consideration of the causes of dystocia.

Pelvic obstruction by the distended urinary bladder

Jackson (1972) has described a type of porcine dystocia in which the birth canal was obstructed by the distended urinary bladder being forced back by straining in the form of a mound under the vaginal floor, where it acted like a ball-valve in the birth canal; it was associated with a very relaxed birth canal. Schulz and Bøstedt (1995) reported bladder flexion and vaginal prolapse as the third most common cause of dystocia in sows in their survey in Germany. Bladder flexion, which is probably caused by straining, will cause kinking of the urethra resulting in urinary obstruction and distention of the bladder. Careful catheterisation of the bladder relieves the condition, ensuring that the catheter is not forced through the urethral wall at the point where it is bent.

Other abnormalities

Remnants of the Müllerian ducts often persist in the anterior vagina of cattle. They generally have the form of one or more 'bands' passing from the roof to the floor just caudal to the cervix and are usually broken during parturition. Sometimes they are laterally situated, and the fetus passes to one side of them. Occasionally, however, a remnant is of such size and strength that it forms an effective barrier to the birth of the fetus. The forelimbs may pass on either side of it. It is important that the obstetrician shall recognise what he or she is dealing with, and not confuse the condition with a partially dilated cervix. To examine the vagina satisfactorily, it is often an advantage to induce caudal epidural anaesthesia and repel the fetus into the uterus. The obstruction can be cut without risk, using a hook-knife or a guarded fetotomy knife of the Colin's or Roberts' type.

Cases of bifid and double cervix are occasionally seen on random post-mortem examination of bovine genitalia, and there is generally plentiful evidence that the animal involved has had one or more calves. The condition is unlikely to be a cause of dystocia, although the author has seen dystocia in which both canals had dilated and one forelimb had passed into one canal, and the head and the second forelimb had entered the other.

Torsion of the uterus

Torsion of the uterus, or part of it, is seen as a cause of dystocia in all domestic species. However, there is a wide variation in its frequency between species which is generally considered to be due to differences in suspension of the tubular genital tract which affects the 'stability' of the gravid tract.

Cattle

Rotation of the uterus on its long axis, with twisting of the anterior vagina, is a common cause of bovine dystocia. It has variously been reported to account for 6% (Tutt, 1944) and 5% (Morton and Cox, 1968) of dystocias, while in the New York State Ambulatory Clinic, Roberts (1972) reported an incidence of 7.3% among 1555 dystocias attended over a 10-year period. In veterinary hospitals to which the more severe types of dystocia are referred, irreducible uterine torsion is the indication for the caesarean operation in from 13.8 to 26.5% of cases (Pearson, 1971).

Aetiology. Uterine torsion is a complication of late first-stage or early second-stage labour. It is probably due to instability of the bovine uterus which results from the greater curvature of the organ being dorsal, and the uterus being disposed cranially to its subilial suspension by the broad ligaments. However, there must be some contributory factor additional to instability that operates during first-stage labour; otherwise torsion would be more frequently seen during advanced pregnancy than at parturition. The precipitating parturient factor is probably the violent fetal movements which occur in response to the increasing frequency and amplitude of uterine contractions during the first stage of parturition, as it assumes the normal disposition for normal birth (see Chapter 6). Excessive fetal weight is also a predisposing factor; Wright (1958) recorded an average calf weight of 48.5 kg in torsion cases, and Pearson (1971) a comparable figure of 49.8 kg. The final factor which allows the uterus to rotate about its longitudinal axis occurs when the cow is attempting to rise to her feet from sternal recumbency, particularly when she is in a confined space. She first flexes her forelimbs so that she bears her weight on both knees (carpal joints); this is followed by a foreward lurching movement of the head and whole body so that both hind legs can be extended; she is now resting on her knees and hind feet. At this stage, she may well rest temporarily, before making the final effort to extend the flexed carpal joints and stand on all four feet. When the cow is bearing her weight on knees and fully extended hind limbs, the longitudinal axis of the uterus will be almost vertical, thus allowing it to rotate quite easily about this axis if violent fetal movements occur at this stage (Figure 10.1).

The presence of bicornually disposed bovine twins would appear to stabilise the parturient uterus, and this view is supported by the great

Fig. 10.1 The longitudinal axis of the gravid uterus of the cow is almost vertical for a period of time when regaining the standing position from recumbency.

rarity of torsion in twin pregnancy. However, in ewes the anatomical attachment of the mesometrium is sublumbar rather than subilial as in cattle and bicornual gestation is very common, yet uterine torsion occurs. In 10 cases recorded by Pearson (1971), five were in bicornual twin pregnancies. Neither breed nor parity appears to affect the incidence of the condition. Regarding the aetiology of bovine torsion, Vandeplassche (1982) observes that uterine instability can be accepted as a cause of torsions of up to 180° but it cannot account for torsions of 360° or more.

Clinical features. The consensus of veterinary opinion is that torsion in an anticlockwise direction (as viewed from behind the cow) is more common than in the other direction, and accounts for about 75% of cases. In a recent study involving cases referred to all 24 veterinary schools in the USA from 1970 to 1994, 635 of the torsions were anticlockwise (Frazer et al., 1996). Although the uterus rotates about its longitudinal axis the actual twist in the majority of cases involves the anterior vagina; in the minority of cases in which the twist affects the posterior part of the uterus there is minimal distortion of the vaginal walls. In the survey by Frazer et al. (1996), 345 of the torsions were precervical and did not involve the vagina. Wright (1958) considered the most common degree of torsion to be of the order of 90–180°. However, in a series of 133 cases which were possibly more severe because they were referred by practising veterinary surgeons to a veterinary clinic, Pearson (1971) found that in only

37 was the amount of rotation 180° or less, while in the majority (88) the torsion was 360°. Frazer et al. (1996) found that 57% of cows had 180–270°, and 22% 271–360° torsions. Williams (1943) maintained that many dystocias, diagnosed as due to lateral and ventral positions of the fetus, were actually uterine torsions of low magnitude. The severity of the twist does not directly affect the survival of the fetus, fetal death being caused by loss of fetal fluids or separation of the placenta.

The most constant feature of uterine torsion is its association with parturition; Frazer et al. (1996) reported 81% of their cases were at term. It is generally believed to occur during the first stage of labour, because immediately after it has been corrected the cervix is found to be dilated to a variable degree. However, if after correction the cervix is found to be fully dilated, or if before correction the membranes are ruptured and portions of them or the fetus are protruding through the cervix, the inference should be that the torsion occurred during early second-stage labour. Roberts (1972) believes that torsions of less than 180° cause little interference with gestation, and that they often arise during advanced pregnancy and may persist for weeks or months, being recognised only when they cause dystocia at term. He further contends that torsions of 45–90° are often detected at pregnancy diagnosis and that they probably undergo spontaneous correction.

Symptoms. Up to the onset of parturition the animal has been normal, and when it enters the first stage of labour the usual signs of restlessness due to subacute abdominal pain associated with myometrial contractions and cervical dilatation are shown. In the typical case, the only real symptom is that the period of restlessness is abnormally protracted or that it wanes and does not progress into second-stage labour. If the torsion does not occur until early second-stage labour, then a short period of straining will have followed the restlessness, but will have ceased abruptly. In severe cases of torsion there may be increasing restlessness, but more probably all parturient behaviour will cease and, unless the animal has been closely observed, there may be no knowledge that parturition has begun. Pearson (1971) has noted slight depression of the lumbosacral spine as a frequent symptom. In the study by Frazer et al. (1996), there was pyrexia (23%), tachycardia (93%), tachypnoea (84%), straining (23%), anorexia (18%) and a vaginal discharge (13%).

If the condition is unrelieved, the placenta will separate and the fetus will die. There will develop persistent low-grade abdominal pain, progressive anorexia and constipation. Because the fetal membranes often remain intact, secondary bacterial infection of the fetus will develop later than with other forms of dystocia.

Diagnosis. Diagnosis is readily made by palpating the stenosed anterior vagina, whose walls are usually disposed in oblique spirals which indicate the direction of the uterine rotation. The cervix may not be immediately palpable, but by carefully following the folds into the narrowing vagina, the lubricated fingers can usually be pressed gently forwards and through the partially dilated cervix. Where the site of the twist is precervical, the vagina is much less involved, and diagnosis is assisted by palpating the uterus per rectum. In torsions of less than 180° portions of the fetus may enter the vagina and the dystocia may be wrongly ascribed to faulty fetal position (lateral or ventral).

Treatment. There are records of spontaneous recoveries, but it is generally believed that unrelieved uterine torsion will progress to fetal death, putrefaction and fatal maternal toxaemia. Fetal maceration with maternal survival is possible. With the adoption of prompt treatment, prognosis is favourable for mother and fetus. Delay leads to fetal death and makes treatment more difficult, but there is still a high rate of maternal recovery. At the New York State Ambulatory Clinic between 1963 and 1968, Roberts (1972) recorded a 4.3% maternal mortality. In Pearson's (1971) series of 168 more severe cases treated in a veterinary hospital, only 67 calves were born alive, but it is certain that a better rate of survival would be obtained in the less severe cases treated more promptly on farms. In the series by Frazer et al. (1996), cow survival was 78% and calf survival was 24%. The possible forms of treatment are as follows.

Rotation of the fetus per vaginam. The aim of this method is to reach the fetus by insinuation of the hand through the constriction of the anterior vagina and partially dilated cervix and then to apply a rotational force to the uterus through the medium of the fetus. Its likelihood of success depends mainly on two factors: whether the cervix is sufficiently dilated to admit the hand and whether the fetus is alive. Pearson was successful in 64 of 104 cases attempted by this method, 39 live fetuses being obtained from the 64 reducible, and 31 dead fetuses from the cases which were irreducible and subsequently treated surgically. Care must be taken not to rupture the fetal membranes, for this markedly reduces the fetal viability. When the fetus is reached, purchase is obtained on its shoulder or elbow region in order to rotate it in the opposite direction to the twist, but the first manoeuvres are designed to generate a gently swinging motion in the fetus before attempting to reduce the torsion. The most difficult part of the procedure is rotation through the first 180°; after this, replacement is spontaneous. It is helpful to have the rear of the cow at a higher level than the front, and epidural anaesthesia should be beneficial. Studies have shown that the use of the spasmolytic clenbuterol hydrochloride facilitates correction (Sell et al., 1990; Menard, 1994). The latter author used it at a dose rate of 0.6 to 0.8 μg/kg body weight intravenously in 70 cases, and reported that it made the task of correction much easier, resulting in a success rate of 77%. When the head of the live calf is readily accessible, pressing on its eyeballs will cause a convulsive reaction which can be translated into a rotation by applying a sufficient torque. Auld (1947) recommended abdominal ballottement to assist swinging the calf prior to reduction per vaginam.

Torsion of the uterus anterior to the cervix cannot be treated by vaginal manipulation, nor can the rare cases of twists of 720°C or more.

Rotation of the cow's body: correction by 'rolling'. This was the most popular method of correction, but because it requires the assistance of at least three people it is being replaced by the previous method. The aim is to rotate quickly the cow's body in the direction of the torsion while the uterus remains relatively steady. The mechanics of the method may be questioned but it is often successful. The cow is cast by Reuff's method on the side to which the torsion is directed. One assistant holds down the head while first the two front feet and then the two hindfeet are tied together with separate 2.5–3 m lengths of rope, each of which is held taut, preferably by two assistants on each rope. At a given signal a sudden smart coordinated pull is made on the leg ropes so that the cow is rapidly turned over from one side to the other. A vaginal examination is then made to ascertain whether correction has occurred, in which case there is ready manual access to the cervix and probably to the fetus in the uterus. If there is no relief the cow is slowly restored to her original position, or the legs can be flexed under her body and she can be turned 180° over her legs on to the original side. The same procedure of rapid turning is repeated, and to check that the rolling is in the correct direction the operator should try to retain a hand in the vagina during the manoeuvre. If there is no success on this occasion and the spiral folds are felt to tighten, one infers that the rolling is in the wrong direction, and sharp rotation in the contrary manner is carried out. Otherwise, repetition of the original procedure is applied until correction is achieved. If a calf's extremity can be grasped and partially flexed whilst the cow is rolled, this will help to fix the uterus and allow correction of the torsion to occur.

A modification of the foregoing traditional technique described by Schäfer (1946) entails the application of a wide plank of wood or ladder, 3–4 m long and 20–30 cm wide, to the flank of the cast cow, the one end resting on the ground. An assistant stands on the plank while the cow is slowly turned over by pulling on the leg ropes. The advantages of this technique are that the plank fixes the uterus while the cow's body is turned and, because the cow is turned slowly, less assistance is required and it is easier for the veterinary surgeon to check the correct direction of the rolling by vaginal palpation; moreover, the first rolling is usually successful.

Surgical correction. If the case cannot be corrected by either of the previous methods, a laparotomy should be performed on the standing cow through the left or right sublumbar fossa and an attempt made to rotate the uterus by intra-abdominal manipulation. Because a caesarean hysterotomy may also be required before the torsion can be corrected – or after the torsion is corrected when the cervix will not dilate – a left flank approach is preferable, although it should be remembered that in cases of uterine torsion there are often loops of small intestine displaced on the left side of the abdomen. Under paravertebral or field infiltration anaesthesia a 15–20 cm incision is made in the left sublumbar fossa. A hand is inserted, the omentum pushed forwards and the direction of twist confirmed. For a twist to the left, the hand is passed down between the uterus and the left flank and a fetal hand-hold sought, whereby an attempt is made first to 'rock' the uterus and then to rotate it by strongly lifting and pushing to the right. For a twist to the right, the hand is passed over and down between the uterus and the right flank, and as before a swinging manoeuvre is followed by pulling upwards and to the left. Owing to oedema of its walls the uterus is unusually friable and there is copious peritoneal transudate.

In some cases, it is impossible to rotate the uterus by abdominal taxis, and a caesarean operation must then be carried out before the torsion can be corrected. In other cases, despite abdominal relief of the twist, the cervix will not dilate and a caesarean operation must be performed to deliver the calf. Where the fetus has to be removed before the uterus can be turned, it may be found that the uterine wound is relatively inaccessible for suturing.

Whatever method is used to correct uterine torsion a decision has to be made on the subsequent management of the case. Because some placental separation and a degree of uterine inertia will have developed in many cases, and because there is a tendency in other cases for the cervix to close quickly after the uterus is replaced and not to dilate again (Pearson, 1971), it is wise to deliver the cow at once, per vaginam if possible or, if failing that, by caesarean operation. Where the cervix is found to be open after correction of the torsion and provided there is no inordinate fetopelvic disproportion, delivery of the cow by judicious traction on the calf will present no problem. If the cervix is only partially dilated, rather than resort to immediate caesarean operation, Pearson (1971) has recommended sectioning of the cervix per vaginam if the following clinical features are present:

- The birth canal caudal to the cervix is dilated sufficiently to allow delivery.
- The remaining cervical rim is thin and stretches like a sleeve on the fetus when traction is applied. Section is contraindicated if the cervix is thick and indurated.
- The fetus does not feel excessively large.

The technique of cervical section is simple and painless. The fetus is pulled backwards so as to engage the cervix fully and the stretched cervical rim is incised deeply at one point. This incision gives immediate relief and allows delivery to proceed.

The caesarean operation is indicated if the torsion is irreducible, or if the cervix is insufficiently dilated, or fails to dilate further after reduction. In the 168 cases of uterine torsion referred to the Bristol Veterinary School Clinic, Pearson (1971) reported that a caesarean operation was carried out on 137 animals, with a maternal recovery rate of 95%. It was noted that the fetal membranes were either already detached at the time of the operation, or were passed soon afterwards and that uterine involution was rapid. Other surgical features related to laparohysterotomy for uterine torsion are discussed in Chapter 20.

Horse

Torsion of the uterus is a rare condition in riding horses in Britain; Day (1972) recalled seeing only three cases over some 30 years in a practice where approximately 1000 mares foaled annually. In a more recent study involving 517 spontaneously occurring foalings on eight stud farms where there were 58 (11.2%) dystocias, no cases of uterine torsion were identified (Ginther and Williams, 1996). It appears to be less rare among draught horses in Europe, although the incidence is difficult to measure in the horse population as a whole, since many of the reports on the disorder emanate from referral clinics where only the more difficult cases are seen. For example, Leidl et al. (1993), in a study from the Munich Veterinary School, found uterine torsion present in 17 out of 100 dystocia cases. Similarly, Skjerven (1965) discussed 15 cases of surgical correction of uterine torsion and Vandeplassche et al. (1972) reported on 42 cases (four of which were included in Skjerven's previous review). The latter authors found that more than half their cases occurred before the end of gestation, but that 5–10% of all serious dystocias in Belgian horses were due to torsion; twisting in an anticlockwise direction was more common and in the majority the extent of rotation was 360° or more.

The possibility of uterine torsion should be considered in cases of colic during late pregnancy. Diagnosis is readily established by rectal palpation of the crossed broad uterine ligaments, which also provides information on both the direction and degree of the torsion. The circulatory disturbance in the uterus is greater than with the same condition in cattle, with consequent risk to the survival of the fetus and the development of shock in the dam.

After trying other methods of treatment for the antepartum case, including rolling the mare, Vandeplassche and his colleagues (1972) recommend laparotomy and rotation of the uterus by direct taxis, the mare being tranquillised in stocks and operated on under epidural and field infiltration anaesthesia. A high flank incision is made on the side of the torsion and a hand passed into the abdominal cavity and under the uterus. By carefully grasping the uterus, or the fetus through the uterine wall, and using the minimum of rotational force, the uterus is easily restored to its normal position. In cases where the foal is alive and the uterus not too congested, there is a good chance of progression to a normal parturition, especially if isoxsuprine is given for 24 hours after the operation (Vandeplassche, 1980). Skjerven (1965) recommended correction of the torsion in the recumbent mare under general anaesthesia. He incised the flank opposite to the direction of twist and then inserted a hand into the abdomen to identify a suitable part of the fetus in the proximal aspect of the uterus. To this fetal component, sufficient pressure was applied in a ventral direction to restore the normal position of the uterus. By pressing ventrally from the proximal side rather than by pulling dorsally from the distal side, there is less risk of rupturing the uterus. In the antepartum case, where the foal is dead or the uterus severely congested, hysterotomy should be performed (Figure 10.2).

When dystocia is due to uterine torsion an attempt should be made to pass the hand through the cervix and to rotate the uterus by manipulating the fetus. According to Vandeplassche (1980),

Fig. 10.2 Uterine torsion in a mare, as exposed by midline laparotomy. Note the congested uterus (u). Correction by rotating the uterus was impossible, and a dead foal was removed after hysterotomy; thereafter, correction of the torsion was possible.

this is facilitated by the use of caudal epidural anaesthesia, and raising the hindquarters of the mare. In addition, because of its value in correcting uterine torsion per vaginam in the cow, clenbuterol might be used. Rolling is rarely successful. If these methods fail, a caesarean operation must be performed.

In Vandeplassche's series of 42 cases, 60% of the mares and 30% of the foals survived. Skjerven's review indicated a favourable prospect for fertility in mares which recovered after torsion.

Sheep and goat

It is generally assumed that the frequency of uterine torsion in the ewe and doe goat is very low, as it does not appear to be a significant cause of dystocia in any of the published surveys. The low frequency of occurrence has been used to try and explain the aetiology of the disorder in cattle, since the suspensory apparatus of the genital tract is similar in all three ruminant species. One suggestion is that singletons are much more common in the cow than in the ewe and doe goat, which if they are distributed between both horns will make the uterus more 'stable'. However, if this hypothesis is true, then it would be much more common in those breeds of sheep that have a large proportion of singletons. One possible explanation for the species difference is the greater athleticism of the sheep and goat in rising to their feet from recumbency.

The condition closely resembles bovine torsion in its clinical signs, but because of the smaller size of sheep and goats it is much more difficult to insert a hand into the constricted vagina. The ewe or doe should be given caudal epidural anaesthesia, and with the hindlimbs held so that the animal is almost vertical, a relatively modest rotatory force on a fetal appendage or a rotatory movement of the dam's body is usually sufficient to correct the torsion. Clenbuterol may be used. If this fails then a caesarean operation must be performed.

Pig

Torsion is rare in sows; there was no case in 200 porcine dystocias attended by Jackson (1972). However, where it does occur, it can be difficult to diagnose and frequently this is only done at necropsy. Torsion can involve one whole horn or, more frequently, part of one horn, thereby trapping a fetus or fetuses proximal to the stricture; in time the uterine wall will rupture and the fetus or fetuses will become pseudoectopic. It is one of the differential diagnoses to consider if a sow has not completed farrowing, and yet piglets can either be palpated or identified using transabdominal ultrasonography. The only method of treatment is correction following a laparotomy, or probably a hysterotomy.

Dog and cat

Uterine torsion is uncommon in the bitch. In a series of 182 dystocias examined at a veterinary hospital in Stockholm over a 4.5-year period two cases of uterine torsion (1.1%) were diagnosed (Walett-Darvelid and Linde-Forsberg, 1994). One was in a 1-year-old mastiff, which had two macerated fetuses in which the complex torsion involved both horns and cervix, whereas the other case was in a 4-year-old giant schnauzer with 13 pups where there was 180° torsion; it is not clear from the description if it involved one or both horns. Both cases were treated by a caesarean operation. The clinical signs will be of an obstructive dystocia where pups remain in the uterus; however, it is very difficult to determine the precise cause of the obstruction. The author is concerned that the use of oxytocin may cause uterine rupture.

In pregnant bitches, a few instances have been discovered on post-mortem examinations where there were torsions of up to 2160°, while the rare finding of encapsulated fetal bones in a bitch's abdomen may be a legacy of uterine torsion and rupture.

If a uterine torsion is promptly diagnosed, a caesarean operation should be successful.

In the cat, torsion of 90–180°, involving either one horn or the uterine body, in near-full-term pregnancies (Young and Hiscock, 1963; Farman, 1965) and a cornual torsion of 360° in a 4-month pregnancy (Boswood, 1963) have been described in association with sudden illness. As in the bitch, a precise diagnosis of uterine torsion as a cause of an obstructive dystocia is difficult, even with good-quality imaging techniques; often a diagnosis is made only at laparotomy, in which case a prompt hysterotomy or hysterectomy should be performed. Occasional instances of extrauterine abdominal fetuses have been recorded (Bark et al., 1980). These probably result from uterine rupture during pregnancy, possibly associated with uterine torsion rather than from an ectopic pregnancy.

Displacement of the gravid uterus

Ventral hernia in the mare, cow and ewe

Occasionally in all three species, hernia of the gravid uterus occurs through a rupture of the abdominal floor (Figure 10.3). The accident is one of advanced pregnancy, occurring at the ninth month or later in the mare, from the seventh

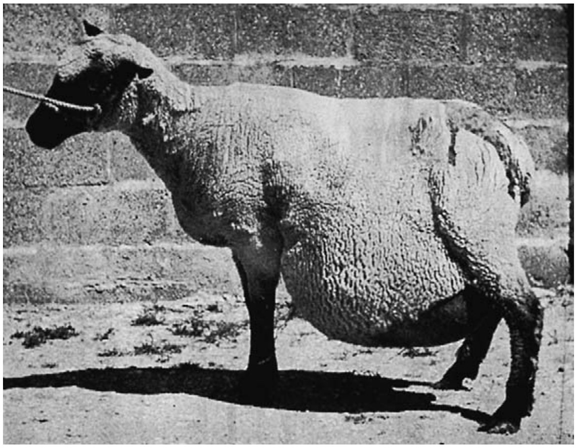

Fig. 10.3 Ventral hernia in the ewe.

month onwards in the cow and during the last month in the ewe. It is probable that in the majority of cases a severe blow to the abdominal wall is the exciting cause, although many observers have stated that it may occur without traumatic influence, the abdominal musculature becoming in some way so weakened that it is unable to support the gravid uterus. The site of the original rupture is the ventral aspect of the abdomen, a little to one side of the midline (left in the case of the mare and right in the cow and ewe) behind the umbilicus. It generally commences as a local swelling about the size of a football but rapidly enlarges until it forms an enormous ventral swelling extending from the pelvic brim to the xiphisternum. It is most prominent posteriorly, where it may sink to the level of the hocks. By this time, practically the whole of the uterus and its contents have passed out of the abdomen to occupy a subcutaneous focus. In cattle the bulk of the swelling is often situated between the hind legs, the udder being deflected to one side. Generally, the condition is complicated by gross oedema of the abdominal wall due to pressure on the veins; in fact this oedema may be so great that it is impossible to palpate either the edges of the rupture or the fetus.

As a rule, gestation is uninterrupted but the condition becomes grave for both mother and fetus when parturition commences, particularly in the case of the mare, although there are records of affected cows calving normally. Nevertheless, it is important to consider whether it is in the interests of the dam's welfare that the pregnancy should continue, or whether it might be preferable for euthanasia to be performed. In the mare, if the foal is to be saved, it is essential that aid shall be forthcoming the moment the expulsive forces of labour commence. Delivery of the foal by traction despite the downwards deviation of the uterus is possible; however, cases can be visualised in which displacement of the uterus places the fetus beyond reach. In these, it is advised that the mare is anaesthetised and placed in dorsal recumbency, and the hernia reduced by pressure. Attempts at delivery should be made with the animal in this position. After parturition and involution of the uterus, the hernia will become occupied by intestine. It is improbable, however, that strangulation will occur and the mare may be able to suckle the foal. At the end of this period she should be killed.

Cows and ewes may give birth spontaneously despite severe ventral hernia, but affected animals should be closely watched during labour in case artificial aid is needed.

Downward deviation of the porcine uterus

Downward deviation of the uterus has been described by Jackson (1972) as the cause of 19 of 200 cases of porcine dystocia. Affected animals strained vigorously despite an empty vagina, and at a point about 15–22 cm in front of the pelvic brim the uterus deviated sharply in a downwards and backwards direction. It was very difficult to extract the obstructed piglet manually, and insertion of the arm up to the shoulder was necessary so that the obstetrician's elbow could be flexed within the sow's abdomen. Affected sows were deep-bodied and pregnant with large litters.

Retroflexion of the mare's uterus

During the previous 10 years at the Ghent Veterinary Clinic, Vandeplassche (1980) reported that he and his colleagues saw 18 cases of severe colic in mares near term in which the foal occupied the maternal pelvis. Per rectum, it could be pushed forwards into the abdomen, although this manipulation provoked renewed colic, and the fetus soon regained the intrapelvic position. It was found that the injection at intervals of the muscle relaxant isoxsuprine lactate, in doses of 200 mg, relieved the colic and allowed the foal to move forwards in front of the pelvis. Normal parturition followed in due course.

Inguinal hernia in the bitch

Acquired inguinal hernia is common in the bitch and not infrequently the incarcerated uterus becomes the focus of pregnancy; it can also occur in the cat, but it is rare in this species. The hernia is generally unilateral and it may contain one or both uterine cornua.

Often the history is that an inguinal swelling the size of a hen's egg has been recognised for months, but that during the last few weeks it has rapidly become larger. In other cases, the recent development of a progressive swelling is the story. There may or may not be a history of recent oestrus and mating.

The lesion is obvious; it is unlikely that it will be confused with a mammary neoplasm or a local abscess if careful examination is made. The condition is painless and there is no systemic disturbance. Although it is tense and irreducible there is little tendency for strangulation provided intestine is not involved. The latter complication is rare. In those cases in which pregnancy is advanced, it will probably be possible to detect fetuses on palpation.

The course of the condition depends primarily on the degree of tension in the sac and this will be influenced by its size and the number of fetuses involved. Sometimes, the fetuses will develop normally up to a certain point and then die, probably because of impaired blood supply to the herniated parts of the horns, and then undergo resorption. The majority of cases will be presented when pregnancy has advanced about 30 days and each fetal unit is about the size of a golf ball, for by this time the size of the swelling is becoming alarming to the owner (Figure 10.4). It is very unlikely, but not impossible, for such a pregnancy to go to term with subsequent dystocia.

If in a pregnant bitch an inguinal hernia is diagnosed, then the following options should be considered:

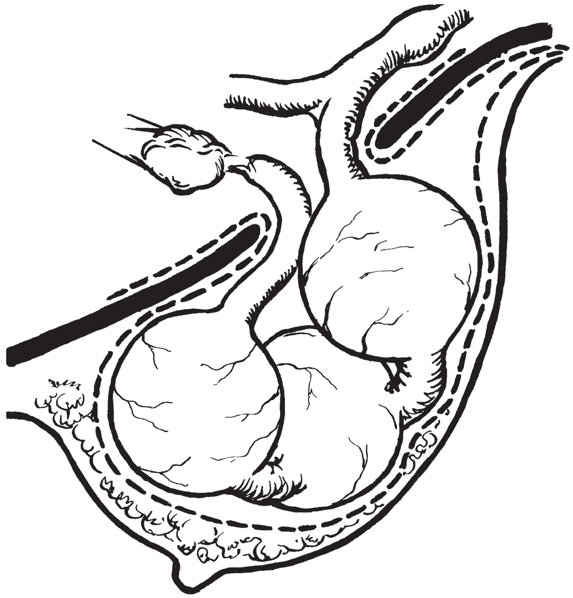

Fig. 10.4 Inguinal cystocele in a bitch gravid with three embryos of about 30 days.

- Reduce the hernia, obliterate the sac and allow pregnancy to take its normal course. In the great majority of cases it will not be possible to reduce the hernia by simple means.
- Enlarge the hernial ring by incision of the abdominal wall and later close by suture after reduction of the hernia. Obliterate the sac; allow the pregnancy to continue. From the strictly ethical viewpoint this is the operation to select. Pregnancy is uninterrupted and the animal's full breeding powers are conserved. It presents, however, several technical difficulties; precise incision of the abdominal wall forwards from the inguinal orifice is not easy owing to the presence of the large and tensely filled sac. Moreover, effective closure of the neck of the sac may be difficult after incision of the parietal peritoneum. At the same time cases will be encountered in which, after assessment of all the individual factors, this operation is selected.
- Dissect out the hernial sac; incise its apex and expose the herniated uterus. Amputate the horn involved. Obliterate the hernial sac. If it happens that the animal is also pregnant in an abdominally situated horn this should not be interfered with. If, however, an abdominally situated horn is empty and it is desired that the

bitch shall be sterilised, it is an easy matter after location of the bifurcation to draw this horn into the hernia and remove it. As a rule it is not possible to draw the ovaries through the inguinal ring. This is the operation most often performed. It presents no particular difficulties and cure of the hernia is certain.

- In those cases in which fetal development is at or approaching term, it may be decided to proceed as described above but, instead of amputating the involved horn, to perform hysterotomy and extract the fetuses with their membranes. In the one case in which the author has performed this operation it was possible to return the uterus to the abdomen after extraction of the fetus.

EXPULSIVE DEFICIENCY

The expulsive force of labour comprises the combined forces of the myometrial contractions and straining induced by the contraction of the abdominal muscles with a closed glottis. Because the abdominal muscles do not come into play until the myometrium has forced the fetus and fetal membranes into the pelvic canal and stimulated the pelvic sensory nerve receptors, it is logical to consider first the expulsive deficiencies that may arise in the myometrium; these may occur spontaneously or dependently, and are called, respectively, primary and secondary uterine inertia.

Primary uterine inertia

Before proceeding further, the reader is advised to refer back to Chapter 6, in particular the sections entitled 'Myometrial contractions', 'Effects of progesterone and oestrogens on myometrial activity' and 'Role of prostaglandins and oxytocin'. Primary uterine inertia implies an original deficiency in the contractile potential of the myometrium, thereby removing or reducing this component of the expulsive force and delaying or preventing the completion of the second stage of parturition. It is a common cause of dystocia in polytocous species, where it has been shown to be responsible for 37% of dystocias in sows (Jackson, 1972), 48.9% in the bitch (Linde-Forsberg and Eneroth, 1998) and 36.8% in the queen cat (Linde-Forsberg and Eneroth, 1998). Not infrequently, it occurs in the cow, where it is usually due to hypocalcaemia/hypomagnesaemia, as well as being a likely cause of incomplete cervical dilatation (see above).

The following factors may be involved in the cause of primary uterine inertia:

- The progesterone:oestrogen ratio is important as it influences uterine contractility in a number of ways. These are discussed in detail in Chapter 6; however, it is appropriate to mention them here. Oestrogen increases the synthesis of contractile protein; the number of agonist receptors for oxytocin and prostaglandins; the activity of myosin light chain kinase (MLCK) which is involved in the phosphorylation of myosin and hence the biochemical changes involved in contraction; calmodulin synthesis which increases MLCK activity; and the number of gap junctions. Progesterone has the opposite effects, thereby reducing myometrial contractility. The changes in the progesterone:oestrogen ratio will be determined by the endocrine cascade that initiates parturition (see Figure 6.3).
- Oxytocin and prostaglandins are involved directly and indirectly in myometrial contractions. Any deficiencies in these hormones, or their receptors through which they exert their action, will prevent or reduce myometrial contractions.
- Calcium and related inorganic ions such as magnesium have a critical role in smooth muscle contractions. Any deficiency will impair these contractions, causing uterine inertia. This is a particular problem in dairy cows, particularly those at pasture, since most cows experience a transient decline in food intake around the time of calving which will result in reduced calcium intake. It is important to control feeding carefully during this transitional period since, not only will hypocalcaemia cause uterine inertia resulting in dystocia, but also there is evidence that it can have a profound influence on feed intake well into lactation, thereby depressing fertility (McKay, 1998) (see Chapter 22).

- Overstretching of the myometrium due to the presence of a large litter or excess fetal fluids (hydrallantois), or understretching due to a small litter in polytocous species can cause reduced uterine activity.
- There is anecdotal evidence that fatty infiltration between the layers of the myometrium can reduce its contractile efficiency.

The diagnosis of primary uterine inertia is made from the history and by an examination of the birth canal and presenting fetus. The dam is at or near term, as denoted by mammary changes and ligamentous relaxation in the pelvis (where this is normally apparent), while the psychological manifestations, coupled with restlessness due to abdominal discomfort, will have indicated the first stage has passed. There may have been a few feeble abdominal contractions but no progress has been made; or in the polytocous species, after an adequate beginning of second-stage labour, all further activity has ceased. Linde-Forsberg and Eneroth (1998) refer to this as ‘primary partial uterine inertia’ to differentiate it from ‘primary complete inertia’ where second stage fails to commence at all. It is difficult to distinguish this from secondary inertia, which is always a sequel to some other factor such as an obstructive dystocia. Examination of the birth canal in the larger animals reveals a patent cervix, beyond which a fetus normally can be palpated contained within its membranes. In the bitch and cat, it is likely that no fetus or membranes will be felt.

It is essential that treatment should be attempted as soon as possible, once other causes of dystocia have been eliminated as being responsible. In the large monotocous species, treatment is generally simple. By vaginal manipulation the membranes are ruptured, and if the fetus is in normal disposition, it should be delivered immediately by traction. In cows, calcium borogluconate should be given even if there is no clinical evidence of hypocalcaemia. In the sow, there is evidence that hypocalcaemia is associated with some cases of uterine inertia (Framstad et al., 1989) but it is difficult to administer large volumes of calcium borogluconate as can be done in cattle. Treatment involves a combination of manual removal of any piglets that can be palpated in the vagina or uterus, together with the use of repeated doses of oxytocin. It is important to stress that oxytocin is a potent ecbolic, and doses of 10 IU i.m. or 5 IU i.v. should be used initially. Large doses tend to cause myometrial spasm, rather than peristaltic contractions; in addition, the myometrium becomes refractory to repeated dosing so it is important to provide an opportunity for an incremental dose regimen.

For the bitch and queen cat, where primary uterine inertia is the main cause of dystocia, Linde-Forsberg and Eneroth (1998) suggest the following treatment regimen:

- Vigorous exercise of the dam will sometimes stimulate uterine contractions.
- Digital stimulation of the vagina (feathering) will stimulate endogenous oxytocin release, and may induce uterine contractions.
- *Slowly* inject 10% calcium borogluconate solution i.v. (0.5–1.5 ml/kg body weight). This is in response to the long-held belief that subclinical hypocalcaemia is a common cause of inertia (Freak, 1962), although a more recent study has failed to support this hypothesis (Kraus and Schwab, 1990).
- Leave the bitch for 30 minutes; if straining commences then repeat the calcium borogluconate treatment. If not, administer oxytocin at a dose rate of 0.5–5 IU i.v. or 1–10 IU i.m. in the bitch depending on size, and 0.5 IU i.v. or i.m. in the queen.
- Perform a vaginal examination and remove any pups or kittens by gentle traction.
- Oxytocin treatment can be repeated, particularly if small numbers of the litter remain.
- If calcium or oxytocin therapy is not successful, or if the litter is very large or small (a single pup or kitten), then a caesarean operation is indicated.

Nervous voluntary inhibition of labour

In 17 of 272 canine dystocia cases (Table 10.1) recorded by Freak (1962), labour did not begin or, having begun, did not proceed. The factor common to all of the affected bitches was the pro-

Table 10.1 Classification of 272 canine dystocias (after Freak, 1962)

Dystocia	*Number of cases*
Obstructive dystocias, fetal	
Relative oversize of one or more fetuses	77
Absolute oversize	15
Fetal monstrosity or gross abnormality	2
Malpresentation other than posterior	12
Posterior presentation of first fetus	35
Obstructive dystocias, maternal	
Abnormality of maternal soft structures	4
Abnormality of maternal pelvis (addidental)	1
Slackness of abdominal wall	3
Inertias	
Primary inertia	41
Secondary inertia	44
Nervous voluntary inhibition of labour	17
Slow initiation of labour (query hormonal in origin)	1
Slow initiation of labour (due to subclinical eclampsia)	7
Abortion near to term of dead fetuses	2
Death of some fetuses prior to parturition	10
Coincidental illness	1

vision of a special parturition environment. When the bitches were returned to their accustomed quarters they proceeded to whelp. Occasionally, bitches appear to be frightened by labour pains and voluntarily inhibit straining; tranquillising drugs are helpful in such cases.

Hysteria

In the study of 200 porcine dystocias previously referred to (Jackson, 1972), there were six cases in which the sows were so excitable and aggressive that they were apparently unable to continue normal parturition. The use of the sedative azaperone was followed by a resumption of normal farrowing. This is also recognised to be a greater problem in gilts; thus if a large number are scheduled to be in the farrowing house at a particular time then it is customary to include some older farrowing sows at the same time, as they seem to exert a calming effect.

Secondary inertia

This is the inertia of exhaustion and is essentially a result, rather than a cause, of dystocia due to some other cause, usually of an obstructive nature. Nevertheless, in polytocous species, prolonged unsuccessful efforts to deliver one fetus may result in dystocia from inertia with regard to the remainder. Secondary inertia is frequently followed by retention of the fetal membranes and retarded involution of the uterus, factors which predispose to puerperal metritis.

Secondary inertia is met with in all species and, speaking generally, is a preventable condition. Its prevention depends on the early recognition that labour has ceased to be normal, and the application of the appropriate assistance. Sometimes in the bitch and queen cat, normal parturition will commence but after expulsion of a few pups or kittens will then cease, even though there is no obstruction. Linde-Forsberg and Eneroth (1998) refer to this as 'primary partial inertia', and identify it as a major cause of dystocia responsible for about 23% of the cases in both species in their study. It is very similar to the classical uterine inertia associated with large litters, and the author finds it difficult to distinguish between the two. If there has been an obstructive dystocia, which has been corrected and normal parturition has failed to resume, then this is clearly secondary uterine inertia.

In the monotocous species, correction of the dystocia which provoked the inertia is the essential feature of treatment. If this involves correction of faulty disposition, then the fetus should be removed by traction immediately. In the polytocous species, management of the case will depend on the duration of labour, the number of fetuses still unborn and their condition. In an early case, delivery of the fetus causing the primary dystocia may be followed after a few hours by a return of uterine contractions and parturition may proceed without further hindrance. Such is often the case in the sow and occasionally in the bitch and cat. When the case is of longer duration, and there are still several young to be born, it is best to proceed with the delivery of the remainder. In the sow, it is often possible to do this with the hand inserted into the uterus per vaginam. In the bitch it may be decided to attempt forceps delivery, but the protracted use of forceps when three or four fetuses remain unborn has very little to commend it. Calcium borogluconate and oxytocin therapy, as recommended for the treatment of primary uterine inertia, should also be tried despite the cause of the inertia apparently being due to 'myometrial exhaustion'. This is because there may be other underlying factors involved of which we are unaware. Since the fetuses will soon die, or may already be dead, an early decision on performing a caesarean operation or hysterectomy is important.

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11 Fetal dystocia: aetiology and incidence

The two broad divisions of fetal dystocia are fetomaternal disproportion and faulty fetal disposition (see Figure 8.1). Traditionally, the former type of dystocia was referred to as fetal oversize, with relative oversize being considered to occur when the fetus was of normal size for the species/breed but the birth canal was inadequate, and absolute oversize when the fetus was excessively large, including some fetal monsters (see Chapter 4). The reason for the change is that sometimes it is difficult to differentiate between the two catagories of oversize, or the dystocia is due to a combination of both.

FETOMATERNAL DISPROPORTION

Fetomaternal disproportion is a common cause of dystocia which is highly species- and breed-related. In Chapter 8, under the section entitled 'Types of dystocia within species', you will have seen that, whilst fetomaternal disproportion is a major cause of dystocia in cattle and to a lesser extent the dog and cat, nevertheless it can occur in all species if the circumstances are right. Simplistically, fetomaternal disproportion occurs if the fetus is larger than normal – it might simply be one of increased mass or conformation – or the pelvic canal is too small or the incorrect shape.

Cattle

Since fetomaternal disproportion is the commonest cause of dystocia in cattle, particularly in heifers, it is not surprising that there is a very extensive literature on the subject extending over many years. Despite having dismissed the use of the traditional divisions of fetal oversize in favour of the all-embracing concept of fetomaternal disproportion, in discussing the aetiology of the disorder we will firstly consider those factors that are associated with the development of a larger-than-normal fetus, and secondly those factors that influence the ability of the dam to give birth to a normal fetus.

Calf birth weight

In a fundamental consideration of fetal development it must be remembered that the fetus grows by both hyperplasia and hypertrophy of its constituent tissues. Prior and Laster (1979) have shown that in cattle, growth by hyperplasia is more important in early gestation, but decreases rapidly towards the end of pregnancy, whereas growth by hypertrophy continues to increase with advancing gestation. Retardation of growth at any stage of gestation would have a permanent effect on postnatal development, but because the relative proportion of growth by hyperplasia gets smaller as fetal age increases, retardation of growth in late gestation has less effect on subsequent postnatal development. Actually, the growth by hyperplasia that does occur in late gestation is mainly in muscle. Prior and Laster (1979) and Eley et al. (1978) found that bovine fetal growth was fastest at 232 days of gestation, but the two research groups' findings differed in the amount of the daily increase, 331 g and 200 g, respectively. By the end of gestation, the increase in fetal weight had declined to 200 g daily. The first group also ascertained that, when pregnant heifers were fed varying diets to produce low, medium and high maternal weight gains there was no resultant difference in fetal birth weights among the three categories.

Calf birth weight is the single most important factor affecting the incidence of dystocia (Meijering, 1984; Morrison et al., 1985; Johnson et al., 1988). Each kilogram increase of birth

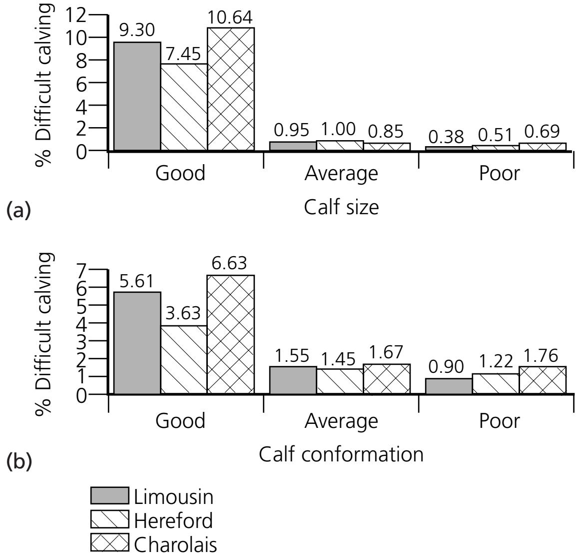

Fig. 11.1 The relationship between the incidence of difficult calvings and (a) calf size and (b) calf conformation, for calves sired by Limousin, Hereford and Charolais bulls (from McGuirk et al., 1998b).

weight increased the rate of dystocia by 2.3%. The larger the calf, the greater the chance of a difficult calving (Figure 11.1 and Table 11.1). A number of factors have been shown to affect calf birth weight; they are as follows.

Breed of sire. In cross-breeding programmes, where beef sires are used on dairy heifers and cows, the selection of the most appropriate sire breed is important for ease of calving and low calf mortality rates. There are some interesting effects of cross-breeding which are shown in some classical studies reported nearly 50 years ago. In general it has been found that when the parents are of disparate size, e.g. Friesian bull and Jersey cow, the birth weight of the cross-bred Friesian–Jersey calf is near the mean of the body weight for the purebred Friesian and purebred Jersey calves. When the reciprocal crosses are made, however, it can be seen that the dam exerts an influence towards its own birth weight. Hilder and Fohrman (1949) demonstrated this influence on calf birth weight for Friesian–Jersey crosses (Table 11.2), and Joubert and Hammond (1958) demonstrated it for South Devon–Dexter crosses (Table 11.3). Some more recent examples are cited below.

In the USA, Laster et al. (1973) surveyed dystocia rates and subsequent fertility following the mating of 1889 Hereford and Angus cows to bulls of the Angus, Charolais, Hereford, Jersey, Limousin, Simmental and South Devon breeds. Calves sired by the Simmental, South Devon, Charolais and Limousin bulls caused significantly more dystocia – 32.66, 32.34, 30.9 and 30.78%, respectively – than calves sired by Hereford,

Table 11.1 Degree of dystocia in 220 2-year-old Hereford heifers, according to yearling pelvic area, calf birth weight and pelvic area:birth weight (from Deutscher, 1985)

	Degree of calving difficulty[a]				
	1	*2*	*3*	*4*	*5*
Yearling heifer pelvic area (cm^2)	146	141	138	142	132
Calf birth weight (kg)	31.4	32.7	34.1	37.7	36.8
Pelvic area:birth weight[b]	2.1	1.9	1.8	1.7	1.6

[a] Calving difficulty scoring system: 1 = no assistance; 2 = slight assistance; 3 = moderate; 4 = much assistance; 5 = caesarian operation.
[b] Yearling pelvic area divided by calf birth weight equals ratio

Table 11.2 Influence of parent on birth weight (after Hilder and Fohrman, 1949)

Parent	*Female calves (kg)*	*Male calves (kg)*
Purebred Friesian	43.4	
Purebred Jersey	25.0	
Calculated mean birth weight	34.2	
Observed Friesian bull X Jersey cow	33.9	34.5
Observed Jersey bull X Friesian cow	34.7	37.1

Table 11.3 Influence of parent on birth weight (after Joubert and Hammond, 1958)

Parent	*Calf weight (kg)*
Purebred South Devon	45.4
Purebred Dexter	23.7
Calculated mean	34.5
Dexter bull X South Devon cow	33.2
South Devon bull X Dexter cow	26.7

Angus and Jersey bulls, 15.78, 9.9 and 6.46%, respectively. In the study by McGuirk et al. (1999), the easiest-calving sire breeds in heifers were the Belgian Blue and Aberdeen Angus, and the most difficult were the Blonde d'Aquitaine, Simmental and Piedmontese whereas for cows the easiest were the Hereford and Aberdeen Angus and the most difficult were the Blonde d'Aquitaine, Simmental and Charolais (Table 11.4). The results in heifers for the Belgian Blue sires was very surprising, since muscular hypertrophy or 'double muscling' is commonly seen in this breed; however, the number of sires from this breed were small, and perhaps the dams were selected for good size. For practical animal breeding one would never recommend the use of a sire of this breed on heifers. In this inherited anomaly, there is excessive development of muscles, particularly of the hindquarters but also of the loins and forequarters; the skin is thin and the limb bones tend to be shorter. It is of varying severity, and is favourably regarded by both farmers and butchers because of the greatly increased proportion of meat in the carcass. When marked, however, it is the cause of severe dystocia, particularly in heifers. Muscular hypertrophy has been described in the South Devon breed by MacKellar (1960), and it is well known in the Belgian Blue, Charolais, Piedmontese and White Flanders breeds. Mason (1963) has described it in the grandsons of a Friesian bull imported into Britain. Vandeplassche (1973) has stated that 50% of oversized calves in Belgium are due to double muscling, and that the condition is a frequent indication for the caesarean operation in Holland, Belgium and France.

Table 11.4 Actual incidence of difficult calvings according to breed of sire and parity of dairy cows and heifers (after McGuirk et al., 1998b)

Sire breed	*% Incidence*	
	Heifers	*Cows*
Aberdeen Angus	3.5	2.8
Belgian Blue	1.1*	3.1
Blonde d'Aquitaine	8.1	3.8
Charolais	5.8*	3.8
Hereford	5.0	1.3
Limousin	6.3	2.1
Piedmontese	10.2	2.8
Simmental	8.3	3.8
Mean	**6.0**	**2.9**

*Relatively small number of data

Parity of dam. A very simple rule is: the bigger the dam, the bigger the calf. This is very apparent between breeds, but it also occurs within breeds with heifers giving birth to smaller calves than parous cows (Table 11.5). This is well illustrated in a study involving Holsteins over an 18-year period by Sieber et al. (1989), in which the mean ± standard deviation of calves born to first-parity animals was 37.9 ± 4.4 kg, compared with 39.7 ± 5.8 kg for second-parity animals; other body dimensions were also lower Table 11.6).

Sex of calf. Many studies have shown, irrespective of breed, that the birth weights of male

Table 11.5 Average birth weights (kg) of various breeds of cattle (after Legault and Touchberry, 1962)

	Ayrshire	*Brown Swiss*	*Guernsey*	*Holstein*	*Jersey*
Number	213	163	154	587	117
Average	36.4	46.4	32.5	42.9	24.7
Males	38.2	48.4	34.4	44.4	25.7
Females	34.6	44.2	30.6	41.6	23.4
First calving	35.2	44.2	31.7	40.7	22.6
Second calving	36.6	48.3	32.5	44.2	25.7
Third calving	38.1	47.7	31.6	44.6	25.9
Fourth and subsequent calvings	38.1	48.1	33.3	43.2	24.8

Average weights for Aberdeen Angus, Charolais and Hereford calves are 27.1, 47.5 and 32.6 kg, respectively

Table 11.6 The effect of parity on calf birth weight and ease of calving (after Sieber et al., 1989)

Parameter	*Parity*				
	1	*2*	*3*	*4*	*>5*
	% Of cases				
Body weight of cow (kg)	49.4	53.2	60.8	59.3	62.1
Birth weight of calf (kg)	4.4	5.8	5.3	6.2	5.4
Types of calving assistance					
None	48.3	79.9	82.7	82.8	86
Manual	3.9	3.4	4.8	4.7	2.7
Manual with chains	42.9	16.5	12.6	11.8	10.7
Mechanical	4.9	0.2	0	0.6	0.7

calves are greater than female calves (Table 11.2). The increased birth weight is associated with an increased incidence of dystocia and an associated increase in calf mortality (Table 11.7).

Seasonal and climatic factors. Several studies have shown the influence of season of year and environmental factors such as mean air temperature on birth weights and hence the incidence of dystocia. In a retrospective study over 3 consecutive years involving cross-bred heifers, Colburn et al. (1997) found that the mean spring birth weights of calves born after a warmer than normal winter were 4.5 kg lower than those following a cold winter; the corresponding levels of calving difficulty were 35% and 58%, respectively. One hypothesis for this finding is that, during cold winters, there is increased uterine blood flow which results in an increased nutrient supply to the fetus.

Table 11.7 Effect of calf birth weight and sex on the incidence and severity of dystocia, and calf mortality in American Angus heifers (after Berger et al., 1992)

*Dystocia/Mortality scores**		*1*	*2*	*3*	*1*	*2*	*3*
Birth weight	*Sex*	*% Dystocia*			*% Calf mortality*		
20 kg	M	86.4	11.4	2.2	95.7	1.0	3.2
	F	92.4	6.5	1.1	97.1	0.7	2.2
21–25 kg	M	92.9	6.1	1.0	94.7	0.6	4.8
	F	95.5	4.0	0.5	96.6	0.5	2.8
26–30 kg	M	89.3	9.3	1.3	96.9	0.4	2.6
	F	92.7	6.5	0.7	97.7	0.3	2.0
31–35 kg	M	79.9	16.5	3.5	96.9	0.8	2.2
	F	86.6	11.2	2.1	97.5	0.6	1.8
36–40 kg	M	61.1	28.3	10.6	94.9	2.2	2.9
	F	69.0	23.8	7.2	96.2	1.8	2.0
40 kg	M	38.3	32.4	29.4	87.2	7.7	5.1
	F	48.6	30.7	20.7	90.6	5.5	3.8

*Dystocia scores: 1 = no assistance; 2 = some assistance; 3 = major difficulty.
Mortality scores: 1 = weaned or sold alive; 2 = dead within 24 hours; 3 = dead preweaning

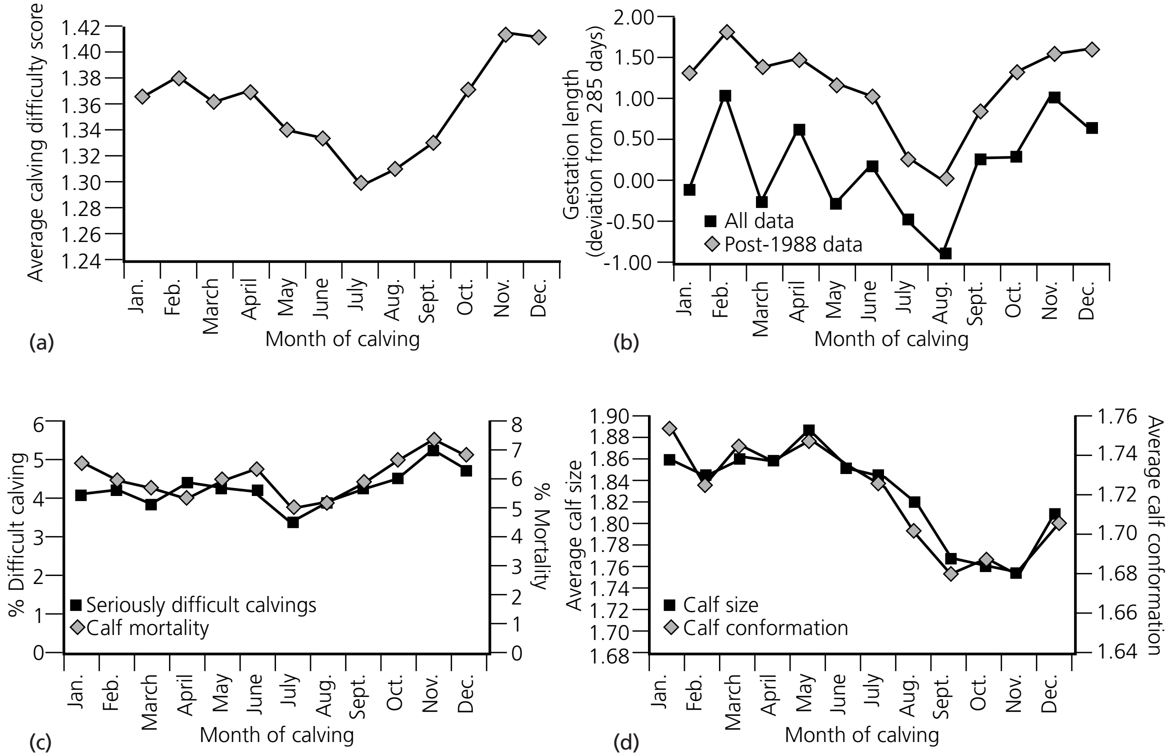

Fig. 11.2 Month of calving effects on (a) calving difficulty score; (b) gestation length; (c) the incidence of seriously difficult calvings and calf mortality; (d) calf size and conformation (from McGuirk et al., 1998a).

This may explain the results of McGuirk et al. (1998a), who found when evaluating data on the effect of beef sires on dairy cows that calf size and calf conformation declined in autumn and early winter, which showed some correlation with the average calving difficulty score and gestation length (Figure 11.2). A similar trend was also observed in dairy herds where Holstein–Friesian sires were used (McGuirk et al., 1999) (Figure 11.3). The reduction in gestation length and increased calving difficulty were slightly out of phase with, and preceded, increase in calf size (Figure 11.3).

Nutrition of the dam

During the last decade, there has been considerable interest in all species, including man, concerning the influence of maternal nutrition during pregnancy on development and health after birth, as well as on birth weight; surprisingly, much of this is associated with the influence of under nutrition during the early stages of gestation when the placenta is developing. Since the placenta controls the transfer of nutrients from dam to fetus, anything that impairs its function will inevitably result in reduced fetal growth and development. There is evidence that in ruminants, for example, the conformation of the placentome changes in an attempt to compensate for the undernutriton and to provide the fetus with adequate nutrients for normal growth and development.

It is difficult to evaluate the literature concerning the effects on fetal weight of variations in the maternal nutrition, because much of it is contradictory. The motivation for this research is mainly economic because birth weight is positively correlated with postnatal weight gain and with the subsequent achievement of commercially desirable slaughter weights of food animals. In the obstetrical context, the concern over birth weight is twofold; firstly, large fetuses contribute to dystocia and, secondly, undersized offspring are more prone to neonatal death and disease. Therefore, while it is reasonable to explore how birth weight may be controlled so as to reduce dystocia, any severe reduction in fetal birth weight, achieved by

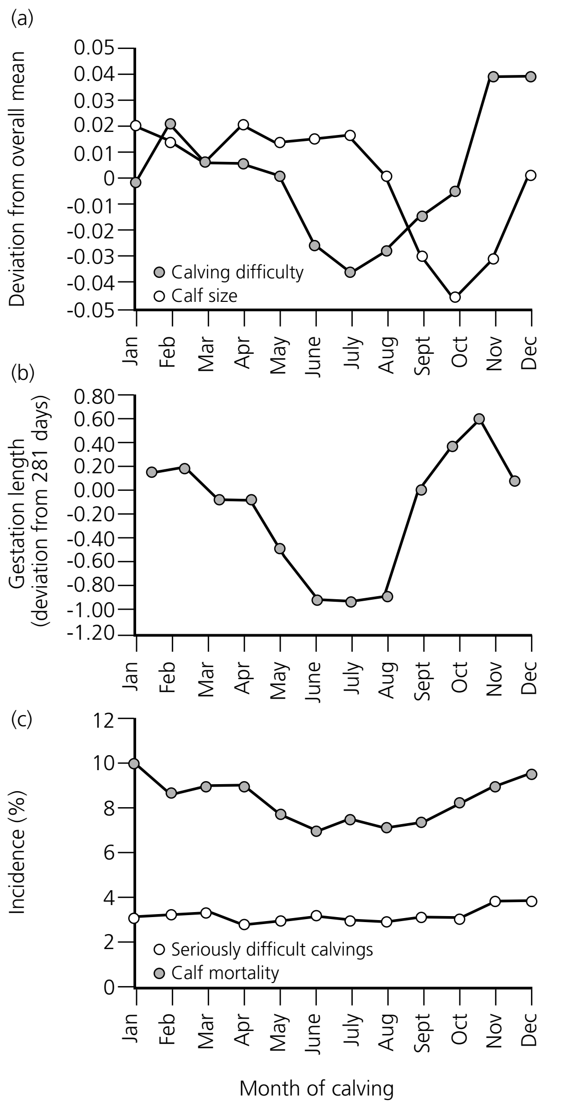

Fig. 11.3 Month of calving effects on (a) calving difficulty and calf size scores; (b) gestation length; (c) the incidence of difficult calvings and calf mortality (from McGuirk et al., 1999).

manipulation of the maternal diet, may place the neonate in jeopardy. It is perhaps best summarised in the statement by Eckles (1919) that the weight of the calf at birth is not ordinarily influenced by the ration received by the dam during gestation, unless severe nutritional deficiencies exist. It is only during the last 90 days of gestation that severe restriction of maternal nutrition, resulting in failure of the dam to maintain body weight, reduces fetal birth weight, the reduction in weight being due to a reduction in fetal muscle mass. Studies have investigated the effects of both energy and protein deprivation of the dam, either alone or together, and have found the results to be variable, with some evidence of both having an effect. Energy intake appears to have a greater influence than protein. An example of one such study is that of Tudor (1972), who fed two groups of cows from 180 days of gestation to term so that one group gained and the other group lost weight. Mean calf birth weights were 30.9 and 24.1 kg for the high- and low-nutrition cow groups. In another experiment, in which cows lost 17.5% of their body weight during the last trimester, the birth weight of the calves was on average 12.9% less.

Length of gestation. Certain fetal calf developmental abnormalities, such as hypophyseal and adrenal-cortex hypoplasia or aplasia, have been associated with prolonged gestation for reasons related to the initiation of parturition, as described in Chapter 6. However, even with normal calves there are substantial variations in gestation length. Many of these are breed-dependent (Table 11.8) and the influence is also seen when cross-breeding occurs (Tables 11.9 and 11.10); the increased gestation length is associated with higher birth weights and an increased chance of dystocia. Male calves, which are heavier than female calves (see above), are usually associated with a longer gestation period of a few days. A mean difference of 1.4

Table 11.8 Gestation length and birth weights of different breeds of cattle (from Noakes, 1997)

Breed	*Average gestation length (days)*	*Average birth weight(kg)*
Aberdeen Angus	280	28
Ayrshire	279	34
Brown Swiss	286	43.5
Charolais	287	43.5
Friesian/Holstein	279	41
Guernsey	284	30
Hereford	286	32
Jersey	280	24.5
Simmental	288	43
South Devon	287	44.5

Table 11.9 Gestation length and birth weights of calves of purebred and reciprocal crosses of Angus and Hereford cattle (after Gerlaugh et al., 1951)

Breed	*Gestation length (days)*	*Birth weight (kg)*
Calves from Angus cows		
Male purebred	277.2	28.3
Male crossbred	282.7	29.8
Female purebred	275.7	25.4
Female crossbred	281.1	28.4
Calves from Hereford cows		
Male purebred	287.5	31.3
Male crossbred	283.1	30.3
Female purebred	285.2	30.7
Female crossbred	283.5	28.4

Table 11.10 Variations in gestation length in several cattle breeds (after Gerlaugh et al., 1951)

Breed	*No. of gestation periods**	*Average gestation length**
Purebred Angus	101	276.47
Purebred Hereford	100	286.28
Hereford bull X Angus cow	94	281.98
Angus bull X Hereford cow	102	283.30

* Male and female calves

days was seen in the study by McGuirk et al. (1998b) involving beef sires and dairy dams. However, when the values were examined in relation to breed of sire, in Aberdeen Angus and Hereford cross-breeds the sex difference was 0.64 and 1.04 days, respectively, whereas in Blonde d'Aquitaine, Limousin, Charolais and Simmental cross-breeds the differences exceeded 1.5 days. In this study, gestations were shorter in summer and longer in winter. The relationship between gestation length and calving difficulty and calf mortality is shown in Figure 11.4. Minimum incidences of difficult calvings occurred in gestations that were shorter than the overall average but then increased with longer gestations. In a similar study involving Holstein–Friesian sires and dairy dams, longer gestations were associated with larger calves (negative regression coefficient – $P < 0.05$) and the optimum gestation length for low calving difficulty was 3 days below the overall average. See also Figure 11.5.

In vitro maturation and fertilisation. The use of in vitro maturated (IVM) and in vitro fertilised (IVF)-derived embryos has increased

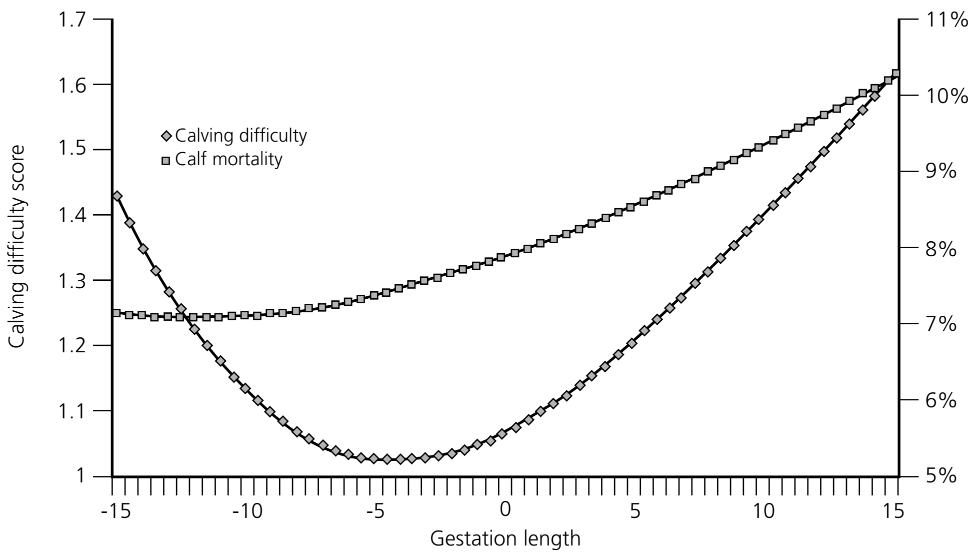

Fig. 11.4 The relationship between calving difficulty score and calf mortality with gestation length. The predictions for calf mortality have been converted to percentages (from McGuirk et al., 1998b).

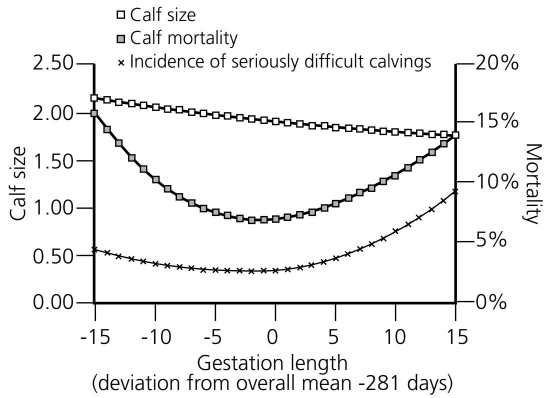

Fig. 11.5 The effect of gestation length on calf size, the incidence of seriously difficult calvings and calf mortality (from McGuirk et al., 1999).

substantially in recent years. These have been obtained following aspiration of oocytes from follicles in vivo or after slaughter. There are numerous reports that the birth weight of calves originating from this source is greater than those following normal artificial insemination (AI): for example, 51 kg vs. 36 kg (Behboodi et al., 1995), a 4.5 kg higher birth weight (Kruip and den Haas, 1997), a 10% increased birth weight (Van Wagtendonk de Leeuw et al., 1998). Some of the increase appears to be due to a longer gestation period: for example, +3 days (Van Wagtendonk de Leeuw et al., 1998), +2.3 days (Kruip et al., 1997). The result of this is an increase in the dystocia rate: for example, +25.2% (Kruip et al., 1997) and 62% (Behboodi et al., 1995) compared with 10% for AI-derived calves. Associated with the increased dystocia rate was a rise in calf mortality rate. Others have not identified such a problem (Penny et al., 1995). The reason for the large calves derived from IVM and IVF is probably related to the constituents of the media used in the procedure.

Body condition score of the dam. There is a direct relationship between body condition score and calf birth weight (Spitzer et al., 1995); this is discussed below in relation to maternal factors.

Fetal numbers. Cattle are normally monotocous, with twinning occurring in about 1–2% of births, although in some instances up to 8% has been recorded. The birth weights of twin calves are on average 10–30% lower than the single-born contemporaries, with a greater reduction in those born to heifers.

Calf conformation

Many studies have identified the influence of calf birth weight on ease of calving (see above). However, the ability of a calf to be expelled unaided through the birth canal at parturition is dependent on its shape or conformation. This is seen in the most extreme situation of some fetal monsters (see Chapters 4 and 17), such as fetal duplication, schistosomes, ascitic and anasarcous calves, where the weight of the fetus is low but the conformation prevents normal expulsion.

Attempts have been made to assess the conformation of normal calves, and to correlate this with ease of calving. Such methods have involved asking the farmer to assess the conformation of the calf as good, average or poor, and then applying a numerical score from 1 to 3 to each subjective value (McGuirk et al., 1998a). Others have made a large number of fetal anatomical measurements, such as head circumference, foot circumference, width of shoulders, width of hips, depth of chest, body length, cannon bone length and diameter (Nugent et al., 1991; Colburn et al., 1997). Using the simple approach, McGuirk et al. (1998a) found a statistically significant difference between calf conformation and incidence of difficult calvings and calf mortality (Figure 11.1). In summary, well-muscled calves born from a beef sire and dairy cow or heifer resulted in more difficult calvings and increased calf mortality.

Using the more sophisticated measurements, the results have been disappointing and contradictory. Meijering (1984) and Morrison et al. (1985) found that there were no differences in the effect of calf body measurements, independent of birth weight, on ease of calving. Nugent et al. (1991), in investigating the relationship between calf shape and sire expected progeny difference (EPD) or ease of calving found that at constant birth weight calves from higher birth weight EPD bulls tended to have larger head and cannon bone circumferences. However, at constant birth weight, body measurements were not associated with calving ease. In conclusion, they stated that calf shape seemed to add no information for the prediction of dystocia, other than that provided by birth weight EPD.

Maternal factors

Parity of the dam. Withers (1953), in a British survey, reported that dystocia was almost three times as common in heifers as in cows. In 6309 pregnancies in cows, difficulty in calving occurred in 1.38%, and in 2814 in heifers difficulty occurred in 3.8%. In a study of 345 bovine dystocias in the USA, 95% of which were in beef cattle, Adams and Bishop (1963) found that 85% of all the dystocias were in heifers, and they were classified as follows: excessive calf size 66%, small maternal pelvis 15% and combination of the two 19%. The younger the heifer, the higher is the dystocia rate (Lindhé, 1966). As would be expected, the stillbirth rate was much higher in heifer (6.7%) than in cow parturitions (2.4%). In a survey involving 75 000 calvings following the use of 685 Holstein–Friesian dairy bulls as AI sires in the UK (McGuirk et al., 1999), the following data were obtained. Calves born to heifers compared to those born to cows had higher calving difficulty scores (1.35 vs. 1.16), a higher incidence of serious difficult calvings (4.80 vs. 1.64), shorter gestations (280.4 vs. 281.3) and higher mortality (9.5% vs. 7.2%). Similarly, when comparisons were made between heifers and cows (88 000 calvings) when beef sires were used, then the mean predicted incidences of seriously difficult calvings were 6.64% and 2.12%, respectively (McGuirk et al., 1998a). After the transition from first to second, the differences between subsequent parities were very small (Sieber et al., 1989), with the percentage of unassisted calvings 48.3% in heifers, and 79.9%, 82.7%, 82.8% and 86% in second, third, fourth and fifth or more parities, respectively (Table 11.6). Similar results were obtained by Legault and Touchberry (1962 – Table 11.5).

Condition score of the dam. It is generally accepted that heifers or cows in a very high condition score are more likely to suffer from dystocia than those that are moderate to poor, the reason being that those in very good condition will have a substantial amount of retroperitoneal pelvic fat, which will reduce the size of the birth canal. Studies in beef heifers have shown that body condition score had no influence on the dystocia rate (Spitzer et al., 1995). However, in this study, comparisons were made between heifers with scores of 4, 5 and 6; given that 1 = emaciated and 9 = obese, the heifers were all in mid-status, and thus it is not possible to extrapolate to the extremes. One noticeable feature about this study was that condition score at calving influenced birth weight, although this might have been a direct effect of nutritional intake; at condition scores 4, 5 and 6 the mean ± sem (standard error of mean) body weights of the heifers were 338 ± 4, 375 ± 3 and 424 ± 424 kg, and birthweights for the calves were 28.9 ± 0.5, 30.4 ± 0.4 and 32.4 ± 0.7 kg, respectively.

Pelvic capacity of the dam. In dystocia due to fetomaternal disproportion, as well as fetal birth weight the other variable is maternal pelvic size, i.e. the area of the pelvic inlet (dorsovental × widest bisiliac dimensions), which was, according to Wiltbank (1961), a much better parameter for the prediction of dystocia than any fetal measurement. There are variations between the breeds in respect of the ratio of the calf weight at birth to maternal weight as follows: Friesian 1:12.1, Ayrshire 1:12.6 and Jersey 1:14.6. When a Friesian bull was used on Friesian, Ayrshire and Jersey cows the ratios of calf weight to maternal weight were Friesian 1:12.1, Ayrshire 1:11.3 and Jersey 1:11.1. Although the Friesian–Jersey calves were larger in proportion to their dams than purebred Friesian calves, the incidence of dystocia with the purebred Friesian calves was about three times the incidence for the Friesian–Jersey calves. These data indicate that the Jersey cow has a more favourable pelvic capacity than the Friesian.

Since then, a number of reports have advocated the value of measuring the pelvic area as a method of predicting the ease of calving both in the short term and in relation to genetic selection (Derivaux et al., 1964; Rice and Wiltbank, 1972; Deutscher, 1985). Measurements are made transrectally using callipers, which can be difficult in some circumstances. For this reason, the validity of the measurements and hence the whole concept has been criticised (Van Donkersgoed et al., 1990). In a study involving Hereford heifers, selection of suitable animals for breeding was made following the measurement of pelvic

Table 11.11 Estimated deliverable calf birth weight using pelvic measurements (from Deutscher, 1985)

Time of measurement	*Heifer's age (mo)*	*Heifer's weight (kg)*	*Pelvic area (cm^2)*	*Pelvic area:birth weight ratio*	*Estimated calf birth weight (kg)*
Before breeding	12–14	250–318	120	2.0	27.3
			140	2.0	31.8
			160	2.0	36.4
At pregnancy detection	18–19	318–386	160	2.5	29.1
			180	2.5	32.7
			200	2.5	36.4
Before calving	23–24	364–432	200	3.1	29.5
			220	3.1	32.3
			240	3.1	35.0

dimensions transrectally, and the calculation of the pelvic area (Deutscher, 1985). Table 11.1 shows the degree of dystocia in 220 Hereford heifers in relation to yearling pelvic measurements, calf birthweight and pelvic area:birth weight ratio. If pelvic area measurements are made before service, then those with a small pelvic canal can be rejected for breeding or inseminated with semen from an easy calving bull, whilst those with a larger pelvis can be bred to an average calving bull. Table 11.11 shows the estimated deliverable calf birth weight using pelvic measurements.

Pelvic area is moderately to highly heritable (about 50%), and thus can be used as a measurement in the genetic selection of breeding stock. There is also interest in the use of pelvimetry in bulls in an attempt to select sires who have a large pelvic area which might then be inherited by their female progeny. Results obtained so far have been equivocal (Kriese et al., 1994; Crow et al., 1994). In recent years in the USA, dairy replacement heifers have been fed growth promoters, which has increased their pelvic area dimensions.

Prevention of dystocia due to fetomaternal disproportion

Since we are aware of most of the reasons for fetomaternal disproportion as a cause of dystocia in cattle, good veterinary practice should attempt to prevent it occurring. The following guidelines have been proposed by Drew (1986–87) in relation to the breeding of Holstein–Friesian heifers in the UK.

Management at service

- Ensure body weight at the time of service is more than 260 kg.
- Take care when selecting the service sire:
  - If artificial insemination bulls:
    Select a well-proven bull of high genetic merit.
    Select a bull which has been used successfully on heifers on several farms or, if this is not possible, one with a below average incidence of calving difficulties and gestation length when used on cows.
  - If natural service bulls:
    Avoid bulls of large breeds.
    Select a bull with a record of easy calvings or, if this is not possible, one with a sire with a good record.

Management before calving

- Adjust feed levels to avoid calving in an overfat condition.
- Restrict energy intake in the last 3 weeks of pregnancy.
- Check iodine and selenium levels if calf mortality has been high in previous years.
- Ensure supplementary magnesium is provided.
- Ensure that an adequate exercise area is available.
- Observe the heifers at least four or five times daily during the last 3 weeks of pregnancy, especially if short-gestation-length bulls are used.

- If possible, run as a heifer group or with dry cows. If fed with the milking cows ensure 'parlour feed' is restricted to the amount required to acquaint the heifer with her postcalving diet.

Management at calving

- Calve grazed heifers in their field or paddock if possible. Housed heifers should calve in familiar surroundings. Avoid moving them to a calving box unless essential for adequate assistance.
- Ensure the field is well fenced to avoid the possibility of heifers rolling into positions from where it is difficult to assist.
- Observe hourly (approximately) when calving starts. Too frequent observations (more frequently than half-hourly) can delay calving.
- Be a good stockperson. Watch for signs of fear, abnormal pain or distress and be ready to assist if these are noted or if calving is prolonged.
- Ensure that the stockpersons are trained to identify potential problems and know when to call professional help. If calving aids are used, instruction should be given as to the correct method of application.
- Call professional advice if an unusually high percentage of the first heifers to calve require assistance – there may be a herd problem which will affect the whole group.

In the case of cross-breeding or pure-breeding calves for beef production, the same principles apply. Thus:

- With well-grown heifers, when breeding purebred replacements, select sires on their ease-of-calving records and normal (i.e. not unduly long) gestation lengths for the particular breed.
- In cross-breeding for beef production from dairy herds: avoid sires of the larger breeds such as Simmental and Charolais for the heifer inseminations, and use instead a known 'easy-calving' Aberdeen Angus or Hereford bull. For second and later parities choose a bull of a larger breed on his ease-of-calving record and gestation length.
- In beef production from beef breeds. For heifer pregnancies use either a sire of a smaller beef breed or a within-breed sire of good ease-of-calving record and gestation length. For later parities use a bull either of the same or larger breed – both based on the calving ease and gestation length.

While applying the above principles in the production of offspring for beef, whether purebred, or cross-bred, it should be noted that the weight of the calf at birth, assuming equal gestation lengths, bears a direct relationship to its weaning weight and to its subsequent slaughter weight, on which the profitability of the enterprise largely depends. On the other hand, unduly large calves at birth predispose to calf deaths and to maternal morbidity, mortality, reduced milk yield and infertility. Thus a breeder must consider how much increase in birth weight can be tolerated in return for increases in growth rate and weaning weight.

If dystocia due to fetomaternal disproportion is anticipated, then gestation can be shortened by the premature induction of calving; this is described in Chapter 6.

Sheep

You will have seen in Chapter 8 (Table 8.9) that dystocia due to fetomaternal disproportion is an important cause of dystocia in sheep. Despite this, there is far less published on the topic in comparison with cattle; this is probably a reflection of the relative values of both dam and newborn offspring. As in cattle, fetomaternal disproportion occurs as a result of a large lamb or a small pelvis, and sometimes the simultaneous combination of both.

Lamb birth weight

Similar factors influence lamb birth weight as those described above for cattle. There is a substantial variation in the birth weights of the different breeds of sheep; these are shown in Table 11.12. The average weights (singletons and twins) ranged from 2.9 kg for Welsh Mountain to 5.8 kg for the Border Leicester. The effect of cross-breeding is shown in the study by Hunter (1957). The results he obtained by reciprocal crossing between one of the heaviest breeds, the Border Leicester, with one of the lightest breeds, the Welsh Mountain, are shown

Table 11.12 Mature ewe and newborn lamb mean body weights of some different breeds of sheep

Breed	*Mating weight of ewe (kg)*	*Birth weight of lamb* (kg)*
Scottish Blackface	54	3.8
Welsh Mountain	35	2.9
Clun Forest	60	4.3
Dorset Horn	72	4.3
Romney Marsh	71	4.7
Border Leicester	83	5.8
Texel	79	5.0
Suffolk	83	5.15
Oxford	89	5.6

* Unweighted averages of single, twin, male and female lambs

in Table 11.13. The influence of the uterine environment on fetal development was shown by means of reciprocal transfers of fertilised eggs between sheep breeds of disparate size. Hunter (1957) and Dickenson et al. (1962) have been able to show the relative influence on birth weight of prenatal environment (phenotype) and the genotype of the lamb. In Hunter's work on Border Leicester and Welsh Mountain breeds, the mean birth weight of Border Leicester lambs born to Welsh Mountain ewes was 1.13 kg less than that of Border Leicester lambs born to Border Leicester ewes; also, the birth weight of Welsh Mountain lambs born to Border Leicester ewes was 0.56 kg more than that of Welsh Mountain lambs born to

Table 11.13 Effect of breed on birth weight (from Hunter, 1957)

Ewe	*Ram*	*Weight of lambs (kg)*	
		Singles	*Twins*
Border Leicester	Border Leicester	Male 6.6 Female 5.9	Female 5.2
	Welsh Mountain	Female 5.9	Male 5.2 Female 4.3
Welsh Mountain	Border Leicester	Male 4.9 Female 4.9	Male 4.3 Female 3.8
	Welsh Mountain	Male 3.8 Female 3.7	Male 4.0 Female 3.4

Welsh Mountain ewes. Thus the maternal influence can limit the size of a genetically larger lamb, as well as increase the size of a genetically smaller lamb. Also, the size limitation imposed on Border Leicester lambs by the Welsh Mountain maternal environment was greater than the size increase produced in Welsh Mountain lambs by the Border Leicester maternal influence. The use of tups of the Welsh Mountain breed as sires for ewe lambs of breeds such as the Texel in their first breeding season can reduce the incidence of dystocia, and at the same time produce a lamb with hybrid vigour and good survival rates.

In reciprocal crossing between the (large) Lincoln and (small) Welsh Mountain breeds, Dickenson et al. (1962) found that no lambing difficulties occurred in Lincoln ewes, but in 13 Welsh ewes carrying Lincoln lambs, eight needed assistance at birth. In another experiment, fertilised eggs from pure Lincoln and from pure Welsh donors were transferred to Scottish Blackface ewes. Of 36 Lincoln lambs 16 required obstetric assistance, while only one of 28 Welsh lambs was associated with dystocia. The results of the egg transfer experiments showed that:

- Lambs of the same breed (genotype) differed in birth weight according to whether their uterine environment (phenotype) was Lincoln or Welsh.
- Lambs reared in the same uterine environment differed in birth weight according to whether their genotype was Lincoln or Welsh.
- Both genotype of lamb and maternal environment had significant effects on the birth weight of the lambs.
- The genotype influence was three or four times as great as the maternal influence on lamb birth weight.

As in cattle, male lambs are heavier than female lambs, the difference being about 5%, and twins are about 16% lighter at birth than singletons (Starke et al., 1958). The effect of selective breeding, based on line breeding to a particular strain of Romney sheep which the owner considered produced lambs of low birth weight with less difficulty at lambing, and the culling of ewes that repeatedly suffered from dystocia, substantially reduced the incidence of dystocia (McSporran et al., 1977). Until 1970, between 20% and 31% of ewes required assistance at lambing; this fell to 18% in 1971, 11% in 1972, 3.3% in 1973, and 4.0% in 1974.

The influences of dietary restriction of the ewe during pregnancy on fetal growth and lamb birth weight are variable and the results from studies often contradictory. Whereas dietary restriction during the last trimester, when fetal growth is greatest, has been shown to reduce birth weights particularly if dietary intake falls below that required by the ewe for maintenance, dietary restriction during the first and second trimesters has resulted in conflicting results. These have been summarised by Black (1983) as having no effect on birth weight, increasing it, or decreasing it. The reason is that severe undernutrition during early and mid-gestation reduces the number of placentomes, but they increase in size and alter their shape. Thus if nutrient intake is increased in the last trimester, then the placenta is probably more efficient in nutrient transfer and the fetus grows more rapidly. Russel et al. (1981) also found a different response to different dietary intakes depending on the body weights of the ewe at the time of mating (Table 11.14). Some interesting data from a study by Faichney (1981) are shown in Table 11.15, in which feed intake was varied during pregnancy and the effects on fetal and placental weights were studied.

It is well recognised that ewes kept in tropical and subtropical environments produce small, weak lambs at birth. Continuous daily exposure for 8 hours of ewes to an ambient temperature of 42°C, followed by 16 hours at 32°C from the 50th day of gestation, can result in a 40% reduction in birth weight. The effect of the high ambient temperature is probably due to reduction in placental weight and function.

Some infectious diseases such as *Brucella ovis* and *Toxoplasma gondii* can cause reduced birth weights.

Table 11.14 Effect of ewe live weight and feeding level from day 30 to day 98 of gestation on the birth weight of lambs (from Russel et al., 1981)

Flock	*Mating weight (kg)*	*Nutrition in mid-pregnancy**	*Lamb birth weight (kg)*
A	42.5	High	3.83
		Low	3.32
B	54.5	High	4.23
		Low	4.95

*High level of nutrition was sufficient to maintain the conceptus-free body weight of the ewe; low level of nutrition resulted in an estimate loss of 5–6 kg in ewe body weight

Table 11.15 Effect of varying feed intake of ewes during gestation on fetal and placental weight at 135 days after fertilisation (from Faichney, 1981)

Treatment	*Feed intake (g/day)*		*Fetus weight (kg)*	*Placenta weight (g)*
	55–99 days	*100–135 days*		
MM	900	900	3.3	321
MR	900	500	3.3	437
RM	500	900	3.7	463
RR	500	500	3.0	413

M = sufficient food intake to maintain ewes at conceptus-free body weight; R = restricted food intake. Mean lamb birth weights for RM and RR were significantly different ($P < 0.05$).
Mean placental weights for MM vs. MR, RM and RR, and RM vs. RR were significantly different ($P < 0.05$)

Pelvic capacity of the dam

In New Zealand, McSporran and Wyburn (1979) and McSporran and Fielden (1979) were able to assess the pelvic area by means of radiographic pelvimetry, and found that variations in the incidence of dystocia between different groups of Romney ewes were related to the pelvic area. Attempts to correlate external bodily measurements with internal pelvic dimensions have been shown not to be particularly useful. Because the particular ovine dystocia studied by these authors was largely due to fetomaternal disproportion, they recommended selective breeding of ewes and rams for freedom from dystocia.

In the cow, attempts have been made to correlate external pelvic dimensions with the pelvic area (Hindson, 1978). In a study in sheep, external pelvic dimensions were measured in a large number of different breeds including several rare breeds; the latter have not been subjected to selection pressures for growth traits and carcass quality (Robalo Silva and Noakes, 1984). Table 11.16 shows the wide variation in pelvic size, with breeds such as the Soay, North Ronaldsay and Shetland having small pelvic dimensions, whereas the Scottish Blackface, Clun Forest and Suffolk having larger pelvic dimensions. However, when the pelvic dimensions are compared with the body weights of the ewes for the different breeds, it is noticeable that the former breeds have relatively larger pelves than the latter group (Table 11.17).

Table 11.16 Mean external pelvic dimensions and body weights of mature ewes of different breeds (from Robalo Silva and Noakes, 1984)

Breed	*TC (cm)*	*TCI (cm)*	*MTI (cm)*	*RL (cm)*	*Weight (kg)*
Soay	13.08	11.3	5.6	17.4	21.7
North Ronaldsay	15.1	12.4	6.3	18.6	24.8
Shetland	16.6	12.7	6.4	19.5	34.6
Scottish Blackface	21.0	16.9	9.0	23.4	66.4
Clun Forest X	26.4	16.5	8.8	23.6	61.6
Suffolk	22.1	18.3	8.8	25.5	79.5

TC = Intertuber coxal dimension; TCI = lateral intertuber ischial dimension; MTI = medial intertuber ischial dimension; RL = rump length

Table 11.17 Ratio of pelvic dimensions: body weight of adult ewes of different breeds (from Robalo Silva and Noakes, 1984)

Breed	*MTI: body weight*	*Sum of pelvic measurements: body weight*
Soay	1.0	1.0
North Ronaldsay	0.984	0.953
Shetland	0.717	0.720
Scottish Blackface	0.525	0.478
Clun Forest X	0.554	0.507
Suffolk	0.429	0.426

MTI = medial intertuber ischial dimension

Since fetal weight is between 6 and 8% of maternal body weight, then the relatively smaller pelves of those breeds that have been subject to genetic selection to produce large lambs at birth are more likely to have dystocia than the rare breeds, that have largely been left to the influences of natural selection.

Pigs

Fetomaternal disproportion is not a major cause of dystocia in pigs. It will be a greater problem in gilts with small litters.

The average birth weight of commercial breeds of pig with an average litter size of 10–11 is about 1 kg; in the case of some ‘pet’ breeds such as the Vietnamese pot-bellied pig the average birth weight is about 0.5 kg with an average litter size of about 4–6. It has been known for some time that when the numbers of piglets per horn is more than five then there is a decrease in piglet birth weight, with those in the middle of the horns being the smaller due to competition for placental space, those at the tip of the horn being the largest at birth. Various studies have shown that the amount of uterine horn space for optimal fetal growth is between 35 and 45 cm.

There is a substantial literature on the influence of nutrition on ovulation rates, embryo survival rates and other reproductive parameters which are discussed in Chapter 27. However, there is little information on the direct effect of nutrition on fetal size, other than the fact that lower ovulation rates will result in smaller litters, and thus larger piglets. In varying the diets of pregnant sows, Pike and Boaz (1972) have shown that variable feeding from conception to 70 days' gestation exerted no effect and only in the last 45 days did maternal nutrition influence birth weight. The latter finding corresponds with the observation that there is a 10-fold increase in porcine fetal weight during the last 45 days.

Dog and cat

The incidences and causes of dystocia in the dog and cat have been discussed in Chapter 8. In the bitch the overall level is about 5%, but it is recognised that in certain breeds which have both achondroplasia and brachycephaly it may approach 100% (Eneroth et al., 1999).

Puppy and kitten size is dependent on a number of factors, particularly breed and litter size; there appears to be no information on the influence of nutrition during pregnancy. In the larger breeds of dog, pups are 1–2% of the bitch's weight, whereas in smaller breeds the figure is 4–8% with normal whelping occurring if the pups are 4–5% of the dam's weight (Larsen, 1946). In the study by Eneroth et al. (1999), the Boston terrier pups' mean weights were 2.5% and 3.1% for normal whelpings and dystocias, respectively, and the corresponding figures for Scottish terriers were 2.1% and 2.5%.

In the achondroplastic breeds, and also in some terrier breeds such as the Aberdeen (Scottish) terrier, Sealyham and Pekinese (Freak, 1962 and 1975), the dorsovental or sacral-pubic dimension is small, thereby reducing the size of the pelvic inlet and causing obstructive dystocia due to fetomaternal disproportion. In an interesting study involving the Boston and Scottish terriers, data were collected from breeders on litter size, pups' weights, height of head, breadth of head and breadth of shoulders for groups that whelped normally and for those that had dystocia due to fetomaternal disproportion. All of the bitches in the study were radiographed in dorsovental and lateral projections (Eneroth et al., 1999). Fetomaternal disproportion in the Scottish terriers was due to dorsoventral flattening of the pelvis, whereas in the Boston terrier it was due to combination of the same pelvic deformity and also the circumference of the head; there was a strong positive correlation ($r = 0.743$) between body weight and head circumference in the Boston terrier. This study demonstrated the value of radiographic pelvimetry as a means of predicting dystocia and in the selection of bitches for breeding, together with a critical evaluation of pup conformation in the selection of both sire and dam.

FAULTY FETAL DISPOSITION

In describing the disposition of the fetus at birth it is important to use the terminology first described by Benesch and outlined on page 211. Frequently the incorrect terminology is used, particularly the word 'presentation', which has a precise obstetrical meaning in relation to the disposition of the fetus. During pregnancy, the fetus assumes a disposition that occupies as little uterine space as possible; however, during parturition it must assume a disposition that enables it to be expelled through the birth canal. Since these dispositions are incompatible, changes must occur during the first stage of labour; you might like to read Chapter 6 in which these are described.

Presentation

About 99% of foals and 95% of calves are presented anteriorly; when sheep are parturient with singletons they show a similar percentage of anterior presentations to cattle, but with twins there is a considerable proportion of posteriorly presented lambs. The polytocous sow and bitch deliver 30–40% of fetuses in posterior presentation. In posterior presentation, the hindlimbs may be extended or flexed beneath the fetal body. When the hindlimbs are extended in polytocous births, dystocia is only slightly more common than with anterior presentation; however, when the hindlimbs are flexed (breech presentation) in polytocous births the incidence of dystocia is increased. In the monotocous species, serious dystocia always occurs with posterior presentation if the hindlimbs are flexed; even when they are extended there is a greater likelihood of difficult birth than with anterior presentation. Because of the relatively long limbs of the fetuses of monotocous species, and the large space required for hindlimb extension, there is obviously a high probability that a fetus presented posteriorly in late gestation will fail to extend its hindlimbs before second-stage labour begins. In ovine twin births, breech presentation causes dystocia, although the twin lamb is smaller than the singleton.

There is a consensus of opinion that both dystocia and stillbirth are much more likely to occur if the calf is presented posteriorly rather than anteriorly. Ben-David (1961) found that 47% of posterior presentations in Holsteins were accompanied by dystocia. Also the likelihood of dystocia in equine posterior presentations is exceptionally high. It is therefore important to enquire into the factors that determine fetal polarity. Arthur and Abusineina (1963) made post-mortem studies on this problem in cattle, while Vandeplassche (1957) has carried out similar investigations in horses. With respect to cattle, during the first 2 months of gestation no definite polarity was evident, but during the third month there were equal numbers of anterior and posterior presentations. From then to the end of gestation, there were only three transverse presentations out of 363 pregnancies. Throughout the fourth, fifth and first half of the sixth months a majority of fetuses were in posterior presentation, but during the sixth month the situation began to change so that at the end of that month, anterior and posterior presentation frequencies were equal. By the middle of the seventh month, the majority of fetuses were in anterior presentation. Beyond the seventh month, only one of 17 fetuses was posteriorly disposed, a situation closely similar to that observed at term. To recapitulate: between $5\frac{1}{2}$ and $6\frac{1}{2}$ months of gestation the polarity of the bovine fetus becomes reversed, and by the end of the seventh month the final birth presentation is adopted. Attempts, using post-mortem pregnant uteri, to alter the presentation beyond the seventh month were unsuccessful because by that time the fetal body length greatly exceeds the width of the amnion, while successful efforts to change the presentation between $5\frac{1}{2}$ and $6\frac{1}{2}$ months required definite manipulative force. Similar attempts carried out under paravertebral anaesthesia on the standing cow were successful with a $6\frac{1}{2}$-month fetus, but unsuccessful with an 8-month calf.

The natural forces which bring about these changes in polarity are not understood, but presumably reflex fetal movements occur in response to changes in the intrauterine pressure due to myometrial contractions, to movements of adjacent abdominal viscera or to contraction of the abdominal musculature. Fetal movements are often felt during rectal palpation of the uterus. The preponderance of posterior presentations in early gestation would be the expected result of suspending an inert body with the same centre of gravity as the fetal calf. With the development of the fetal nervous system, and a consequent appreciation of gravity, the fetal calf would begin to execute righting reflexes which would tend to bring up the head from the dependent part of the uterus. If these assumptions are true, then posterior presentation, rather than being regarded as an obstetric accident, could be caused either by a subnormally developed fetus or by a uterus deficient in tone. Obviously size of fetus and uterine space must influence the ease with which a fetus can change its polarity in utero; there is a much higher percentage of posterior presentations in bovine twin births, while an above average percentage of posterior presentations occurs with excessively large fetuses.

With foals, 98% assume an anterior longitudinal presentation between $6\frac{1}{2}$ and $8\frac{1}{2}$ months of gestation (Vandeplassche, 1957). A small proportion of the remaining 2% – possibly about 0.1% – are transverse presentations, in which the extremities of the fetus occupy the uterine cornua while the uterine body is largely empty. This presentation causes the most serious of all equine dystocias. It probably arises at about 70 days of gestation, when the uterus normally changes from a transverse to a longitudinal direction in front of the maternal pelvis as a result of the allantochorion passing from the pregnant horn into the uterine body. In the abnormal situation, either the allantochorion does not intrude into the uterine body or the major, rather than the normally minor, branch of the allantochorion passes into the non-pregnant horn and is followed by the amnion, containing a fetal extremity. Normally neither the amnion nor the fetus passes into the non-pregnant horn. Other, less serious, equine transverse presentations occur across the uterine body; it is not known when they occur, but they could occur during birth.

Transverse presentations are very uncommon in cattle and sheep, but in the polytocous species a fetus is not uncommonly found to be disposed across the entrance to the maternal pelvis; such presentations undoubtedly arise during birth.

The lack of a marked difference in frequency between anterior and posterior presentations in pigs and dogs may be due to the horizontal disposition of the long uterine horns as compared with the sloping uteri of the monotocous species.

Position

As regards position of the fetus, the natural tendency is for it to lie with its dorsum against the greater curvature of the uterus so as to occupy as little space as possible; thus the equine fetus is upside down and the bovine fetus is upright during late gestation. The latter maintains this relationship during birth, but in the mare the fetus changes from a ventral to a dorsal position during the course of labour. Therefore, as might be expected, ventral as well as lateral positions are much commoner in equine than in bovine dystocias; they arise during birth.

Posture

As regards posture, the arrangement of the bovine fetus during the final 2 months of gestation is one of anterior presentation and dorsal position with flexion of all joints of the movable appendages. The appendages of the equine fetus are similarly flexed on the inverted fetus. This postural disposition of 'universal flexion' achieves the maximum economy of space. The fascinating and unsolved problem is the nature of the parturient mechanism whereby the occipitoatlantal and cervical joints become extended, while the forelimbs become straightened in front of the fetus. The extended forelimb posture necessary for normal birth in cattle is the more remarkable because it is a posture which is never repeated postnatally. In his studies of the first stage of labour in cattle Abusineina (1963) noticed that the flexed knees of the calf first occupied the dilating cervix; 30 minutes later the digits were felt in the cervix. It can be postulated that the limb extension occurs while the fetus is practising righting reflexes in its attempt to 'stand up in utero'. No doubt such active fetal movements are provoked by the myometrial contractions of first-stage labour. In this connection, the observation by Jöchle et al. (1972) that progesterone given to parturient cows caused a high incidence of postural dystocia could be due to it maintaining the 'progesterone block' on the myometrium (see Chapter 6), thereby reducing the stimulation of the fetal calf to initiate its righting reflexes. It is also well known that there are increased frequencies of postural aberrations in premature births, where uterine inertia is more prevalent and with twins, where there is also an increased likelihood of uterine inertia but also reduced space, thereby interfering with the ability of the limbs to extend.

Lateral deviation of the head is a postural abnormality which deserves special mention. It may be due to the same factors as those noted above, but lack of uterine space may be more important and it may arise during late gestation rather than during birth. A congenital deformity known as *wryneck*, in which the head and neck are fixed in flexion due to ankylosis of the cervical vertebrae, arises during the peculiar bicornual gestation of solipeds (Williams, 1940). In 27 difficult equine dystocias treated by Vandeplassche (1957), the majority of which were associated with bicornual gestation, 10 of the foals were affected with a degree of wryneck.

In the monotocous species, the dimensions of the maternal bony pelvis are just sufficient for the normal full-term fetus to negotiate the birth canal; any fetal disposition other than anterior presentation, dorsal position, extended posture is likely to result in dystocia. In the polytocous species the fetomaternal relationship is not so exact, with the result that the disposition of the comparatively small fetal limbs is less important and many piglets, puppies and kittens are delivered normally with their limbs in postures which would have caused dystocia in the foal and calf. However, if a female of a polytocous species is parturient with an abnormally low number of fetuses there is likely to be some degree of fetomaternal disproportion and in these circumstances malposture of the limbs may cause dystocia.

From the above account, the causes of faulty fetal disposition might appear to be due more to chance; however, there are some indications that there may be an inherited predisposition. For example, Woodward and Clark (1959) found that a particular Hereford sire, when used on an inbred line of cattle, produced a high incidence of posterior presentations, while Uwland (1976) reported ranges of between 2 and 9.7% of posterior presentations in the progeny of different bulls; these observations suggest that a hereditary factor may affect the incidence of posterior presentation. More recently, in a study of 3873 calvings over a 20-year period at Colorado State University, of which 155 were dystocias with 72.8% in posterior presentation and dorsal position, posterior presentation heritability estimates for Hereford and Angus breeds were 0.173 and 0.0, respectively. Also of interest in this study was that other non-heritable factors such as year, sex of calf, sire of calf within breed, and age of dam influenced the incidence of posterior presentations (Holland et al., 1993).

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12 Manipulative delivery per vaginam: farm animals and horses

GENERAL CONSIDERATIONS

- Vaginal obstetrical procedures should be performed as cleanly as possible; it is impossible to perform them aseptically, since inevitably there will be some contamination. It is important to sterilise or disinfect instruments and equipment between animals to prevent the spread of infection. Gentleness is of prime importance, so as to reduce the amount of trauma to the dam's genital tract and also to the newborn.
- The prevention of pain and discomfort should be of paramount importance so that caudal epidural anaesthesia, sedation or general anaesthesia should always be considered.
- In monotocous species the aim of any manipulative procedures must always be to ensure that the fetus is in normal disposition before attempting traction. In polytocous species (and in sheep or goats with small multiple fetuses), it is possible for per vaginam delivery to occur with some slight postural abnormality. Correction of defects of presentation, position and posture can be achieved only by intrauterine manipulation of the fetus. Thus an essential prerequisite to treatment is retropulsion of the fetus. This is greatly facilitated by caudal epidural anaesthesia.
- In cases of prolonged dystocia, where fetal fluids have been lost, delivery is expedited by their substitution. Sterile water is the best substitute for allantoic fluid, although non-sterile clean water is perfectly satisfactory. In the cow and mare, volumes of up to 14 litres, instilled into the uterus by gravity using a soft rubber or plastic tube (a stomach tube is satisfactory) and funnel, greatly increase the mobility of the fetus in utero. For actual vaginal delivery, a lubricant substitute for the amniotic fluid is required, and this may be in the form of a water-soluble cellulose-based obstetrical lubricant. In the absence of this, a substitute such as soap, particularly soap flakes, or lard is well tried and effective. The value of fetal fluid supplements cannot be too strongly emphasised.
- After diagnosing the cause of dystocia and deciding on a plan of action, the obstetrician should consider whether the facilities are appropriate, whether there is sufficient professional and other help available, and whether the equipment is adequate to carry out the treatment successfully. In severe forms of dystocia, more especially in mares, the veterinarian should always seek the assistance of a professional colleague and consider whether it might be appropriate to transport the animal to somewhere with hospital facilities, provided that the animal is in a fit state to travel.
- After the successful delivery of the fetus or fetuses, the dam's genital tract should always be examined for the presence of others; remember that monotocous species can have twins and rarely more.
- The dam's genital tract should be examined for signs of injury, and appropriate treatment administered (see Chapter 18).
- The fetus or fetuses should be examined to see if resuscitation is necessary, if there is evidence of respiratory acidosis which should be treated and if there are injuries. These items are discussed in detail in Chapter 7.

OBSTETRICAL EQUIPMENT

The aim should be to possess the minimum of essential equipment, and to be thoroughly conversant with its use. It cannot be stated too often that the best instruments are the *clean and gentle* hands and arms of the obstetrician. Simple instruments that are easy to handle and convenient to sterilise are best. More complex equipment is occasionally required, and the important consideration is to know when the use of such complicated instruments is indicated. For the veterinarian doing ambulatory visits to farms, studs and other livestock units where parturient animals are kept, it is advisable to have a dedicated collection of instruments and other equipment that is always available in an emergency; in addition, a dedicated caesarean operation kit is also important (this will be described in Chapter 20). With the availability of better sedatives and anaesthetic agents, and improved methods for the caesarean operation, many of the long-established items of obstetrical instrumentation have become obsolete and veterinarians have lost the skills to use them effectively. Despite this, many of them can be very helpful at times, and for completeness some of the more useful ones in cattle, and to a very much lesser extent in the horse, are shown in Figure 12.1. These include:

- Obstetric snares, i.e. 1 m lengths, with loops, of cotton rope (clothes line), nylon cord or webbing (A, B, C) – a finer cord for snaring the mandible is essential – and traction bars (D). These items are essential and it is advisable to have at least two sets; they can be sterilised.
- As an alternative to snares one may use Moore's obstetric chains (E) with handles (F). Many veterinarians find these easier to use than rope snares. Their main advantage is that they are heavier and do not move so readily when they are repositioned during intrauterine or intravaginal manipulation.
- A snare introducer (G) is also illustrated. This can be used with ropes as well as chains; the author has found that a bull ring is an effective substitute.
- Obstetrical hooks include Krey–Schottler double-jointed hooks (H), Obermeyer's anal hook (I), Harms's sharp (J) or blunt (L) paired hooks on a fine (farrowing) chain (K), and Blanchard's long, flexible cane hook (M). These are useful when performing fetotomy to enable traction to be applied to various fetal segments.

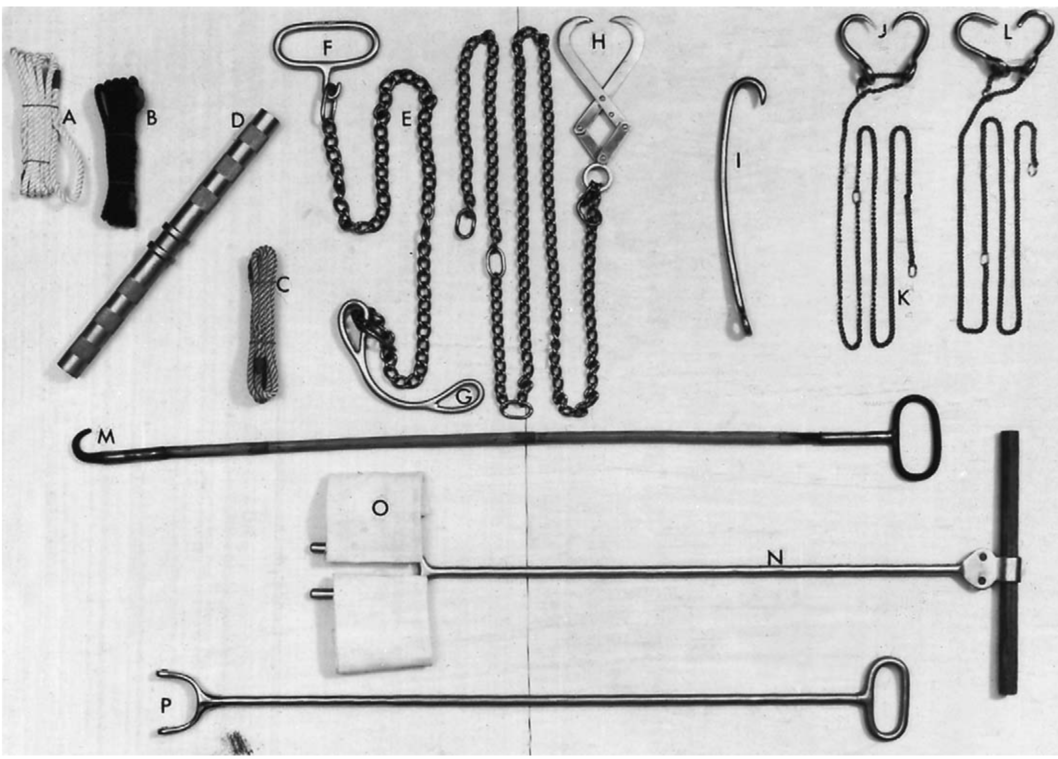

Fig. 12.1 Instruments for manipulative delivery (see text for key).

- Additional instruments are Cämmerer's torsion fork (N) with canvas cuffs (O) and Kühn's obstetrical crutch (P).
- Traction may be applied using a block and tackle, or a calving aid such as an HK calf puller or Vink calving jack (Figure 12.2).

Instruments for fetotomy (Figure 12.3) include:

- Fetotomy guarded knives such as Robert's (A) or Unsworth's (B); the former is particularly useful for performing subcutaneous fetotomy (see Chapter 14) as well as during a caesarean

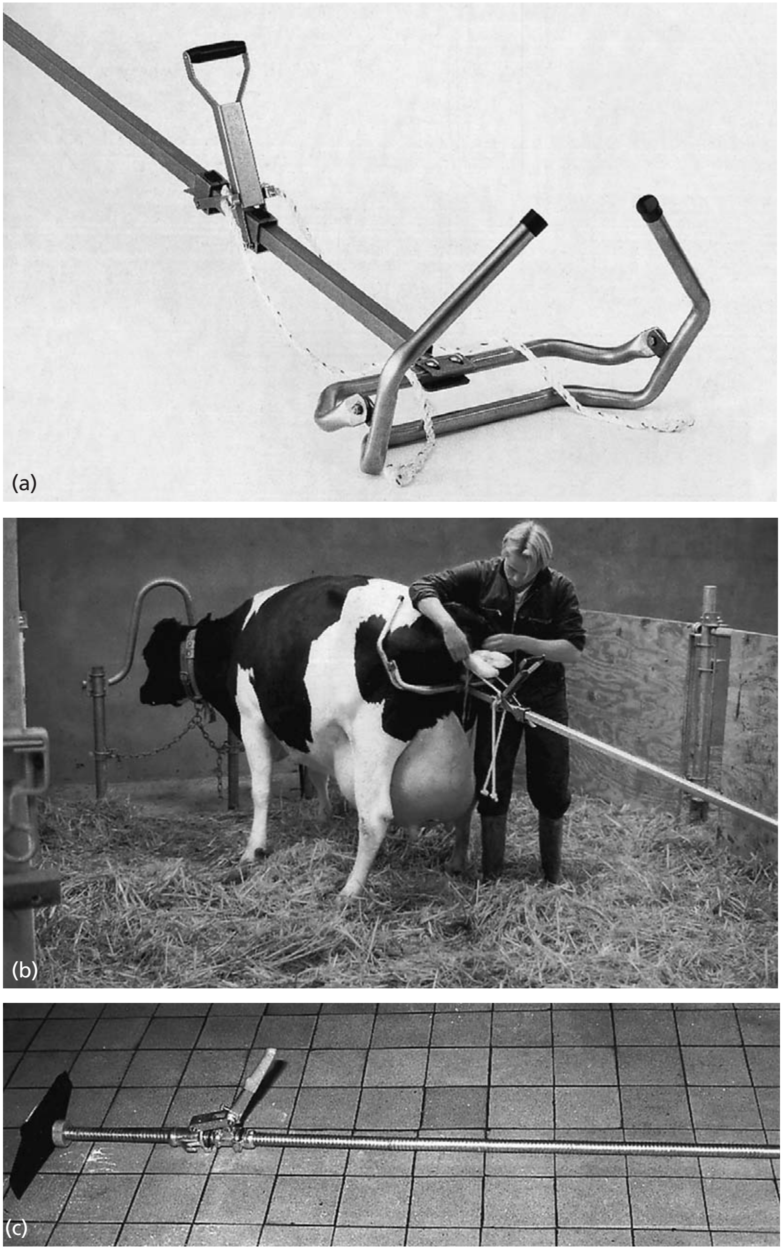

Fig. 12.2 (a) Vink calving jack. (b) Vink calving jack in use to apply traction to calf in anterior longitudinal presentation with snare attached to both forelimbs. (c) HK calf puller.

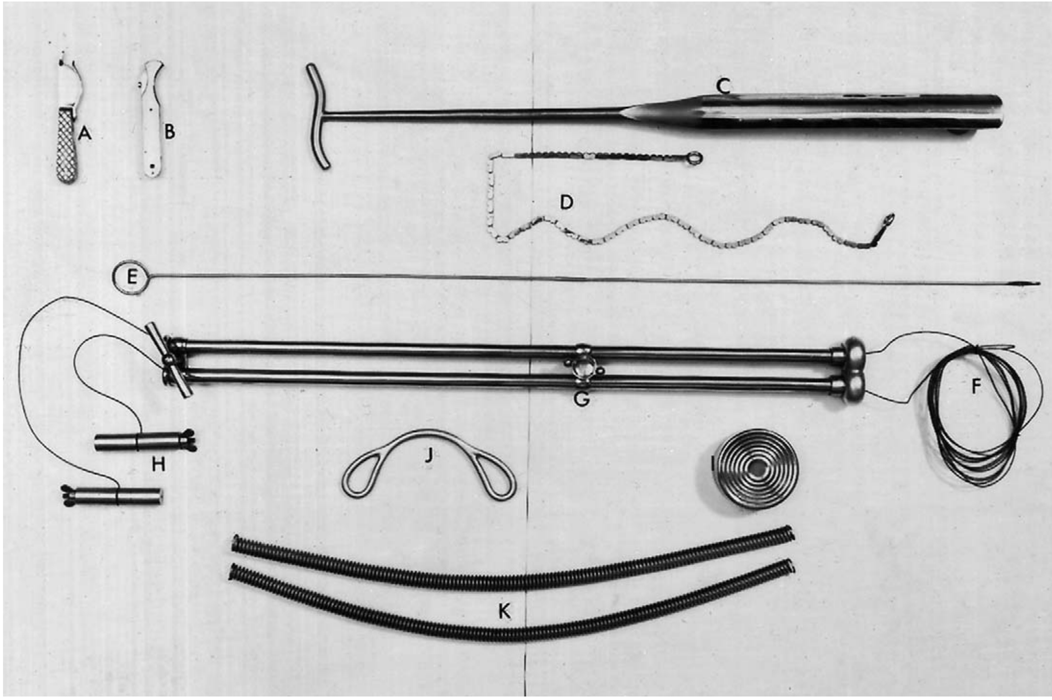

Fig. 12.3 Instruments for fetotomy (see text for key).

operation in the cow when it is impossible to exteriorise the uterus to incise it.
- Spatula (C) for use in subcutaneous fetotomy.
- Persson's chain-saw (D), now replaced by polyfilamentous fetotomy wire.
- Fetotome (Swedish modification of Thygesen's model) (G), with wire introducer (E), wire (F), hand-grips (H) and Shriever's wire introducer (J).
- Gättli's spiral tubes (K), which are a cheaper alternative of protecting the dam's genital tract than the Thygesen's model.

OBSTETRIC MANOEUVRES

The manoeuvres which are practised on the fetus in manipulative obstetrics are as follows.

Retropulsion

Retropulsion means pushing the fetus cranially from the vagina (and the bony pelvic canal) towards the uterus. It is fundamental to all intra-uterine measures, which may be required to rectify defects of presentation, position and posture, since there will be inadequate space to perform even the simplest manipulations. It is effected by pressure with the hand on the presenting bulk of the fetus; in some cases it is convenient for an assistant to repel the fetus while the obstetrician otherwise manipulates it, while in others retropulsion is applied by means of a crutch (see Figure 12.1). As far as possible, the repelling force should be exerted in the intervals between bouts of straining. Alternatively, epidural anaesthesia may be induced to prevent the dam 'straining'; however, it has no effect on myometrial contractions which can be suppressed by the use of a spasmolytic such as clenbuterol.

Extension

Extension refers to the extension of flexed joints when postural defects are present. It is carried out by applying a tangential force to the end of the displaced extremity so that it is brought through an arc of a circle to the entrance of the pelvis. The force is applied preferably by hand or, failing that, by snare or hook(s).

Traction

Traction means the application of force to the presenting parts of the fetus in order to supplement, or in some cases to replace, the maternal forces. Such force is applied by hand or through the medium of snares or hooks. Limb-snares are fixed above the fetlocks, and the head snare may be applied by the Benesch method, in which the loop is placed in the mouth and up over the poll and behind the ears or, alternatively, the centre of a single rope may be pushed up over the poll and behind both ears, leaving both ends of the rope protruding from the vagina. For replacement of the laterally deviated head, where the operator's hand is insufficient, a thin rope snare applied to the mandible is essential. However, this must only be used to correct the postural defect; other traction which might be used to effect delivery must be applied using a conventional Benesch head snare.

A very important consideration is the magnitude of the supplementary force which may be used, since excessive force inappropriately applied can cause severe trauma to dam and fetus. In the cow, it is felt that the well-coordinated pull of four average persons should be the limit. Mechanical devices are now used extensively to apply traction; they must always be used carefully and sympathetically since they can cause severe trauma if used inappropriately. Table 12.1 gives some interesting data compiled by Hindson (1978), comparing the magnitude of the forces used to apply traction, using a hydraulic drawbar dynamometer. This shows that pulley blocks or calving jacks or pullers generate over 5 or 6 times the force associated with a natural calving. However, for the stock person or veterinary surgeon with little or no help the pulley block and tackle, or a calving aid such as the HK calf puller or Vink calving jack (see Figure 12.2) are invaluable. The most important aspect of applying effective traction is to coordinate the supplementary force with the straining effort of the dam. In the case of the cow, the slack in the calving snares is 'taken up', as she strains so preventing the calf from returning to its original site within the birth canal.

Table 12.1 Measurement of maximum tractive effort as shown and recorded on a hydraulic drawbar dynamometer (after Hindson, 1978)

Origin of force	*Tractive effort (kg)*
Cow at natural calving	70
Traction by one person	75
Traction by two persons	115
Traction by three persons	155
Calving jack	400
Pocket pulley blocks	445
Tractor	5000+

In the mare, the use of snares with several persons providing manual assistance is usually sufficient. In the ewe and doe goat traction can be applied using simple fine cord snares or a fixed plastic head snare (Figure 12.4). In the sow, traction is nearly always applied using the hand but fine cord snares and the plastic lambing snare previously described (Figure 12.4) are sometimes very useful.

In the dog and cat, the most appropriate obstetrical instruments are the fingers; whelping forceps are useful but they need to be used with the utmost care since they can cause trauma to both dam and offspring. A much neglected instrument is the vectis; this is shown in use in Figure 13.3. The author has found it to be a very effective method of applying traction whilst virtually ensuring that neither dam nor offspring are injured.

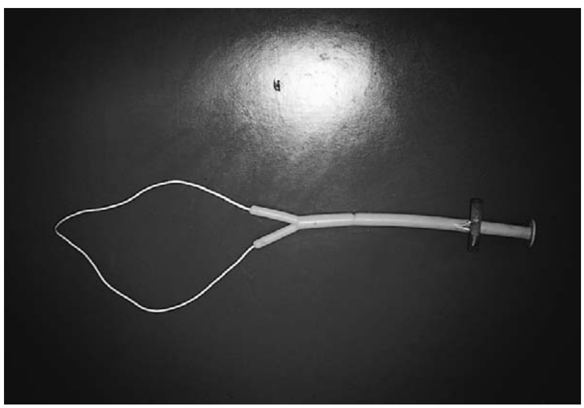

Fig. 12.4 Plastic head snare for use in ewes and sows.

Rotation

Rotation entails alteration of the position of a fetus by moving it around its longitudinal axis: for example, from the ventral to dorsal position. It is more often required in horses than in cattle and is much more easily effected on the responsive live fetus, which may be readily rotated by digital pressure on the eyeballs, protected by the lids; this causes a convulsive reaction, and slight rotational force then completes the manoeuvre. If this fails – and in the case of dead fetuses fetal fluid supplements are indicated – rotational force may be exerted on the crossed extended limbs by hand or mechanically through the medium of Cämmerer's torsion fork or Künn's crutch. Alternatively, by repelling the fetus, crossing the limbs to which the snares are attached and then applying traction, the traction force will tend to rotate the fetus about its long axis. By repeating the process several times it is often possible to rotate it about 180°.

Version

Version means alteration of transverse or vertical to longitudinal presentation.

OBSTETRIC ANAESTHESIA FOR VAGINAL DELIVERY

In order to correct many dystocias more easily and humanely the induction of local or general anaesthesia in the dam should be considered.

General anaesthesia

Deep narcosis, or general anaesthesia, is better suited to the temperament of mares than local analgesia, although in well-chosen cases epidural anaesthesia may be combined with sedatives. Where a complicated correction or fetotomy is required, it is best to use general anaesthesia, preferably in a veterinary hospital. Using hobbles and a hoist, it is relatively easy to place the mare in dorsal or lateral recumbency. Such a change of position may greatly expedite obstetric manoeuvres; in addition, elevation of the hindquarters will allow the foal to fall back into the uterus in the abdomen under the influence of gravity, thereby providing more space for any manipulative procedures. Because of pressure on the diaphragm, this may cause the anaesthetist some concern. General anaesthesia is useful for obstetric procedures in dogs and cats.

Epidural anaesthesia

For a excellent detailed account of all aspects of epidural and other local and regional anaesthetic techniques, the reader should consult Skarda (1996).

Cattle

In cattle, epidural anaesthesia is ideal for obstetric purposes. Its merits were first demonstrated to the veterinary profession by Benesch (1927). It is a form of multiple spinal nerve block in which, by means of a single injection of local anaesthetic solution into the epidural space, the coccygeal and posterior sacral nerves are affected, thus producing anaesthesia of the anus, perineum, vulva and vagina. As a result, painless birth is possible, but an outstanding additional advantage of epidural anaesthesia to the veterinary surgeon is that by abolishing pelvic sensation, reflex abdominal contraction ('straining') is prevented. Thus, intravaginal manipulations are facilitated, retropulsion is made easier, fetal fluid supplements are retained and defaecation is suspended. The patient stands more quietly and, if recumbent initially, often gets up when relieved of painful pelvic sensations; this again makes the obstetrician's task easier and cleaner. This form of anaesthesia is useful whenever straining is troublesome, as in prolapse of the uterus, vagina, rectum or bladder. It is also indicated for episiotomy and for suturing the vulva or perineum.

Provided that the epidural injection is made with due regard to asepsis and that an excessive volume of anaesthetic is not injected (thus causing the animal to become recumbent), the method is free from risk. It should be clearly understood that epidural anaesthesia does not inhibit myometrial contractions; it has no effect on the third stage of labour or uterine involution.

Technique of epidural injection. The site of injection is the middle of the first intercoccygeal space. This is located by raising the tail 'pump-handle' fashion to identify the first obvious articulation behind the sacrum. The sacrococcygeal space can also be used; however, it is smaller than the first coccygeal space and in some older cows becomes ossified. The spinal cord and meninges are cranial to these points, the spinal canal containing only the coccygeal nerves, the thin phylum terminale, vasculature and epidural fat and connective tissue.

The area is clipped, thoroughly washed with an antiseptic solution or surgical scrub and dressed with surgical spirit. Some inject a small volume of local anaesthetic using a fine needle to desensitise the skin over the injection site; others do not. The epidural needle, which is 18 gauge and 5 cm long, is inserted into the middle of the space at right angles to the normal contour of the tail-head exactly in the midline and directed downwards in the mid-sagittal plane; it is easier to ensure that this occurs by standing directly behind the cow whilst an assistant pumps the tail. Some find it easier to direct the needle slightly cranially at an angle of about 10° from the vertical. The needle is passed downwards for a distance of 2–4 cm until it strikes the floor of the epidural space; it is then very slightly withdrawn (Figure 12.5). Confirmation that the needle is correctly placed is obtained by attaching to it the syringe and making a trial injection; if there is no resistance to injection, the needle point is in the epidural space. Alternatively, the hub of the epidural needle can be filled with anaesthetic solution. As the needle is advanced into the epidural space, the anaesthetic solution will be sucked in as a result of the slight negative pressure which exists there. Within 2 minutes of the injection the tail becomes limp, but it takes a slightly longer time interval (10–20 minutes) before the perineum is desensitised and the straining reflex is completely abolished.

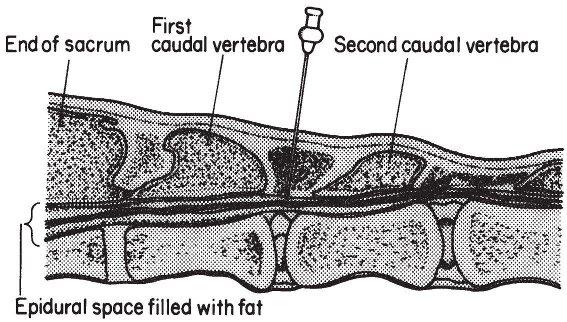

Fig. 12.5 Longitudinal section through the caudal vertebrae of the cow.

A dose rate of 1.0 ml/100 kg of 2% lidocaine or lignocaine hydrochloride injected at a rate of 1 ml per second will produce obstetric anaesthesia lasting about 30–150 minutes (Skarda, 1996); thus heifers and small cows require a volume of 5 ml and large cows 7–10 ml. The addition to the local anaesthetic of 2% of adrenaline prolongs the period of anaesthesia. Recently, the simultaneous injection of xylazine into the epidural space at a dose rate of 0.05 mg/kg diluted to a volume of 5 ml can prolong the duration of anaesthesia for up to 3 hours. Some adverse side-effects frequently occur, which can be controlled by the intravenous injection of tolazoline, an α_2-adrenoceptor antagonist, at a dose rate of 0.3 mg/kg (Skarda, 1996).

Sheep and goats

Caudal epidural anaesthesia is a very useful, if somewhat underutilised technique in ovine and caprine obstetrics, because although in both species there will be straining, the relative forces are much less than in the cow, and the ewe or doe can always be suspended by her hindlimbs. However, the uterus of both species appears to be more susceptible to rupture when manipulative procedures are performed. For this reason, and in the interests of welfare, anasthesia should be used as a routine for all but the simplest of vaginal and uterine manipulations.

The injection can be made into either the sacrococcygeal or the first coccygeal interspace with a 3.5 cm, 20 gauge needle using 2% lignocaine hydrochloride with adrenaline at a dose rate of 1 ml/50 kg body weight. When a mixture of 1.75 ml 2% lignocaine hydrochloride and 0.25 ml 0.25% xylazine is injected into the epidural space at a dose rate of 1 ml/50 kg, the duration of effect can be as long as 36 hours, and this can be extended by repeated doses (Sargison and Scott, 1994).

Horses

The technique of epidural injection in the mare is the same but, because the root of the tail is well covered by muscle and fat, the spines of all coccygeal vertebrae are not so easy to locate; the first coccygeal interspace is the preferred site. This can be located by flexing the tail, since it is at the most angular portion of the bend of the tail and is usually about 5 cm cranial to the origin of the tail hairs. It is important to ensure that the mare is adequately restrained. After the site has been clipped and thoroughly cleaned, a small bleb of local anaesthetic should be injected subcutaneously and into the surrounding tissue over the site. With the mare standing squarely and symmetrically, a 4–8 cm 18 gauge needle (note that it needs to be longer than in the cow) should be inserted at about 10° from the vertical and directed cranially until it strikes the floor of the spinal canal; it should then be withdrawn 0.5 cm before the injection is made.

Traditionally, 2% lignocaine (lidocaine) hydrochloride is effective, using a volume of 6–8 ml in a light hunter-type mare weighing 450 kg; proportionately larger or smaller volumes should be used in larger and smaller animals, respectively. It is important to err on the side of caution, since too large a volume will cause ataxia, which is of much greater concern in horses than in cattle, because of their different temperaments; if the needle is capped and left in place, an additional volume can be given. It also takes longer to take effect in the horse than the cow. Other local anaesthetic agents can be used (in different volumes) as well as α_2-adrenoceptor agonists such as xylazine (0.17 mg/kg) and detomidine (60 μg/kg) in 10 ml 0.9% saline; the latter is used either alone or in combination with local anaesthetics. Using a combination of 2% solutions of lignocaine hydrochloride (0.22 mg/kg) and xylazine (0.17 mg/kg), rapid-onset (5.3 minutes) and long-lasting (330 minutes) caudal epidural anaesthesia was obtained (Skarda, 1996).

Pigs

Epidural anaesthesia is rarely used for obstetric purposes in swine other than for replacing prolapses of the vagina and uterus; it can be used for a caesarian operation. The site of injection is the *lumbosacral* space which can be located as follows. The wings of the ilia are joined by an imaginary transverse line; where this crosses the mid-dorsal line, the needle is inserted at an angle 20° caudal to the perpendicular until it strikes the floor of the vertebral canal. The needle is then withdrawn slightly and the injection made. The size of the needle varies depending on the size of the gilt or sow but over 100 kg body weight a 10–15 cm 18 gauge needle is satisfactory. The sow or gilt needs to be adequately restrained, preferably in a crate to prevent lateral movement, which in the author's experience can be the most difficult aspect of the technique and can make the difference between success and failure. For anaesthesia caudal to the umbilicus, a dose rate of 1.0 ml of 2% lignocaine hydrochloride per 4.5 kg body weight injected at 1.0 ml per 2–3 seconds should achieve anaesthesia by 10 minutes and last for about 120 minutes (Skarda, 1996). Injection at this site affects the nerves of the lumbosacral plexus and produces posterior paralysis.

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13 Vaginal manipulations and delivery in the bitch and queen cat

With the advent of improved anaesthetic techniques resulting in low mortality rates in both dam and offspring, there has been an understandable increase in the use of the caesarean operation to relieve dystocia in the bitch and queen. Despite this, there is still an important place for manipulative obstetrical procedures to treat dystocia, particularly simple ones which, when implemented, can be followed by normal expulsion of the remainder of the litter, or if the affected fetus is the last one of the litter to be born, the preceding ones having been expelled unaided.

DIGITAL MANIPULATIONS

Before resorting to instrumental assistance the use of the finger should be fully exploited. When parts of the fetus have already passed through the pelvic inlet, for instance, it is often possible by insinuating the finger over the occiput, into the intermaxillary space or in front of the fetal pelvis in posterior presentation, to apply sufficient traction to draw these parts into the vulva. Straining on the part of the bitch is of great assistance to one's efforts. Once parts of the fetus are in the vulva, traction delivery is generally simple. In cases of posterior presentation, in the ventral position this form of assistance is also often effective. In breech presentation, it is generally possible to hook the fingers around the retained limbs and draw them upwards and backwards into the maternal pelvis. In vertex posture it is usually a relatively simple matter to insert the finger beneath the fetal chin and, by drawing it upwards, direct the muzzle in line with the birth canal (Figures 13.1 and 13.2). During all these manipulations, it is helpful to fix the position of the fetus in the uterus by gripping it with the left hand through the abdominal wall, and to direct the fetus towards the pelvic inlet.

Fig. 13.1 Vertex posture ('butt' presentation) with bilateral shoulder flexion.

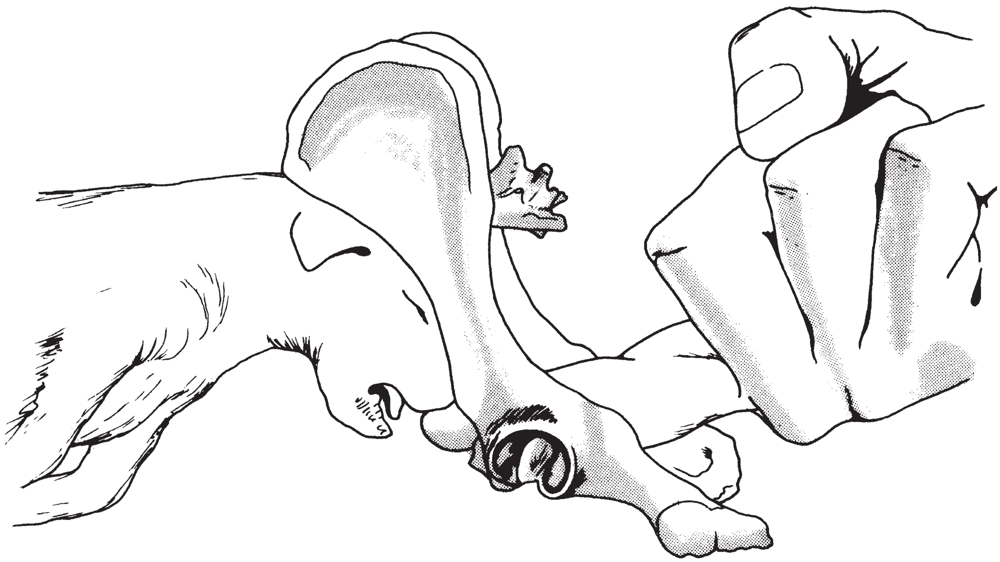

Fig. 13.2 Correction of the vertex posture with the finger.

THE USE OF INSTRUMENTS

When parts of the fetus have already traversed the pelvic inlet and occupy the vagina, Hobday's vectis is a useful instrument. The vectis is passed into the vagina and, according to the presentation, over the dorsal aspect of the fetal head or pelvis and by pressure downwards engaged behind the occiput or tuber coxae. The index finger is then introduced, and pressed upwards into the intermaxillary space or in front of the fetal pelvis; between the opposing grips of the vectis above and the finger beneath it is often possible to apply sufficient traction to the fetus to deliver it without injury (Figure 13.3). The method may even be successful in cases in which the forelimbs are retained and the correction of which is difficult because of the presence of the head in the vagina.

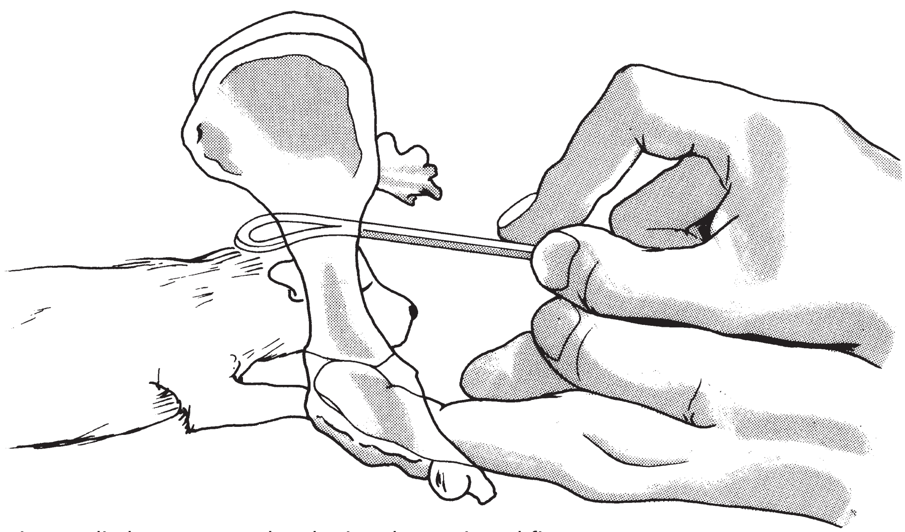

Fig. 13.3 Traction applied to a puppy's head using the vectis and finger.

In cases of fetomaternal disproportion in anterior presentation, in which the fetus is entirely in the uterus and obstruction is caused by the size of the cranium, Roberts' snare forceps are of value, particularly in small bitches and cats. Such cases may also be associated with retention of the forelimbs. Should the latter be the case, it is better to attempt delivery with the posture uncorrected, for the forelegs will cause no greater obstruction lying alongside the chest than they would if extended; moreover, the subsequent traction, applied as it is to the head only, may cause the elbows alongside the head to become impacted at the pelvic inlet. Snare forceps are used as follows. While fixing the fetus at the pelvic brim by holding it through the abdominal wall, the closed forceps carrying the snare are passed into the uterus and over the fetal head until they lie above the neck. The jaws are then opened as widely as possible and depressed downwards until they lie ventral to the neck and then closed. In this way an encircling noose has been applied. By traction on the free ends of the snare the noose is drawn tight and it is held in position by the forceps. Traction is then applied to the forceps and the free ends of the snare (Figure 13.4).

Freak (1948) recommends Rampley's sponge-holding forceps for the application of traction to the living fetus in cases similar to those previously outlined. Using the index finger as a guide to their application, the forceps are lightly fixed to the upper or lower jaw, or even the whole snout. In the case of posterior presentation they may be applied to a hindlimb until the fetal pelvis is drawn into the maternal inlet and then a more secure hold obtained. Points made by Freak in favour of Rampley's forceps over those of the Hobday type, in relatively simple cases, are that, firstly, they can be applied and fixed by means of the ratchet* to comparatively small parts of the fetus, and thus they do not increase the total size of the obstructing part when drawing it through the maternal inlet; and, secondly, consequent on the lightness with which it is possible to apply them, the fetus can be delivered uninjured.

Lateral deviation of the head and nape posture are abnormalities which require special consideration, for the diagnosis may be difficult and attempts to deliver fetuses so presented without correction, even with severe forceps traction, are generally futile, at any rate in the healthy fetus. In lateral deviation, the forelimb on the side opposite to the neck flexion has generally passed through the pelvic inlet (Figure 13.5). Thus, the presence of a single forelimb in the anterior vagina indicates a likelihood of the condition. To verify the diagnosis and also to ascertain the side to which the head is deviated, the fetus must first be repelled cranially. The finger is then directed laterally towards the iliac shaft in order to detect the

* Care should be exercised in the use of ratchet forceps; there is a great temptation to close the forceps completely. Rampley's forceps without a ratchet are preferable.

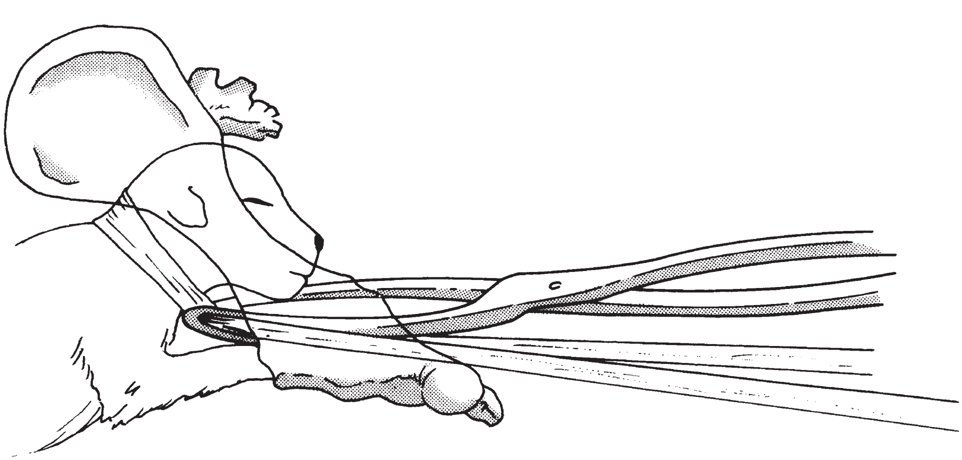

Fig. 13.4 Roberts' snare forceps applied to the fetal neck.

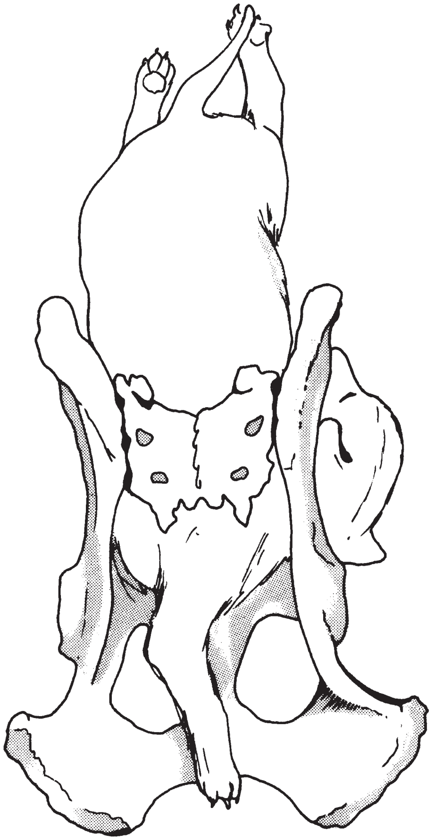

Fig. 13.5 Lateral deviation of the head (shoulder presentation).

fetal occiput or ears. In the small bitch or normal-size queen, this may not be difficult, but in the large one the length of the maternal pelvis and of the fetal neck are often such that it is impossible to make an accurate diagnosis, let alone correct the condition. In a protracted case it may be impossible to obtain the space in front of the pelvis necessary for exploration with the finger. The fetal fluids have been lost and the uterus has contracted firmly on the fetus, the latter often being enlarged by putrefactive emphysema.

Freak (1948) recommends Rampley's forceps both as an aid to diagnosis and to the correction of downward and lateral deviation of the head. It is proposed to quote her excellent description:

> Breast-head posture: The forceps are of great assistance (to diagnosis) since a light grip may be obtained on one foreleg, if present, or on the neck, raising the fetus sufficiently close into the pelvic inlet for a more complete examination to be made with the finger, when foetal ears may be recognized lying just below the pelvic brim. To correct the posture a light grip should be taken on the skin over the occiput and the foetus slightly repelled. Forceps may be left *in situ*, supported by the finger and thumb, while an attempt is made with the other hand on the maternal abdominal wall to raise the foetal head above the pelvic brim. Sometimes the forceps grip and repulsion of the foetus are alone sufficient to bring this about, and the finger can then be inserted into the mouth to hold it in position while the forceps are reapplied on the upper jaw. Frequently correction has to be done in stages, obtaining a grip a little lower on the forehead after each repulsion.
>
> Lateral deviation: Forceps are used to assist in the diagnosis of the posture and the side to which the head is deflected. The shoulder of the opposite side may be recognized by the finger, or again, the position of the ears may assist. When this is decided a grip is taken on that side of the head or neck presented and the foetus is repelled diagonally away from the side to which the head is turned. Again the grip and repulsion may need to be replaced, and again, particularly in a small bitch, great assistance is derived from external manipulation assisted by guidance from the finger in the vagina.

DELIVERY BY TRACTION

Traction may be employed in cases of fetomaternal disproportion when the less drastic methods previously outlined fail. It is used particularly in the case of dead and emphysematous fetuses. The method should always be avoided in the case of a living fetus, for the grip of the forceps generally causes it severe injury. Hobday's forceps are generally employed.

It should always be remembered that a caesarean operation or, in the case of putrid fetuses, hysterectomy, will carry a better prognosis for the bitch or queen than prolonged attempts at forceps delivery.

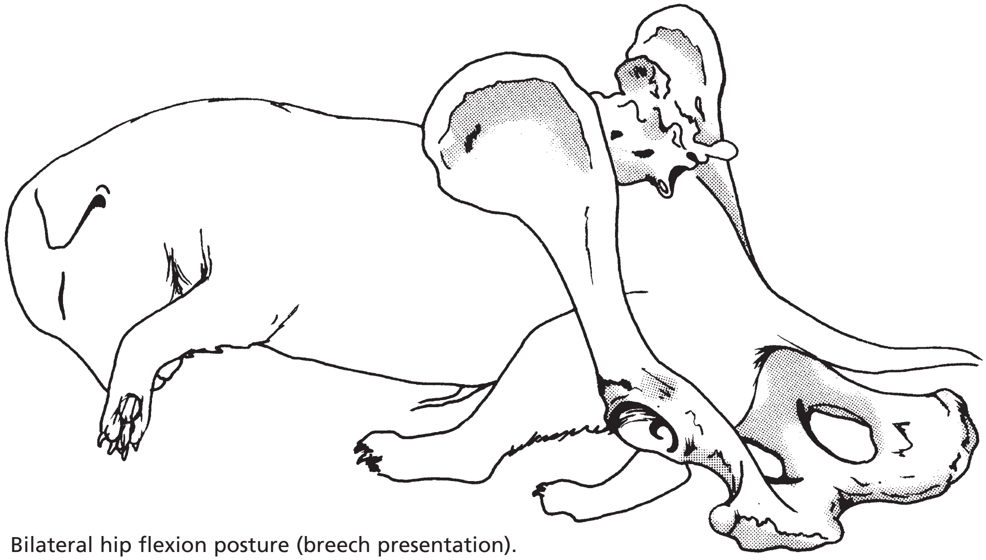

Fig. 13.6 Bilateral hip flexion posture (breech presentation).

In cases requiring traction, the whole of the fetus, with the possible exception of the limbs, lies in the uterus. Occasionally, in cases of posterior presentation (Figure 13.6), the pelvis and hindlimbs have passed into the pelvic inlet. In these it is best to repel these parts into the uterus before attempting to apply the forceps.

The aim is to obtain a secure grip across the fetal cranium or pelvis so that considerable traction can be applied. The application of the forceps to a limb or the lower or upper jaw is generally futile, because the force that is necessary to apply causes either the forceps to slip or the parts to be torn away.

The procedure should be carried out under general anaesthesia with the bitch or queen in breast recumbency. The position of the presented fetus is fixed by gripping it through the abdominal wall. The closed forceps are introduced into the vulva and directed at first upwards until they have reached the pelvic floor, then horizontally forwards through the pelvic canal, and finally slightly downwards and forwards into the uterus. Here the fetal extremity will be felt beneath. The jaws of the forceps are now opened as widely as possible and again depressed downwards. On closing them it becomes clear from the extent to which the handles are apart that the whole width of a fetal head or pelvis has been gripped. On no account should traction be applied until the operator is satisfied that he or she has a firm grip on the cranium or pelvis (Figure 13.7).

Working in the dark, as the method entails, the operator is always fearful lest the uterine wall has been picked up in addition to the fetus. Fortunately, if the forceps are applied within the uterus in the method described, there is little tendency to injury of the maternal soft parts in so doing; nevertheless, as soon as the secured part has been drawn back to a point that can be reached with a finger, the operator will ensure before proceeding that it is the fetus only which is involved.

Steady traction is applied in the upwards and backwards direction until the secured part has passed through the pelvic inlet. From this point, delivery is relatively easy. It will be appreciated that there is a limit to the amount of force which can be safely applied, for severe pulling may cause rupture of the vagina at the pelvic brim. In neglected cases, in which the fetuses are putrid, the application of traction often results in breakage of the fetus, a head or hind parts being torn away. Often in posterior presentation, the fetal trunk is torn away and the head remains in the uterus.

Again in protracted cases, in which complete inertia has supervened, attempts must not be made to extract fetuses from the cornua with the

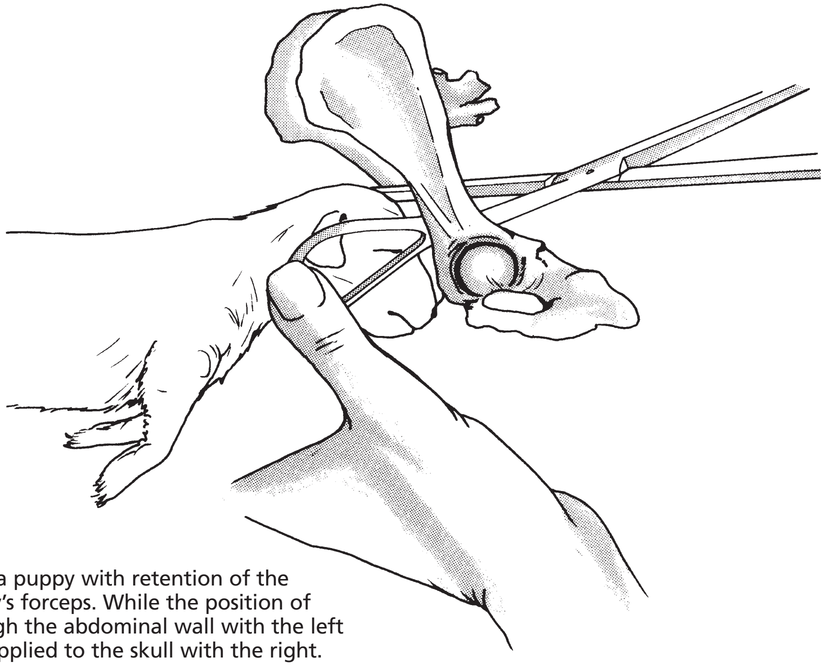

Fig. 13.7 Delivery of a puppy with retention of the forelimbs using Hobday's forceps. While the position of the fetus is fixed through the abdominal wall with the left hand, the forceps are applied to the skull with the right.

forceps, for it is highly probable that by so doing the uterus will be torn. Forceps delivery is only applicable to fetuses the extremity of which has passed into the uterine body.

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14 Dystocia due to fetomaternal disproportion: treatment

FETOMATERNAL DISPROPORTION IN CATTLE

As has been discussed earlier in the book, fetomaternal disproportion is a major cause of dystocia in cattle, with a considerable variation in the degree; it can be marginal or severe, the latter being associated with a very immature heifer or pathological enlargement of the fetus. The latter occurs with fetal giantism which can occur in embryos derived from in vitro maturation (IVM) or fertilisation (IVF), or prolonged gestation, or fetal monsters such as conjoined twins; these are described in Chapter 4 and 17.

Sometimes in cases of dystocia due to fetomaternal disproportion, it may not always be obvious to the obstetrician whether the fetus is too large or the pelvis too small. However, the clinical signs based on clinical history and examination are the same, namely that the dam has been straining unproductively for a time in excess of the normal duration of the second stage of parturition for that species, with the fetus in the normal disposition for birth. In addition, the approach to the case and the technique for the treatment of the dystocia and delivery of the fetus are the same. It may be overcome in one of the following ways:

- The normal expulsive forces may be supplemented by external traction on the fetus. This method is frequently employed successfully by stockpersons and shepherds.
- The diameter of the vulval opening may be increased by episiotomy.
- The fetus may be removed by a caesarean operation.
- The volume of the fetus may be reduced by fetotomy (originally referred to as embryotomy), i.e. dismemberment of its body within the uterus and vagina, and the fetus removed in several parts. Nowadays fetotomy is applied only when the fetus is already dead.

As a guide to deciding which of the foregoing methods to use in a case of fetomaternal disproportion the veterinarian should be influenced by the obstetrician's ideal, which is to render the abnormal birth as near to the physiological as possible, ensuring both the welfare and survival of dam and fetus whilst preserving the dam's subsequent fertility. In the case of a group of animals where dystocia is being caused by fetomaternal disproportion, consideration should be given to inducing early parturition in the remainder of the group (see Chapter 6).

Fetomaternal disproportion: anterior presentation

This is probably the commonest type of bovine dystocia. Modest disproportion is often successfully treated by the stockperson. It occurs in all breeds, particularly in immature heifers and those where there is a tendency for muscular hypertrophy. Although it is much commoner in heifers, many cases occur in mature cows, particularly when there has been a long delay in rendering obstetric aid, with resultant fetal enlargement due to emphysematous decomposition. Unfortunately, this occurs all too frequently. Often, when the veterinarian arrives, the animal has been in second-stage labour for at least 2 hours and there is a measure of secondary uterine inertia. The allantochorion has ruptured and two forefeet are visible as well as, occasionally, the fetal nose. Difficulty seems to be associated with the birth of the fetal head. In heifers this can be due to a failure of the posterior vagina and vulva to dilate; in adult cows it is often associated with too great a bulk of fetal chest and shoulders at the entrance to the maternal pelvis.

Once the head is expelled, the remainder of the calf can usually be delivered, except in the case of calves with muscular hypertrophy which have disproportionately large shoulders and particularly large hindquarters. In these cases, the head, and perhaps the chest, may emerge with relatively little effort, but the calf's hips will not pass into the maternal pelvis. At the initial examination, it is often difficult to be sure of the degree of disproportion, and therefore to decide which of the treatment options should be tried. With increasing experience, and if the degree of disproportion is severe, the veterinary surgeon may be able to make this judgement with considerable accuracy; however, in many cases it can only be made following attempted traction. A useful guide is to apply traction using two persons, or with a calving jack, and if it is possible to bring the head and the elbows of the two forelimbs caudal to the brim of the pelvis, then it is likely that traction will be successful. If it cannot be achieved, then an alternative strategy must be considered since prolonged unsuccessful traction will result in a high calf mortality rate and possible trauma to the cow or heifer.

Delivery by traction

The vast majority of cases of moderate fetomaternal disproportion are successfully treated by the application of manual traction to the presenting feet, but birth is greatly expedited by first applying a head snare so that an axial pull may be put on the fetus. For vaginal delivery, three snares are required, although it is important to stress that only minimal traction should be applied to the head snare.

The animal is suitably restrained. A loop is made in the head snare and this is carried into the vulva where part of the loop is placed in the calf's mouth and the remainder pushed up over the forehead and behind the ears. A simpler alternative, which is easier to apply and less stressful to the calf, is to push the centre point of a rope snare over the forehead and behind the ears, leaving both ends of the snare outside the vulva. A good axial pull, which also tends to depress the calf's poll ventrally, can be achieved by simultaneous traction on both ends of the snare. Each of the other snares is placed above the fore fetlock of the calf. At first, with the head rope held taut, traction is applied to one foot snare with a view to advancing one shoulder at a time through the pelvic entrance (Figure 14.1). Then the other leg is advanced. All three ropes are then pulled on. At all times traction should be synchronous with the expulsive efforts of the cow and, as far as practicable, the initial pulling should be upwards; once the head engages the vulva, however, the direction of traction should be obliquely downward. After each bout of straining, and with each small advance of the fetus, the veterinarian should ascertain by further examination that delivery is proceeding satisfactorily. Frequent applications of lubricant to the vagina and to the fetal occiput are indicated and the veterinarian should be satisfied with very gradual progress.

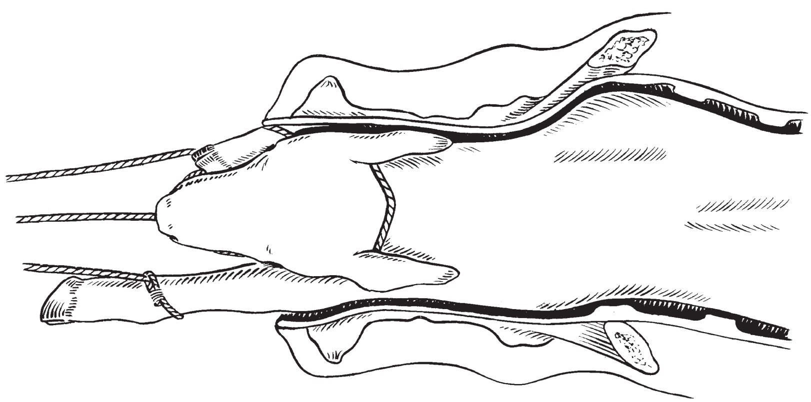

Fig. 14.1 Diagnosis: anterior presentation, dorsal position, extended posture; fetal oversize. Delivery by traction. Alternate traction is first applied to the forelimbs. Note Benesch's head snare for axial traction.

Episiotomy. If it is obvious that the vulva is relatively small (as is commonly the case in Friesian–Holstein heifers) and that further traction on the calf will cause rupture of the vulva and perineum (with subsequent infertility), episiotomy should be performed. Freiermuth (1948) suggested incising, in the shape of an arch and in a dorsolateral direction, the vulval labium in its upper third. Cutting directly upwards into the perineal raphe is contraindicated because, once started, further birth of the calf will cause a traumatic upward extension towards, and sometimes into, the anus and rectum creating a third-degree perineal laceration. It is preferable to cut both labiae in the manner advised by Freiermuth; the requisite depth of the vulval incisions can be decided only by trial on the basis of the minimum amount to allow delivery. By gentle traction on the fetal head so as to cause firm engagement of the occiput in the vulval orifice, it is easy to ascertain the necessary depth of the incisions. Local infiltration, rather than epidural anaesthesia, should be used so as not to interfere with the maternal expulsive efforts. Immediately after delivery the wounds should be sutured, the suture material being passed through all the tissues of the wound except the vulval mucosa.

Birth of the head is facilitated and rupture of the perineum is less likely to occur if, while downward traction is maintained on the head snare, the obstetrician inserts both hands, 'cups' them over the occiput and presses vigorously downwards. When the fetal head is born, all three ropes may be pulled on as the cow strains and the direction of traction should progressively approach the vertical. Obstruction sometimes occurs as the fetal pelvis engages the pelvic inlet; this is sometimes referred to as 'hip-lock' and is due to the greater trochanters of the femurs and the overlying muscle impinging on the shafts of the ilia. At this stage, slight retropulsion and rotation of the calf through an angle of 45° or even 90° is very helpful; this is because the sacral-pubic dimension is greater than that between the two ilia (remember the pelvic opening is oval in shape). The direction of traction should now be vertically downwards until birth is completed. The calf is attended to so as to free its nostrils of amnion or mucus, and respiration is stimulated. The genital tract of the cow or heifer is explored, firstly in order to ascertain that another calf is not present, and secondly to make sure that it has sustained no trauma.

In the case of impacted 'hip-lock' by a dead fetus where it is found impossible to repel and rotate the calf, Graham (1979) has suggested a method of reducing the fetal diameter so that traction may succeed. He uses a long-handled (75 cm) blunt hook which is passed into the fetal abdomen through an incision made just behind the xiphisternum. The hook is advanced to engage the fetal pelvis and abrupt traction on it then fractures the pelvic girdle. One or two repetitions of this procedure to cause further fractures and to ensure pelvic collapse may be followed by easy traction delivery. Another method of treating hip-lock in a dead fetus is to make a transverse bisection of the calf in the thoracolumbar region and then to divide the hindquarters by means of a vertical cut, both cuts being made by means of the wire-saw fetotome. When this has been completed each 'half' of the hindquarters can then be removed with care, which sometimes can be difficult without the use of obstetrical hooks (see Figure 12.1).

At all stages of traction it is important that the veterinarian should determine that the disposition of the calf continues to remain normal, as well as its progress through the birth canal by vaginal examination; the importance of ensuring that there is plenty of lubrication cannot be stressed enough.

Where possible, traction should coincide with the abdominal contractions of the cow, and the veterinary surgeon should be satisfied with very gradual progress. It is not unusual for a cow to go down when heavy traction is applied; this is not necessarily a disadvantage, provided that she does not fall awkwardly and injure herself. In fact, with the patient in lateral recumbency, traction may be applied to better advantage, particularly if manual or by means of a pulley block. In the case of some calving jacks it can be an inconvenience. As you may have noted in Chapter 12 (Table 12.1), the tractive forces exerted by calving aids and pulley blocks are much greater than those associated with natural calvings and the use of people. Despite their obvious advantages there are some important disadvantages:

- The amount of force which in unskilled hands can be applied.

- The fact that the pull is continuous and ungiving, which may lead to damage of maternal soft tissues. (In natural birth the calf would be advanced some way with each contraction, and then go back a little before the next contraction pushes it even further.)
- The fact that the direction of pull has to be at least slightly down towards the udder. If it is horizontal or away from the udder then the rump bar merely slips down the perineum away from the vulva when traction is applied. This means it is very difficult to apply force in the same direction as the expulsion forces of the cow. Ideally, force should be applied in a slightly upward direction until the calf's head is within the pelvis, then in a horizontal direction until the calf's head and chest have been delivered and, finally, in a progressively more downward direction until the calf's hips have been born. This has been overcome in a more recent design of the calf puller, the Vink calving jack. This has a rump frame which fits around the tail head and vulva of the cow, allowing traction to be applied in the direction chosen by the obstetrician.

If after 5 minutes of judicious traction no progress is made, the veterinary surgeon must resort to a caesarean operation if the calf is alive or dead, or fetotomy if the calf is dead. There are cases where it is difficult to assess whether a calf is alive or dead. If there is any question, the calf should be given the benefit of the doubt. If certain of success by the employment of limited fetotomy, such as the removal of a forelimb, or a forelimb together with the head and neck, this would be the method of choice; unfortunately, not infrequently, having embarked on fetotomy the obstetrician finds that total dismemberment will be necessary to effect delivery. Because of the difficulty in assessing the amount of fetotomy required and the knowledge that total fetotomy is a tedious and arduous task, there is an increasing tendency for veterinary surgeons to resort to a caesarean operation in cases of disproportion where the fetus cannot be delivered by reasonable traction.

Assessment of the likely success of traction to relieve dystocia due to fetomaternal disproportion is very much based on trial and error. Several attempts have been made to develop predictive methods of the likely success of traction or whether a caesarean operation should be performed, the objective being to prevent the sequence: attempted traction/failure/caesarean operation/dead calf (Hindson, 1978). In any predictive method the two factors which have to be considered are the size of the calf and the size of the pelvis. Hindson (1978) found a good correlation between the digital diameter of the calf (as measured at the level of the fetlock) and its body weight. Since at the time of dystocia it is likely to be difficult, if not impossible, to measure the size of the pelvic inlet directly attempts have been made to correlate it with external pelvic measurements. Hindson (1978) found a good correlation between the medial interischial tuberosity distance and both the vertical and horizontal pelvic diameters. As a result of this, and a study involving 60 selected calvings, he devised a formula to obtain a figure for the traction ratio (TR). It is as follows:

$$TR = \frac{\text{Interischial distance}}{\text{Calf's digital diameter}} \times \frac{P1}{P2} \times \frac{1}{E}$$

P1 = the party factor of 0.95 for heifers; P2 = a correction factor of 1.05 for posterior presentation; E = a factor for breeds with muscular hypertrophy.

Traction ratios greater than 2.5 are unlikely to have dystocia due to fetomaternal disproportion; between 2.3 and 2.5 traction is likely to be successful; between 2.1 and 2.3 substantial traction may be required which may not be successful; 2.1 or less the method of treatment should be by caesarean operation. In the author's experience, it has some value as a predictive method, but since there are other variables such as the degree of uterine inertia or the dryness of the birth canal, for example, it needs to be used with caution.

The technique of fetotomy for severe fetal oversize in extended anterior presentation will now be described. The method used involves the removal of one or sometimes two forelimbs, with a view to reducing the circumference of the fetal chest. If the head is likely seriously to impede the proposed manipulations it may be returned to the uterus; failing this, it may first be removed (Figure 14.2)

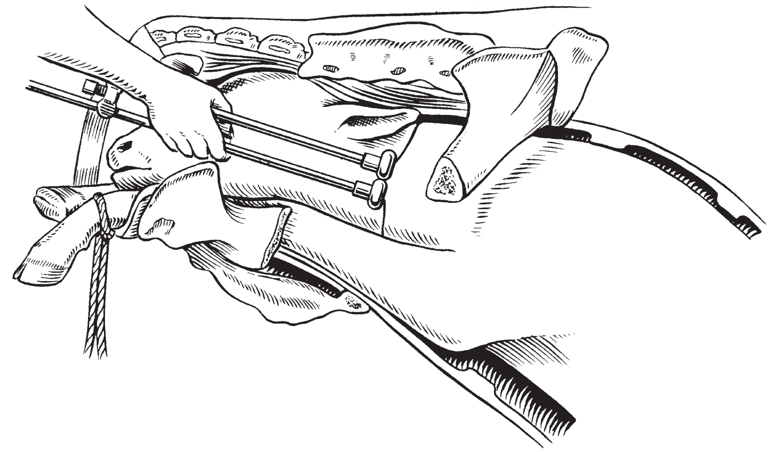

Fig. 14.2 Diagnosis: as in Figure 14.1. Delivery by fetotomy. Amputation of the head using Thygesen's wire-saw fetotome.

but it must be understood that the head is not itself the cause of dystocia due to fetomaternal disproportion.

Subcutaneous fetotomy: removal of a forelimb

A foreleg may be removed by subcutaneous or percutaneous fetotomy. In either case, caudal epidural anaesthesia is employed. The simpler method, which will now be described, is subcutaneous removal, for which the essential instrument is a fetotomy knife. When both forelegs are equally accessible it is immaterial which is removed, but the right-handed operator will find it easier to perform fetotomy on the left foreleg of the calf. This leg is snared – around the pastern rather than above the fetlock – and sustained traction applied to it by one assistant. The obstetrician makes a small incision with a scalpel into the skin in front of the fetlock joint. Into this 'nick' the beak of Roberts' fetotomy knife is inserted, and a longitudinal incision is made up the front of the limb from the pastern to the scapular cartilage (Figure 14.3).

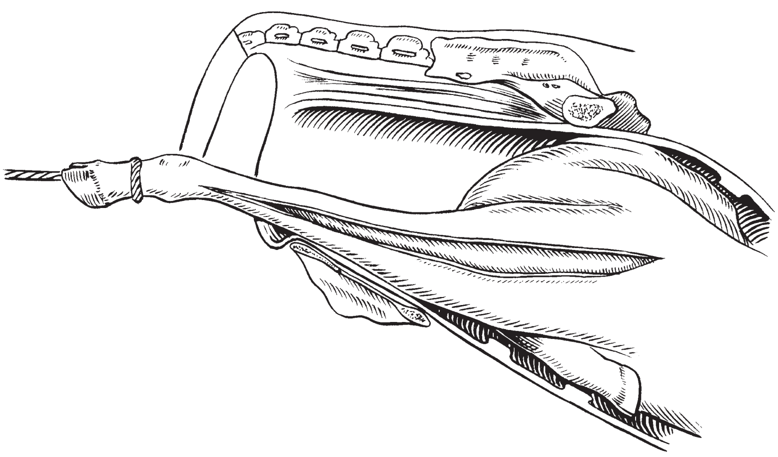

Fig. 14.3 Diagnosis: as in Figure 14.1. The head has been returned to the uterus. Delivery by fetotomy. Subcutaneous removal of the extended forelimb. Stage 1: the skin has been incised from the fetlock to the scapula, using Roberts' fetotomy knife.

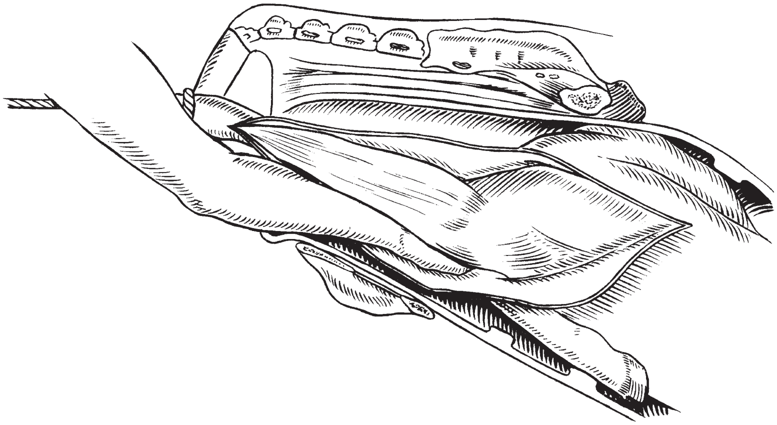

Fig. 14.4 Diagnosis: as in Figure 14.1. Subcutaneous removal of the extended forelimb. Stage 2: finger dissection of the skin around the leg and extending as high as possible in the scapular region.

The knife is now laid aside, and the second step in the procedure is literally the 'skinning' of the limb in situ (Figure 14.4). This operation requires strong fingers, but with diligent application it may be completed in about 10 minutes. (The separation of the skin from the muscles lying over the scapula completes this second step.)

The third step involves the division of the adductor muscles. This is conveniently done by reintroducing Roberts' knife, and, by vigorous probing with the beak of the instrument, the muscle mass is separated into several 'strings'; then each of these, in turn, is engaged and severed by the knife.

The fourth step (Figure 14.5) is to disarticulate the fetlock joint so that the digit is left connected to the detached skin of the metacarpus. A snare is then attached to the cannon bone, and, in order to get a more secure hold, an additional half-hitch is put on above the first loop.

The shank of the snare, with traction bars, is then handed to two assistants, and the final step in the operation consists in avulsion of the denuded forelimb by the forcible traction while the operator applies counterforce to the front of the fetus. In this way, the remaining muscle attachments to the top of the scapula are broken and the limb comes away.

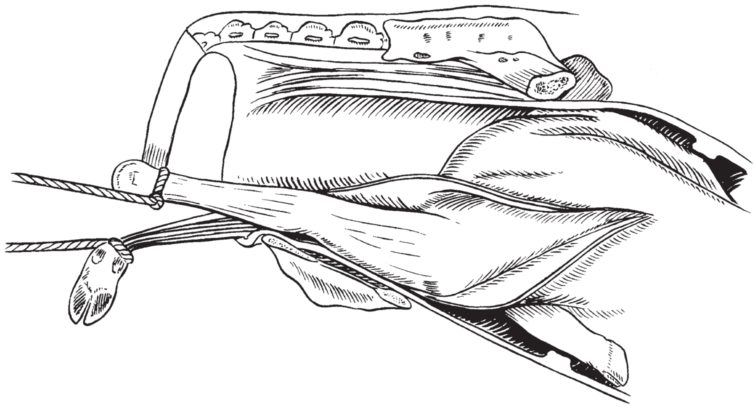

Fig. 14.5 Diagnosis: as in Figure 14.1. Subcutaneous removal of the extended forelimb. Stage 3: after the attachments of the pectoral muscles in the axilla have been broken down and the metacarpophalangeal joint disarticulated, traction is applied to the denuded limb. Note that the foot is still attached to the skin.

In many cases the removal of the one forelimb gives a sufficient reduction in fetal diameter to allow delivery. The principles of traction previously described are applied and in this case the foot and skin of the amputated limb afford a safe hold for a snare. Should delivery not be possible after this operation, the other foreleg must be removed in the same way, after which moderate traction is usually successful. Occasionally, after removal of one or both forelimbs – and despite partial rotation of the fetus – its hindquarters become locked at the pelvic inlet. Now the calf should be withdrawn as far as possible, and the protruding part of the trunk completely severed. The fetal abdomen is eviscerated, following which one of the hindlegs must be removed. There are two ways of doing this, and the one chosen will depend largely on the mobility of the retained extremity. If it is possible, the posterior part of the calf should be repelled and one of the hindlimbs brought forward with the aid of a snare; the limb is then removed by subcutaneous fetotomy (presently to be described). If it is not possible to grasp the limb and bring it forward it must be amputated in the following way. Using a direct cutting fetotomy knife, such as Unsworth's, an incision is made over the hip joint of the leg to be removed. The muscles lateral to the femoral head are also divided and the upper extremity of the femur is isolated. Around this a snare is passed and by vigorous abrupt traction the teres ligament is broken and the articular head freed from the acetabulum. The snare loop is then made secure below the great trochanter and sustained traction applied. This causes the leg to be drawn out from its skin; difficulty occurs over the os calcis but a few strokes of the knife frees this part also. The hind digit should be left attached to the skin and the leg disarticulated at the fetlock joint. After one of the hindlimbs is removed, the remainder of the posterior part of the fetus can be withdrawn by traction through the medium of the double hook – which is attached to the coapted skin of the severed trunk – and the digit and skin of the amputated limb. Amputation of both hindlimbs is rarely needed.

In cases where hip-lock occurs after partial fetotomy of the front extremity, Graham's (1979) method of causing fetal pelvic collapse should be considered as an alternative to further dismemberment of the fetus.

Complete fetotomy as described above is tiring and time-consuming, and requires substantial skill as well as the appropriate equipment. If the fetus is emphysematous and undergoing putrefaction the tissues readily break down even with modest force, thus making the task much easier.

Percutaneous fetotomy

In the opinion of many obstetricians, the delivery of a dead calf associated with dystocia due to fetomaternal disproportion may be more expeditiously accomplished by percutaneous fetotomy, that is, by means of the wire-saw tubular fetotome. For ease of sterilisation the model preferred is the Swedish modification of Thygesen's instrument. Reliable wire, safe handgrips, a wire introducer – such as Schriever's – and a threader are required. Percutaneous fetotomy of a calf in anterior presentation will now be described.

The first operation is the removal, in one piece, of the fetal head, neck and one forelimb (Figure 14.6). To do this the fetotome wire must be looped around the neck and forelimb and pushed back on one side so as to lie behind the posterior angle of the scapula where a deep incision is made with Unsworth's knife to accommodate the wire. The head of the instrument is brought up to the base of the neck on the side opposite to the foreleg being removed. With the wire loop correctly placed, the section is very easily completed by an assistant who makes long sawing strokes, so as to use the maximum length of available wire. The detached segment of fetus is carefully drawn out of the birth canal. An attempt is now made to deliver the remainder of the calf by traction; a snare is placed on the intact limb and, with the aid of the double hook, another point of traction is available on the exposed lower, cervical vertebral column. If birth is not yet possible, the calf is repelled and the fetotome wire is looped around the trunk of the calf with the head of the instrument laterally, and as far back as possible, in the dorsolumbar region (Figure 14.7). Sawing is continued until the vertebral column is severed, when the anterior part of the calf may be delivered. The remainder of the abdomen is eviscerated, and the next step is to bisect, in the sagittal plane, the

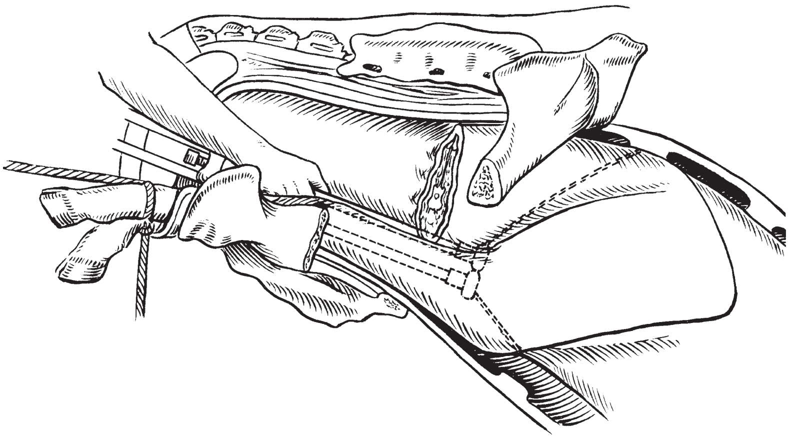

Fig. 14.6 Diagnosis: as in Figure 14.1. Delivery by percutaneous fetotomy. Amputation of the forelimb and neck after removal of the head (as in Figure 14.2). It is sometimes possible to remove the head, neck and forelimb in one operation.

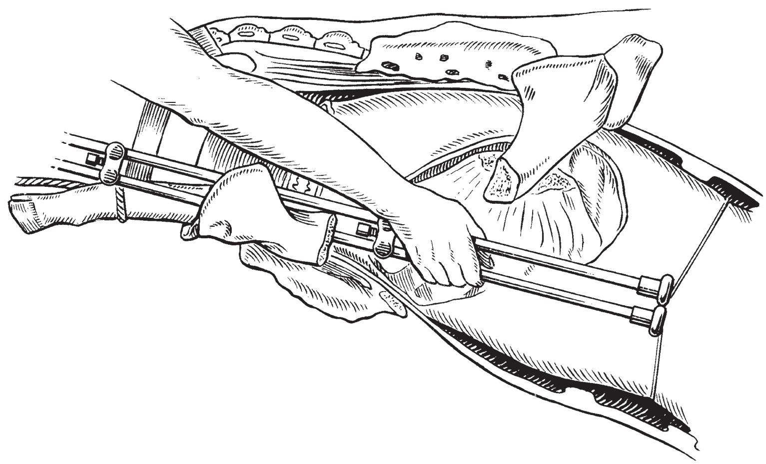

Fig. 14.7 Diagnosis: as in Figure 14.1. Delivery by percutaneous fetotomy. Transverse division through the trunk after removal of the head and forelimb. Note that if the base of the neck had been removed with the forelimb, as in Figure 14.6, the operation would have been simplified.

hind extremity. To do this, the introducer, with wire attached, is passed over the dorsal aspect of the sacrum and down behind the perineum, where the hand, passed in under the calf, reaches it, pulls it out and completes the loop. The head of the instrument is placed against the fetal spine (Figure 14.8) and the hindquarters are divided by direct sawing; then each of the halves can be withdrawn in turn by means of the double hook.

In comparing the facility with which a calf may be removed by subcutaneous or percutaneous fetotomy, it must be clearly appreciated that the troublesome part of the percutaneous method is the correct placing and retention of the wire. Given strong wire, the actual sawing presents no difficulty. Occasionally, the two methods may be advantageously combined, e.g. the subcutaneous procedure for the forelimb(s) and the wire-saw fetotome for the head, trunk and hindlimbs. Many veterinary surgeons now prefer a caesarean operation to total fetotomy. One cannot generalise on which method is preferable, but the subsequent health and fertility of the cow should figure prominently in the reckoning.

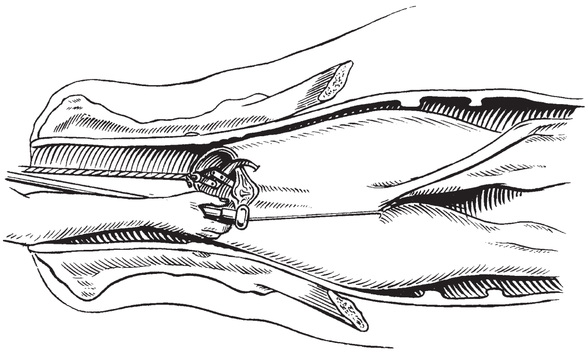

Fig. 14.8 Diagnosis: as in Figure 14.1. Delivery by percutaneous fetotomy. Final stage of total fetotomy: longitudinal division of the hindparts.

Fetomaternal disproportion: posterior presentation

The capacity of the fetus to survive obstructive dystocia is diminished if it is in posterior presentation; such cases therefore require prompt attention. Because of the abruptly presenting buttocks and contrary direction of the fetal hair, a posteriorly presented fetus is more difficult to deliver than a comparable one presented anteriorly. The retroverted tail may also be an impediment.

When confronted with such a dystocia, the obstetrician should first attempt to assess the degree of disparity between the fetus and birth canal. Where oversize is slight, delivery by traction should first be tried.

Delivery by traction

The hindfeet are usually visible at the vulva, and to them snares are applied above the fetlock joints. It should be ascertained that the fetal tail is not retroverted; in delayed cases fetal fluid supplements are essential. With one leg repelled as far as possible (Figure 14.9), the other is pulled on so as to bring its stifle over the pelvic brim. The repelled limb is similarly dealt with. In this way a smaller fetal diameter is presented at the pelvic inlet and, with this simple manoeuvre, traction may succeed. A simple way of assessing the likely success of traction can usually be predicted if both stifle joints can be brought into the pelvis following a moderate amount of traction. If during traction the fetal pelvis becomes 'jammed' in the birth canal, the calf should be repelled a little, rotated through 45° and again pulled on. This latter manipulation, which brings the greater diameter of the fetus into the largest pelvic dimension, is often successful; it may be accomplished by simply bending the protruding metatarsi and using them as levers in a rotary manner. There is a misunderstanding, particularly among some stockpersons, that calves in posterior presentation need to be pulled out very rapidly, otherwise they will die. One must remember that the calf's life will not be compromised until its umbilical cord becomes trapped against the maternal pelvis. In practical terms, therefore, traction should be slow and controlled until such time as the calf's tail-head and anus begin to emerge from

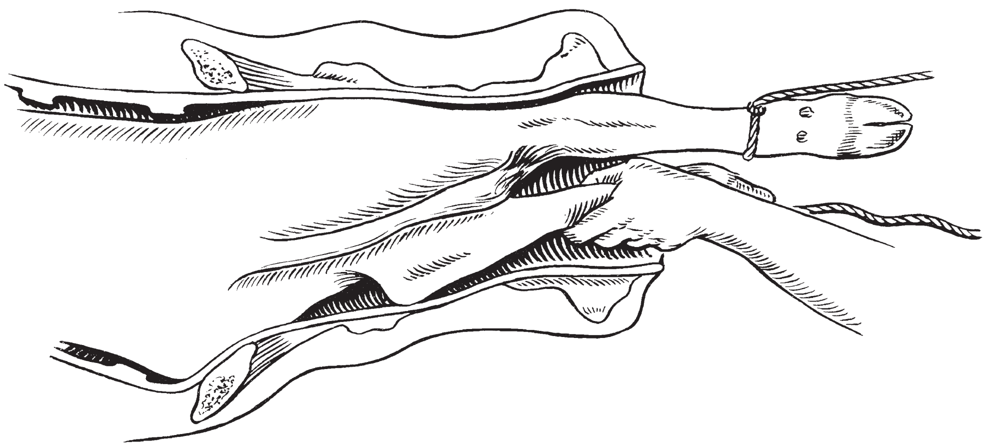

Fig. 14.9 Diagnosis: posterior presentation, dorsal position, extended posture; fetal oversize. Delivery by traction. Alternate traction on the hindlimbs.

the cow's vulva. Once this point is reached, delay should be avoided. If the hindquarters can be delivered the forequarters usually follow, but there are exceptions and they will be considered when discussing total fetotomy in posterior presentation.

In cases of posterior presentation where substantial judicious traction has not succeeded, the fetus must be removed by caesarean operation if the calf is alive, or if dead by caesarean or fetotomy. In the case of an immovable, dead fetus there is a choice about which it is difficult to generalise, but if there is obviously gross oversize a laparotomy is preferable. In many instances of medium oversized and dead fetuses, however, it may be easier to remove one limb, for this relatively simple operation often makes birth possible, and this fetotomy will now be described. The presenting legs can be removed by subcutaneous or percutaneous methods, and the former will be described first.

Subcutaneous removal of the hindlimb

Posterior epidural anaesthesia is induced and a 'nick' made just above the fetlock on the posterior aspect of the extended fetal leg. Into this is placed the 'beak' of Roberts' knife, and with it an incision is made from the fetlock up the back of the limb to the anterior gluteal region. The skin is separated all around the leg, and the muscles above the hip joint, as well as the adductor muscles, are divided. The femoral head is detached from the acetabulum by introducing a traction bar underneath the Achilles tendon and by forcibly rotating the limb laterally. The skin is then cut sufficiently around the fetlock joint to give scope for disarticulation, and a rope snare is placed over the freed end of the metatarsus. Sustained traction on the snare by two assistants, with retropulsion of the calf by the obstetrician, usually causes avulsion of the denuded limb.

Removal of the one leg followed by traction on its foot – connected to the torso by the skin of the leg – and on the other limb often results in extraction of the calf. If it does not, then the other hindlimb must be similarly removed. This will allow complete delivery or birth of the posterior half of the calf.

Should the forequarters become obstructed at the pelvic inlet, then further fetotomy is required as follows. As much of the calf as possible is withdrawn from the vulva and amputated. Evisceration is now carried out. The remainder is repelled and then, with Unsworth's knife, an incision is made in the skin over the scapula cartilage, and the muscles which connect the scapula to the spine are divided. By blunt dissection, the upper end of the shoulder blade is isolated and to it Krey's hooks are fastened and traction applied. In this way, the limb is drawn out of its skin as far as the fetlock joint, at which point it is disarticulated and removed. The digit, with skin attached, together with Krey's hooks gripping the thoracic vertebral column, serve as traction points for extraction of the remainder of the calf. In rare cases, before the anterior half can be withdrawn, the other forelimb must be removed.

Percutaneous removal of the hindlimb

Percutaneous fetotomy in posterior presentation is most conveniently performed with the tubular wire-saw Danish fetotome. The instrument is threaded, and the wire loop placed over one foot and passed up the limb so that laterally it lies anterior to the external angle of the ilium where a cut in the skin, previously made with Unsworth's knife, helps to retain it. The head of the instrument is placed lateral to the anus, and the tail of the calf must be included in the loop; otherwise, during sawing, the wire will slip down the limb and the section will be made through the distal third instead of through the upper extremity of the femur. The severed limb is removed. Traction is then applied to the calf by means of the Krey–Schottler hook attached to the perineum or with the aid of Obermayer's anal hook passed over the calf's pubic brim. If delivery is still impossible, the other hindleg must be removed and the fetus withdrawn as far as possible. If the calf cannot now be removed completely then its trunk must be bisected by means of the wire loop, the division being made as far forward as possible. One, or if necessary both, forelimbs are afterwards amputated by passing the wire, with the aid of Schriever's introducer, forwards between the neck and foreleg and then reaching for the introducer underneath the calf; the wire is withdrawn, the threading of the instrument completed and its 'head' passed up the severed end of the vertebral column where the section may be made by sawing. The severed limb may be brought out by attaching to it Krey's hook.

An attempt is again made to withdraw the anterior portion of the calf and in most cases this is now possible. In the exceptional case the other foreleg must be removed in like manner.

FETOMATERNAL DISPROPORTION IN OTHER ANIMALS

Mare

Disproportion as a cause of dystocia is uncommon in horses. Apart from being more urgent, the occasional case of relative oversize is treated on similar lines to the bovine case, with the exception that because of the late osseous union of the fetal skull, only limited traction should be applied to the fetal head. Although prolonged gestation is not uncommon, excessively large fetuses are rare in horses. When the fetus is alive, the caesarean operation is the first consideration and, with the increasing experience of recent years, it is now preferred to total fetotomy for a dead fetus.

Ewe

Oversize is a common cause of dystocia in ewes carrying single lambs. Ewes of the smaller breeds are often mated to larger rams, and although the fetal size is controlled to a large extent by the dam, bulky body features derived from the ram, such as large head and coarseness of shoulders and buttocks, often cause trouble. Most cases are successfully overcome by the shepherd applying traction to the forelegs. More severe cases may be brought to the veterinary surgery, where they may be conveniently treated as described for the cow. Where judicious traction – using fine snares, copious lubricants and a high standard of cleanliness – does not succeed, a caesarean operation or fetotomy may be employed. Where the fetus is dead, and this is frequently so in cases seen by the veterinary surgeon, fetotomy is often indicated. In this species the subcutaneous methods of limb removal are very easily carried out, but the percutaneous technique, using the wire-saw protected by Glattli's spiral tubes, is quite practicable.

Sow

Although fetal oversize may occur in the multiparous species when pregnant with an abnormally small litter, it cannot be treated by fetotomy; if traction by hand, snare or forceps fails, then hysterotomy is indicated.

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15 Dystocia due to postural defects: treatment

POSTURAL DEFECTS OF ANTERIOR PRESENTATION IN CATTLE

Faulty disposition due to postural defects, of which the commonest are carpal flexion and lateral deviation of the head, are a frequent cause of dystocia in ruminant species. Generally, postural defects are readily rectified by manipulation if treated early in second-stage labour. But in neglected cases associated with secondary uterine inertia, loss of fetal fluids and a dead, emphysematous fetus, tightly enclosed by the uterus, very serious dystocia may occur, for which fetotomy or a caesarean operation may be required.

The mechanics of the correction of postural defects are extremely simple; the secret of success lies in an appreciation of the value of retropulsion. Except for dystocia of short duration, this means that epidural anaesthesia is needed, particularly for the inexperienced veterinarian. Hence, once the posture has been corrected, the cow must then be delivered by traction, since she will not strain to aid expulsion. The obstetrician with relatively thin arms may have a significant advantage in correcting postural defects, in that it is often possible for both arms to be used inside the cow simultaneously – one to push and the other to pull.

Abnormalities of posture will be considered in series – beginning with the simple and proceeding to the complicated – in each example.

Carpal flexion posture

One or both forelimbs may be affected. In the unilateral case the flexed carpus is engaged at the pelvic inlet; the other forefoot may be visible at the vulva. The simple recent case requires retropulsion at the fetal head or shoulder; the retained foot is then grasped and, as the carpus is pushed upwards, the foot is carried outwards and finally brought forwards in an arc over the pelvic brim and extended alongside the other limb (Figure 15.1). More difficult cases require a snare attached to the retained

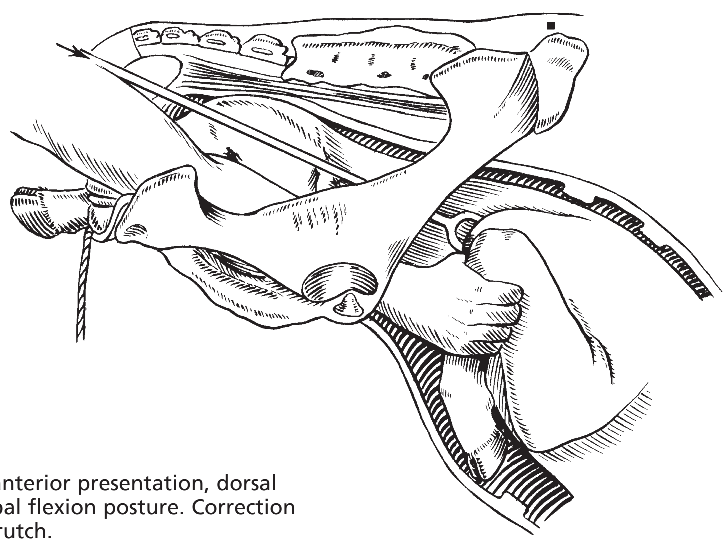

Fig. 15.1 Diagnosis: anterior presentation, dorsal position, unilateral carpal flexion posture. Correction using the hand and a crutch.

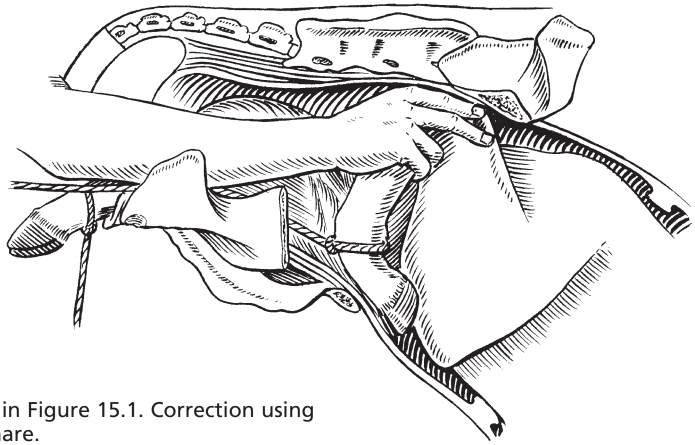

Fig. 15.2 Diagnosis: as in Figure 15.1. Correction using the hand and a digital snare.

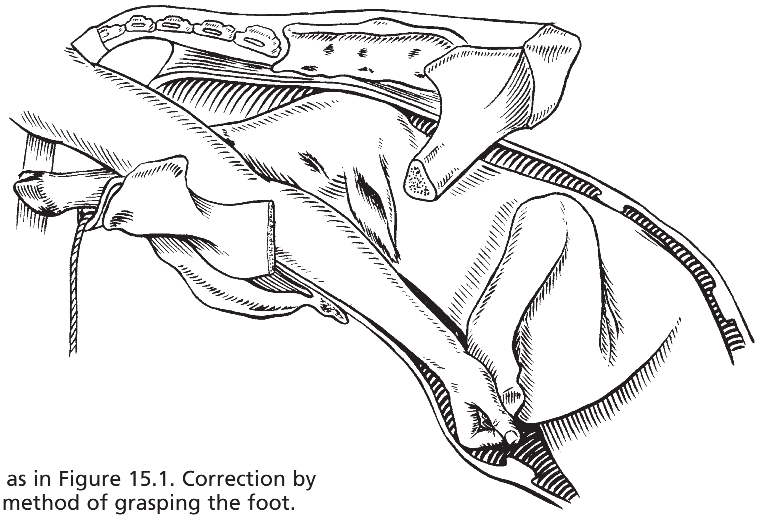

Fig. 15.3 Diagnosis: as in Figure 15.1. Correction by hand alone. Note the method of grasping the foot.

fetlock to help extend the limb (Figure 15.2). The fetal foot should always be carried over the pelvic brim in the cupped hand of the obstetrician (Figure 15.3). An obstinate case may require the introduction of copious warm water to help mobilise the calf. Rarely, in very protracted dystocia and cases of ankylosis, the limb cannot be extended and then it must be cut through at the carpus by means of the wire-saw fetotome.

Incomplete extension of the elbow(s)

This case is diagnosed on vaginal examination, with the digits emerging at the same level as the fetal muzzle instead of being well advanced beyond it. Usually, without the need of epidural anaesthesia, the head is repelled and each limb pulled in turn in an obliquely upward direction so as to lift the olecranon process over the maternal pelvic brim. Delivery is accomplished by traction on the head and both forelimbs, as already described in the chapter on fetomaternal disproportion.

Shoulder flexion posture; complete retention of the forelimb(s)

This type of dystocia may be unilateral or bilateral. The diagnosis of bilateral retention is usually obvious by observing that the head partly or completely protrudes from the vulva, but in the absence of the forelimbs. (In bilateral carpal flexion the head cannot be advanced so far). In a 'roomy' cow, with a small full-term or premature calf, the dystocia may be overcome by traction in the abnormal posture; in such cases, unless there has been much delay, correction of the abnormal posture is usually easy and always should be resorted to.

Retropulsion is a very obvious necessity, and if the extruded head is very swollen and the calf is dead, it should be amputated outside the vulva. To this end, Krey's hooks are placed in the orbits and traction applied, or if downward pressure is applied to the head, the head can be forced beyond the vulva to allow disarticulation at the occipito-atlantal joint using a sharp knife or scalpel. Following this, as the fetus is repelled, the retained forelimbs tend to come forwards; the calf's radius and ulna are then grasped and the defect is easily converted into carpal flexion posture and relieved accordingly (Figures 15.4 and 15.5).

In the more difficult case the limb must be snared, at first proximally, and then the noose passed down until it lies above the fetlock, the shank being placed from before backwards between the claws so as to flex the fetlock and pastern when traction is applied to it. The digits are held in the

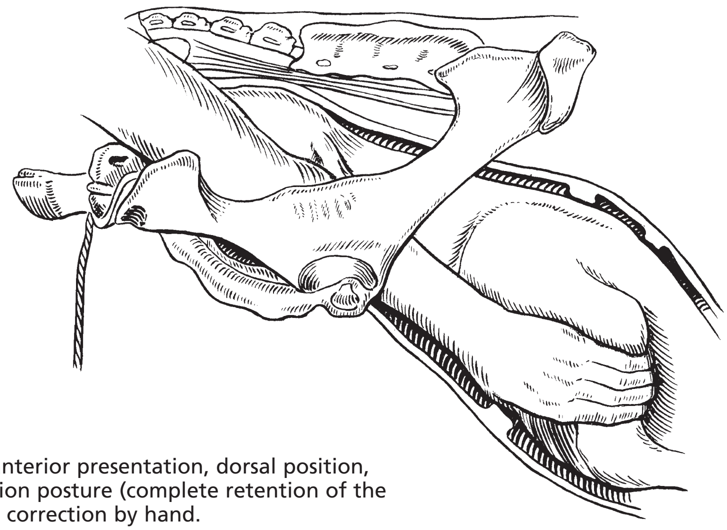

Fig. 15.4 Diagnosis: anterior presentation, dorsal position, unilateral shoulder flexion posture (complete retention of the forelimb). First stage of correction by hand.

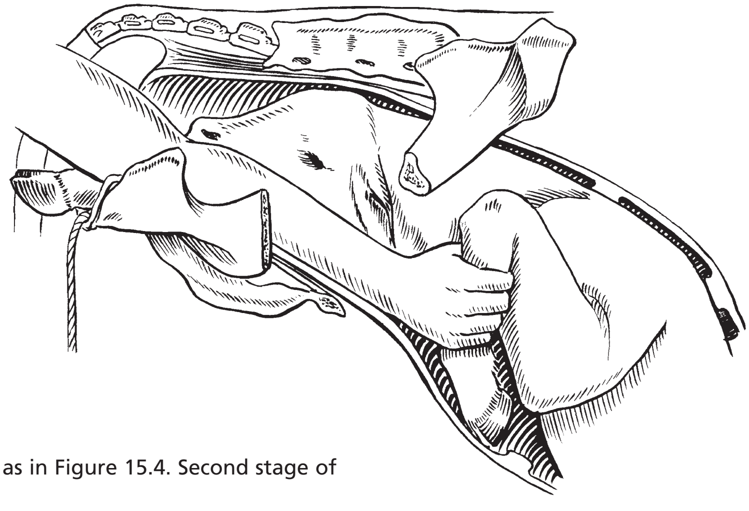

Fig. 15.5 Diagnosis: as in Figure 15.4. Second stage of correction by hand.

cupped hand and the carpus forced upwards while an assistant, pulling on the snare, helps the operator to bring the foot over the pelvic brim. In a delayed case, such a manoeuvre may be impossible, and then fetotomy of the limb is undertaken by the percutaneous fetotomy.

Percutaneous removal of retained forelimb

Epidural anaesthesia is indicated and, if it has not already been done, extravulval decapitation is now essential. At this stage it is wise to employ fetal fluid substitute which is fed into the uterus by gravity flow from an elevated funnel attached to a rubber delivery tube, whose end is controlled by a hand in the uterus. The fetus is now repelled and the fetotomy wire, protruding from one tube of the fetotome and fitted with Schriever's introducer, is passed in dorsally above the neck and down between the thorax and retained limb whence it is sought below by the hand introduced ventrally to the fetal scapula. The introducer and wire are drawn outwards, and the threading of the fetotome is completed. The head of the instrument is passed into the birth canal so that finally it rests dorsal to the posterior angle of the scapula. Some force is required to maintain it thus, while the muscles which attach the limb to the trunk and the skin at the base of the neck are severed by sawing. The detached portion is extracted by means of Krey's hooks. An attempt should again be made to extend the other retained limb and, this being possible, traction on it, and on the neck – through the medium of Krey's hooks – should result in delivery. Alternatively, the fetus may be withdrawn without extension of the other leg. If both these attempts fail, then the other limb must also be removed, the subsequent procedure being that described for fetomaternal disproportion.

Subcutaneous removal of retained forelimb

The operation of subcutaneous detachment of the retained forelimb again requires prior removal of the head so as to allow sufficient space for the hand and arm to carry in Unsworth's knife, which is used to divide the skin and muscles that connect the dorsal border of the scapula to the trunk. Following this division, vigorous blunt dissection is employed in order to expose the upper part of the scapula. To this isolated portion either a snare or Krey's hook is attached and traction applied. The operation is expedited by further vigorous incision of the adductor (pectoral) muscles. In this way the limb is pulled out of its skin until the fetlock joint is exposed, at which point disarticulation is performed and the limb removed leaving the foot attached to the skin of the limb. The fetus is now withdrawn by traction on the intact limb and on the foot and skin of the detached limb. If necessary, additional traction may be produced by fastening Krey's hooks to the exposed end of the vertebral column.

Lateral deviation of the head

The head may be displaced to either side, and this constitutes one of the commonest types of ruminant dystocia. When treated in early second-stage labour, it is easily corrected by hand, without recourse to epidural anaesthesia. The lubricated hand is introduced and, when the provoked straining has ceased, the fetus is repelled by pressing forwards at the base of its neck. The hand is then quickly transferred to the muzzle of the calf, which is firmly grasped and brought round through an arc until the nose is in line with the birth canal (Figure 15.6). In a more inaccessible case, the muzzle may be reached after preliminary traction on the commissure of the mouth (Figure 15.7) or on the mandible (Figure 15.8). A head snare and forelimb snares are now affixed, and traction, synchronously applied with the cow's expulsive efforts, leads to delivery.

In more protracted cases of dystocia due to head displacement, with greater loss of fetal fluid and with the uterus contracted on the calf, it is more difficult to rectify the posture. Caudal epidural anaesthesia is indicated, followed by the instillation of fetal fluid substitute; this renders the calf more buoyant. A special head cord, of smaller calibre than those used on the limbs, is carried in as a running noose and slipped over the mandible of the calf, where it is tightened, and the shank of the snare is handed to an assistant (Figure 15.8). The operator reintroduces a hand,

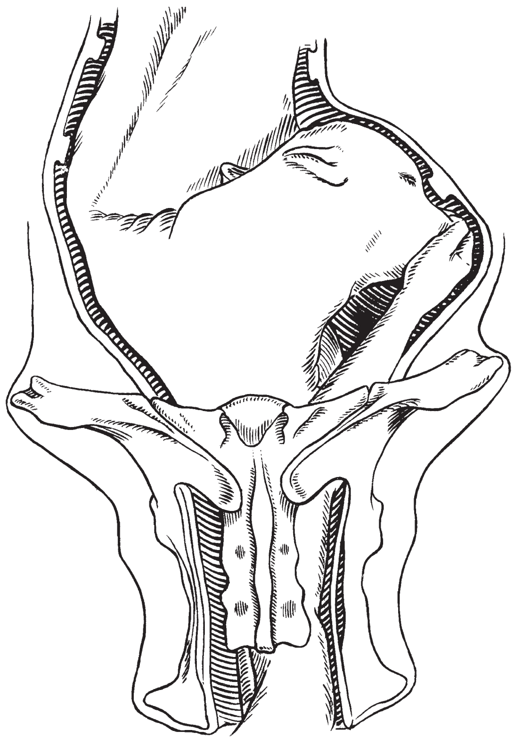

Fig. 15.6 Diagnosis: anterior presentation, dorsal position, lateral deviation of the head. Correction by hand.

grasps the calf's muzzle and, as it is manipulated to extend the neck, the assistant is directed to apply gentle traction. It is obviously important that this head snare should be passed around the greater curvature of the neck to the mandible. Should the snare inadvertently be passed across the concavity of the neck curvature to the mandible, pulling on it will accentuate, rather than relieve, the displacement.

In very obstinate neglected cases of dystocia due to lateral deviation of the head when the fetus is dead, and in the occasional congenital rigid curvature of the neck called 'wryneck', correction is impossible and decapitation is required. This is conveniently performed by means of the wire-saw fetotome, the wire being passed in on an introducer around the flexure of the neck. The severed head is first removed, and the remainder of the calf withdrawn by applying traction on the forelimbs by means of snares and to the neck through the medium of Krey's hook. The correction of this postural defect can also be facilitated by casting the cow in lateral recumbency on the side opposite to the direction of the neck flexion; this allows the gravid uterus to sink slightly to one side, thereby providing more space to correct the deviation.

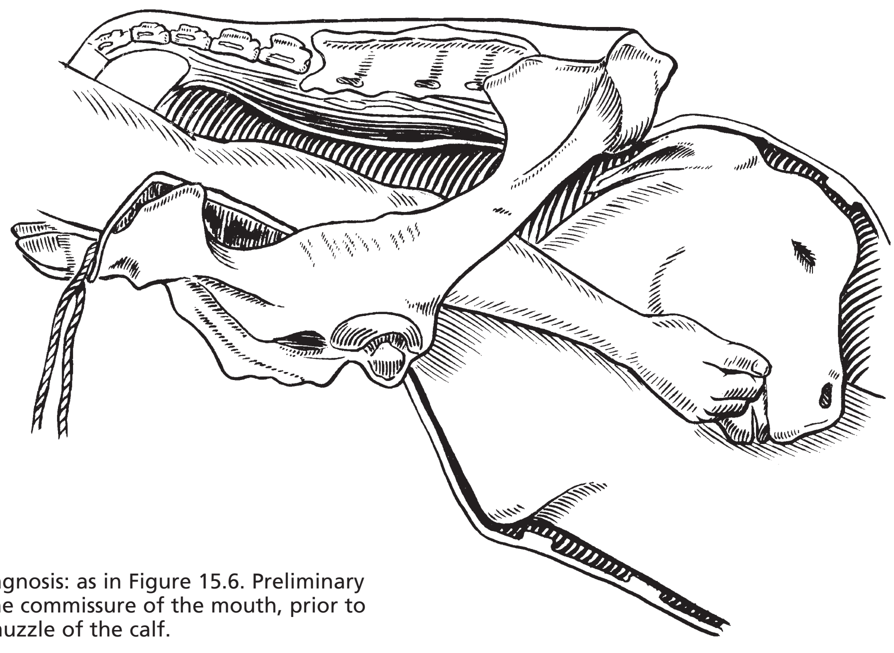

Fig. 15.7 Diagnosis: as in Figure 15.6. Preliminary 'hooking' of the commissure of the mouth, prior to grasping the muzzle of the calf.

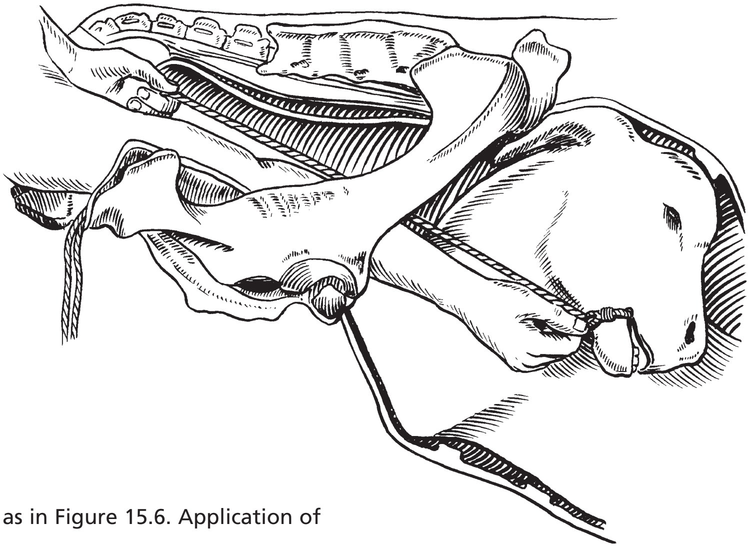

Fig. 15.8 Diagnosis: as in Figure 15.6. Application of the mandibular snare.

Downward displacement of the head

This is an uncommon type of dystocia in cattle. It usually takes the form of 'vertex posture' in which the calf's nose abuts on the pubic brim and the brow is directed into the pelvis (Figure 15.9). The more severe varieties of downward deviation of the head, namely 'nape presentation' and 'breast-head' posture – in which the head is flexed vertically between the forelimbs – are rare in cattle; when present, they have usually been caused by traction on the limbs before the head had extended.

Provided sufficient retropulsion can be achieved, vertex posture is easily overcome. Neglected cases may require caudal epidural anaesthesia and fetal fluid supplement. The calf is repelled by applying pressure to the forehead by means of a thumb, while lifting the mandible over the pelvic brim with the fingers.

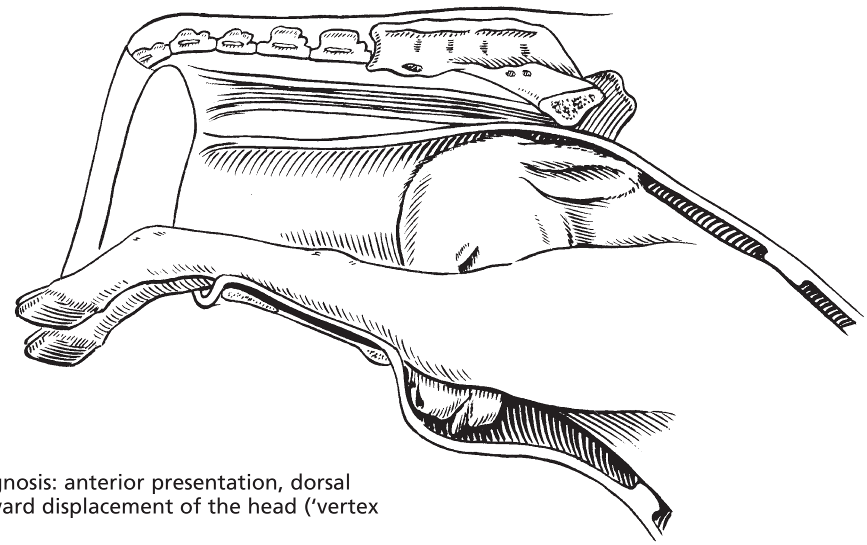

Fig. 15.9 Diagnosis: anterior presentation, dorsal posture, downward displacement of the head ('vertex posture').

More severe degrees of downward displacement of the head are treated in a similar way, but if difficulty is experienced one of the forelimbs should be replaced into the uterus. This gives room for the head to be first rotated laterally and then brought upwards and forwards over the pelvic brim. The leg is then extended and the fetus removed by traction. In very difficult cases, it may be advantageous to replace both forelimbs into the uterus. Casting the cow and placing her in dorsal recumbency may greatly facilitate extension of the fetal head. Another alternative is to rotate the fetus, by means of a force applied to its legs, into a temporary ventral position from which the head may be more easily extended. When manipulative correction fails, fetotomy may be practised; either the head is removed in nape presentation or one forelimb is sectioned in breast-head posture.

In difficult cases of downward deviation in which the calf is still alive, a caesarean operation has much to commend it.

POSTURAL DEFECTS OF ANTERIOR PRESENTATION IN HORSES

Although showing a lower incidence in horses than in cattle, defects of limb posture cause more serious dystocia in mares than in cows. This is due to the severe pelvic impaction that is consequent upon the mare's very strong expulsive efforts, and to the longer limbs of foals. In order to prevent rupture of the uterus or vagina, correction of posture must be done with the utmost care. Where impaction is severe, it may be possible to repel the fetus; traction without correction of posture may then be attempted since it has a better chance of success than in the cow. The obstetrician is ever mindful of the urgency of equine dystocia, but if at the outset an impacted fetus is found to be already dead, the advantage of anaesthetising the mare and placing her in lateral or dorsal recumbency should be considered.

Carpal flexion posture

The principles of correction are the same as for the cow. Adequate retropulsion of the fetus, in order to make sufficient room for the extension of the longer limbs of the foal, is most essential, and a foot snare is a great aid to manual extension of the limb. During the final extension of the carpus, the birth canal must be protected from injury by holding the fetal foot in the cupped hand.

There is a tendency for a foal in carpal flexion posture to become impacted in the maternal pelvis (Figure 15.10); the procedure required will depend on the degree of impaction, on the relative sizes of the fetus and birth canal, and on the duration of second-stage labour. Retropulsion of the fetus, followed by extension of the carpus, should always be attempted. Where there is obviously insufficient room for extension, the flexed carpus

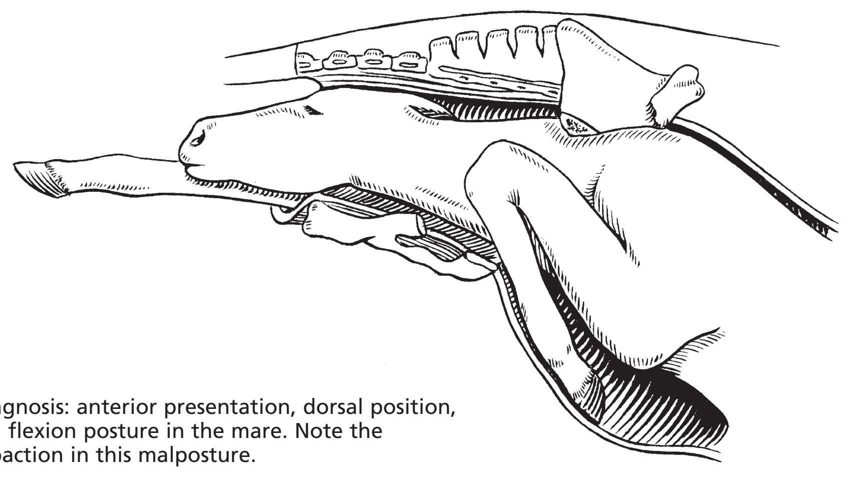

Fig. 15.10 Diagnosis: anterior presentation, dorsal position, unilateral carpal flexion posture in the mare. Note the tendency to impaction in this malposture.

may be pushed forwards into the uterus so that the retained limb lies under the fetal abdomen. Moderate traction applied to the other limb and to the fetal head then often succeeds without injury to the mare. Where it is found impossible to relieve the impaction, there are two alternatives for the veterinary surgeon: either to attempt traction without correction or to section the leg through the carpal joint. The first alternative will be tried on a live foal and when the flexed carpus is well advanced into the maternal pelvis. In addition to snares on the head and the other extended limb, traction is applied to a snare placed around the flexed carpus. Fetotomy for irreducible carpal flexion is easily effected by means of the wire-saw fetotome, section being made through the carpal joint. A snare is then placed above the carpus, and the fetus is removed by pulling on this, as well as on the other limb and head.

In the case of irreducible *bilateral* carpal flexion, affecting a normally developed full-term foal, traction should not be attempted. It is unlikely that the foal will still be alive so that fetotomy is indicated, one or two of the carpal joints being sectioned as required.

Incomplete extension of the elbow

This is uncommon. The treatment is that described for the same condition in cattle.

Shoulder flexion posture

One or both forelimbs may be retained. The more slender head and longer neck of the foal give more room in the maternal pelvis for the hand and arm of the obstetrician than is available in the same type of bovine dystocia; but the retained limb is further away and it is consequently more difficult to pass a snare around the radius and ulna. Copious fetal fluid supplement should be infused and vigorous retropulsion applied. Once the radius and ulna have been snared it should be possible to advance the limb and to convert the posture into one of carpal flexion and then to proceed accordingly.

When it is found to be impossible to extend the limb, traction may be tried. This often succeeds, but the foal is usually dead. Rather than use inordinate force, it is preferable to remove the retained forelimb by means of the wire-saw fetotome as described for the cow. When both forelimbs are retained and attempts at correction fail, traction may be tried, but it is probably better first to try to remove one limb by means of the wire-saw.

Foot–nape posture

This deviation of posture comprises upward displacement of one or both extended forelimbs so that they come to lie above the extended head in the vagina. It is a postural defect peculiar to the horse that is made possible by the more slender head and longer limbs of the foal. It is very likely to lead to serious impaction and carries a great danger of penetration of the vaginal roof by the foot of the foal. The uppermost limb is recognised, and as the foal's muzzle is vigorously repelled in a cranial and upward direction the fetal foot is raised and then pushed, or pulled, to the appropriate side. The other foot is similarly manipulated and, finally, the head is again raised and each foreleg placed underneath it. Traction is then applied to the head and both forelimbs.

If penetration of the vaginal roof has occurred, epidural anaesthesia or general anaesthesia should be induced. Reposition is first attempted, and if it is not possible, amputation of the fetal head or the upper limb – whichever is easier – should be performed. The upper limb is sectioned through the radius by means of the wire-saw, and it should then be possible to replace the other limb under the head; the stump of the radius must be carefully controlled during the final delivery.

Where one foot is already protruding from the ruptured perineum, or rectum, it may be necessary to incise the perineum, extract the fetus and then repair both the lacerated and the incised tissue.

Lateral deviation of the head

This is a more serious malposture in horses than cattle because, owing to the greater length of the neck and head, the foal's nose lies further away near the stifle joint instead of on the middle ribs, as in the calf (Figure 15.11). Thus, except in ponies, the displaced head is beyond the reach of the obstetri-

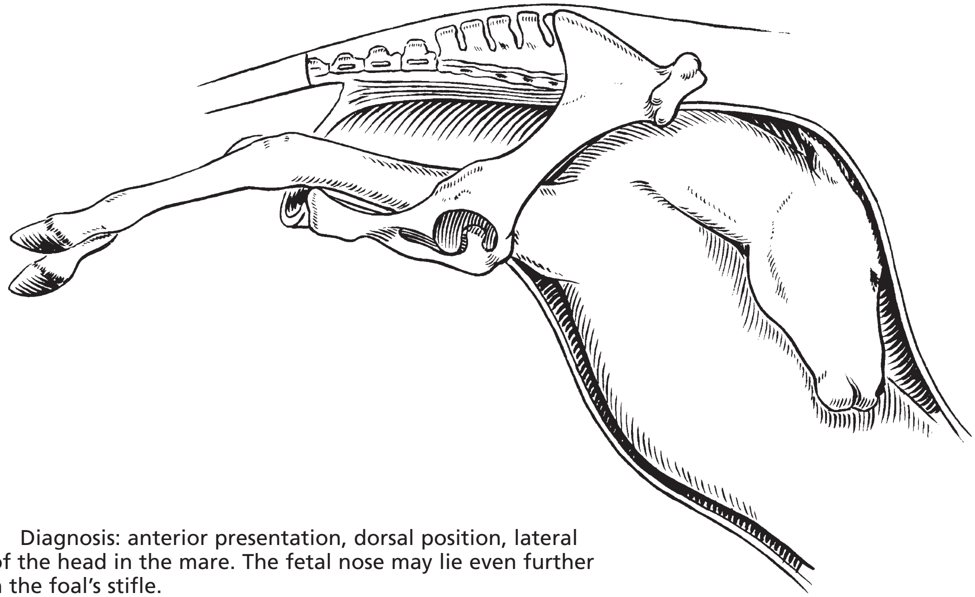

Fig. 15.11 Diagnosis: anterior presentation, dorsal position, lateral deviation of the head in the mare. The fetal nose may lie even further forward on the foal's stifle.

cian's hand. Special instruments are therefore required to help procure the head, and three such are available: Kühn's crutch, Blanchard's long flexible hook and the Krey–Schottler double hook. Their use requires considerable skill, and with the availability of safer general anaesthetic methods they are rarely used now.

In cases of 'wryneck', where it is quite impossible to extend the neck, the head and neck must be amputated by means of the wire-saw fetotome (Figure 15.12), or a caesarean operation performed.

Downward deviation of the head

Downward deviation of the head is not so rare in horses as in cattle; nape posture is the most likely to be encountered. The methods of correction are

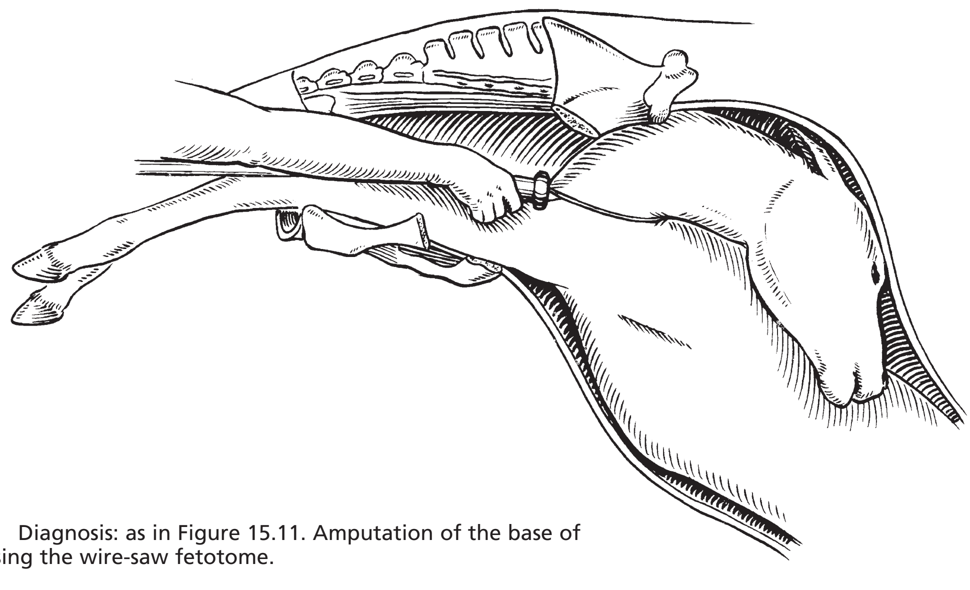

Fig. 15.12 Diagnosis: as in Figure 15.11. Amputation of the base of the neck using the wire-saw fetotome.

the same as for the cow. Extension of the head requires the application of a mandibular snare, and while firm pressure is placed upon the fetal brow with one hand, the snare is pulled upwards and backwards by an assistant. If the operator can apply rotational as well as repellent force to the fetal head, lateral movement of the head – which is a necessary preliminary to its forward extension – is promoted. If this simple method does not soon succeed the mare should be anaesthetised, placed in dorsal recumbency and the hindquarters raised. Retropulsion of the fetus and correction of the head posture are now greatly facilitated.

In view of the fact that spontancous delivery of a foal in nape posture has been observed, it has been suggested that, where the head has projected so far into the vagina that the ears are visible at the vulva, successful traction without reposition has occurred but it is not advisable.

In obstinate cases of nape posture with impaction at the pelvic brim, fetotomy is indicated. However, the introduction and correct placing of the wire between the markedly flexed head and the neck would appear to be very difficult.

If the head is completely displaced between the forelimbs, so that it comes to lie under the chest or abdomen of the foal, reposition should be attempted by means of retropulsion and the application of Krey's hooks to the neck; traction is then applied with a view to raising the head to within reach of the hand. If this fails, a foreleg will have to be removed, preferably by the subcutaneous method, in order to give space for raising the head.

POSTURAL DEFECTS OF ANTERIOR PRESENTATION IN SHEEP AND GOATS

Postural defects are common causes of ovine dystocia. When affected sheep are promptly treated correction is relatively simple, and in many cases is successfully carried out by the shepherd. Manipulation is more difficult in the case of large single lambs, but delay in rendering obstetrical aid is the most frequent cause of difficulty. Repeated ineffectual maternal expulsive efforts cause expulsion of the fetal fluids, impaction of the fetus and close envelopment of the fetus by the uterus. Secondary uterine inertia supervenes, and in protracted cases the lamb dies and undergoes emphysematous decomposition. Thus even a simple postural defect in an unresponsive, inelastic, swollen fetus may be very difficult to correct. The veterinary surgeon is likely to see the more serious instances of postural abnormality in which there has been considerable delay and in which unskilful attempts at correction may have caused damage to the ewe.

Gentleness of manipulation within the ovine genital tract is most essential; otherwise serious contusion or laceration of the vagina, cervix and uterus may result and is especially liable to be followed by fatal shock or infection. The wool should be clipped from around the perineum and tail base, and this area should be thoroughly washed. Except for the simplest manipulative procedure, caudal epidural anaesthesia should always be used. The ewe should then be placed on a bale or if possible a table in lateral or dorsal recumbency (if appropriate), with the hindquarters overhanging one end. Alternatively, the ewe may be gently restrained whilst standing or held by an assistant, so that its head and neck rest on the floor while the hindquarters are raised by grasping the hindlegs above the hocks. The assistant straddles the ewe and maintains her in the supine position with the hindquarters at a convenient height for the operator. Fetal fluid supplements, particularly the cellulose-based obstetrical lubricants as substitutes for amniotic fluid, should be infused. With the advantages provided by raising the ewe's hindquarters and instilling fluid, the majority of postural defects will be readily overcome. The principles of reposition adopted for the several varieties of postural aberration are the same as those used for the cow. Many cases can be rectified by the hand alone, but snares are frequently, useful. Instruments are seldom required although forceps are occasionally employed in very small ewes as is the simple head snare referred to in Chapter 12.

Carpal flexion posture

With the ewe held as previously described, and with the instillation of fluid in delayed cases, retropulsion is easily achieved. The retained foot may then be grasped and gently brought into the pelvic entrance whence it is extended into the vagina. The ewe is then lowered on to her side and gentle traction applied each time she strains. After delivery of the fetus, the uterus is searched for another lamb. Owing to uterine inertia, a second (or third) lamb may fail to be advanced to the pelvic brim. The obstetrician should therefore bring the fore or hind extremity into the pelvis; expulsive efforts will recommence and gentle traction is then applied to help delivery.

Where there has been gross delay and it is found impossible to extend the leg of an emphysematous fetus, the carpus should be sawn through with fetotomy wire. Copious lubrication is indicated and further fetotomy may be required as described for fetomaternal disproportion. As an alternative; unrelieved carpal flexion may be dealt with by the caesarean operation.

Incomplete extension of the elbow

Retropulsion of the fetus, followed by gentle extension of each limb in turn, is easily achieved. Gentle traction is then applied to the head and forelimbs.

Shoulder flexion posture (Figure 15.13)

With adequate retropulsion, and fetal fluid supplement in delayed cases, it is usually possible to reach the forearm and to convert the defect to carpal flexion posture and then to proceed as previously described. In the case of a grossly oversized fetus where it is found impossible to advance the leg, a caesarean operation may be necessary; where the fetus is emphysematous the retained limb may be amputated by means of the wire-saw fetotome. Following the removal of one limb it is usually possible to deliver the fetus. In view of the fact that spontaneous delivery has been seen to occur despite complete retention of a forelimb, it would seem proper where the ewe's pelvis is large and the lamb of small or moderate size to attempt delivery without rectification of posture. In such a case, however, it is likely that correction will be simple, and in any case, inordinate force should not be used.

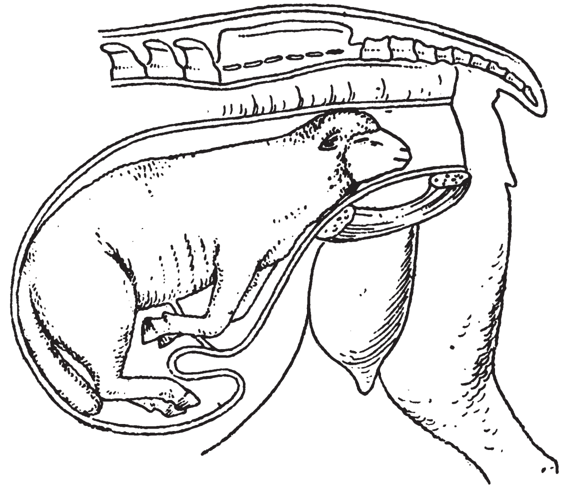

Fig. 15.13 Diagnosis: anterior presentation, dorsal position, bilateral shoulder flexion posture (from a paper by H. Leeney in *Transactions of the Highland Agricultural Society, c.* 1890).

Lateral deviation of the head (Figure 15.14)

This is a very common cause of ovine dystocia. The methods used for correcting it are those described for cattle. Under caudal epidural anaesthesia, with the hindquarters raised and with the instillation of lubricant fluid, retropulsion and manual reposition are possible in most instances. Where there has been delay a mandibular snare

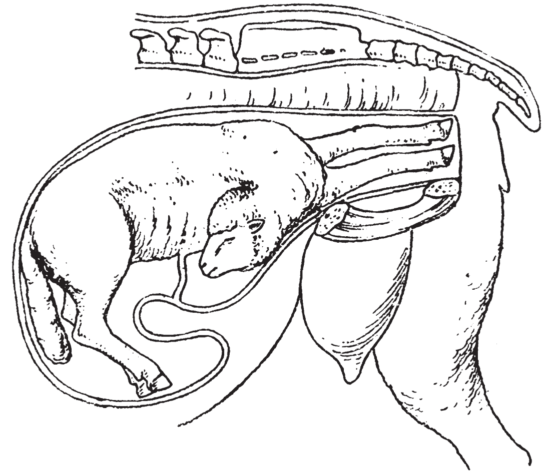

Fig. 15.14 Diagnosis: anterior presentation, dorsal position, lateral deviation of the head (from a paper by H. Leeney in *Transactions of the Highland Agricultural Society, c.* 1890).

may be used to good effect. Where there is insufficient room to correct the deviation, the displaced head of an emphysematous lamb may be amputated with a wire fetotome, but a caesarean operation may be preferred.

POSTURAL DEFECTS OF POSTERIOR PRESENTATION

Faulty posture of the posterior limbs is more difficult to correct than abnormalities of the anterior limbs, particularly in horses. The defects now to be considered concern lack of extension of the hock and hip joints, which may affect one or both limbs. Also, occasionally in calves it is found that the umbilical cord runs between the hindlimbs and over the posterior aspect of one or other. In this case it is necessary to create a hock flexion in order to replace the cord in its correct position. Failure to do this may result in its occlusion, ending in death of the calf. Owing to the difficulty of extending the retained limbs, due to lack of space in front of the pelvis, there are three essential requirements in attempting to correct the cause of the dystocia: namely, epidural anaesthesia, fetal fluid supplementation and retropulsion. All manipulations should be conducted very carefully and gently for the danger of accidental perforation of the uterus is a real one. The variable factor exerting the greatest influence on the relative difficulty of the corrective procedure – as well as on the outcome of the operation – is the duration of dystocia prior to treatment. Cases attended in early second-stage labour may be delivered quite easily, but where there has been considerable delay, with consequent loss of fetal fluid, uterine contraction and death of the fetus, a most difficult and protracted fetotomy or a caesarean section may be necessary. There is a large proportion of stillbirths among fetuses presented posteriorly.

Hock flexion posture

Cattle

The condition is usually bilateral (Figure 15.15). The points of the hock may be felt in front of the pelvic brim or may be firmly engaged in the maternal birth canal. An estimate will be made of the likely degree of difficulty in correction, and a decision made on whether epidural anaesthesia and/or fetal fluid replacements will be needed. The aim of the manipulative procedure is to extend the hock joint(s); the difficulty is in procuring sufficient space for this to be done. In early cases, with or without epidural anaesthesia, the posture may be corrected by hand. The fetus is first repelled by pressing forward in its perineum, and the hand

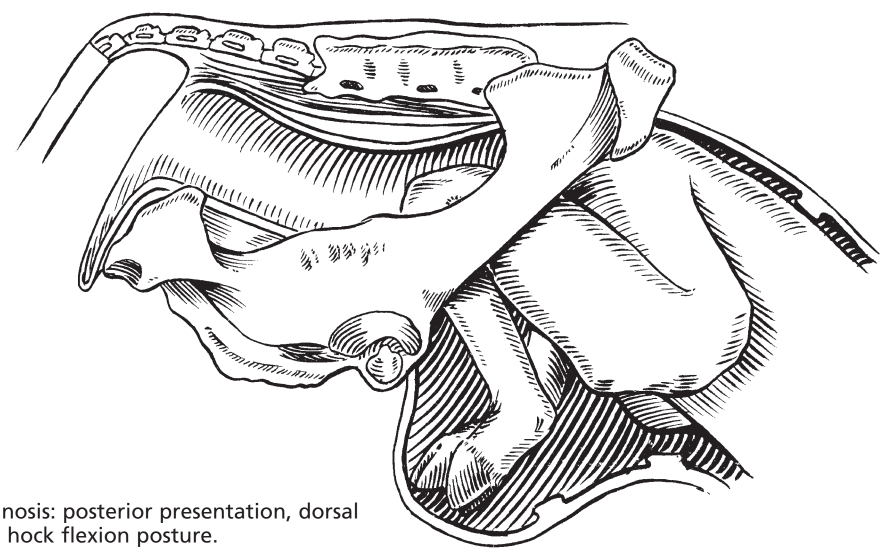

Fig. 15.15 Diagnosis: posterior presentation, dorsal position, bilateral hock flexion posture.

then grasps the fetal foot. As the foot is drawn back through an arc, the hock is firmly flexed and retropulsion maintained as far as possible; eventually, with the points of the digits in the cupped hand, the foot is lifted over the pelvic brim and the limb extended in the vagina. In cases in which it is found to be impossible to extend the hock owing to the lack of space, an assistant is directed to pass in an arm and to press forwards and upwards on the point of the hock while the operator again tries, as before, to bring the foot into the pelvic canal. An alternative method is to supplement manual extension by traction on a snare fixed to the retained foot in the following way. One end of an obstetric snare – to which may be attached Schriever's introducer – is passed into the birth canal, around the hock flexure, brought out and passed through the loop at the other end; the running noose thus formed is applied to the metatarsus. The noose is then manipulated down the limb until it lies in the pastern, the shank of the noose being placed from before backwards between the digits, so that when traction is applied to it the fetlock and pastern joints are flexed (Figure 15.16). After again repelling the fetus the obstetrician grasps the foot, and as the assistant pulls on the snare the extremity is lifted over the pelvic brim. Casting the cow and placing her in dorsal recumbency can also provide more space for the manipulation.

In the occasional case where it is impossible to extend the hock and the calf is dead, simple fetotomy may be performed. Either the Achilles tendon may be severed so as to make possible maximum flexion of the hock and thus allow the limb to be brought into the maternal pelvis; or the limb may be amputated below the point of the hock by means of the wire-saw fetotome (Figure 15.17). A snare may then be applied above the hock and the limb extended. If the calf is alive, then a caesaeian operation will be necessary.

Horses

The methods used are those described for cattle, but owing to the longer limbs of the foal the procedure is much more difficult, and more frequent recourse to fetotomy or a caesarean operation will be required. If the foal should survive the initial unsuccessful manipulative attempt at correction it is worthwhile anaesthetising the mare and again trying to extend the limb with her in dorsal recumbency, preferably with the hind end raised.

Hip flexion posture

Cattle

When both hindlegs are retained in the uterus – a commoner condition than unilateral retention

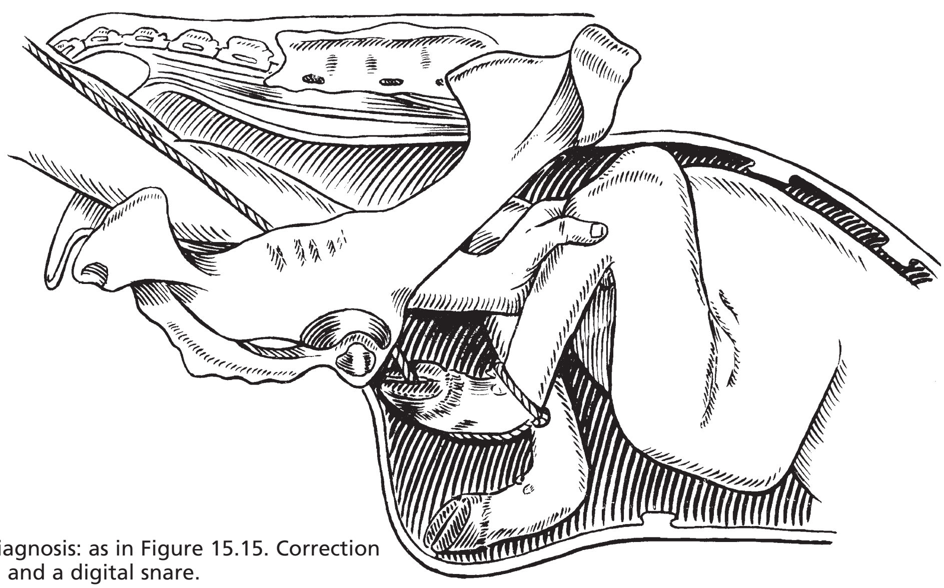

Fig. 15.16 Diagnosis: as in Figure 15.15. Correction using the hand and a digital snare.

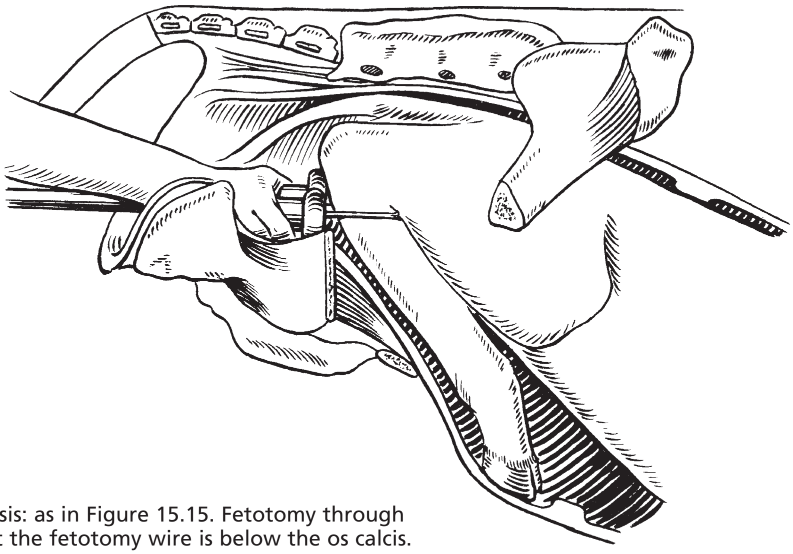

Fig. 15.17 Diagnosis: as in Figure 15.15. Fetotomy through the tarsus. Note that the fetotomy wire is below the os calcis.

– the case is described as 'breech presentation'; where much delay has occurred before correction is attempted, this constitutes one of the most difficult types of dystocia dealt with by veterinary obstetricians. Usually on vaginal examination, the calf's tail is recognised (Figure 15.18). The degree of engagement of the fetus in the maternal pelvis varies, and in some cases the hand cannot be passed to the hocks of the calf. The aim of the treatment is to convert the condition into one of hock flexion posture and then proceed accordingly. Again, the need for epidural anaesthesia and fetal fluid supplement will be primary considerations. In recent cases neither will be needed, but in a protracted case both will be invaluable. The manipulative procedure is to repel the calf's perineum forwards and upwards with a view to bringing the retained limbs within reach, when the leg may be grasped as near to the hock as possible. Traction on the limb converts the posture into

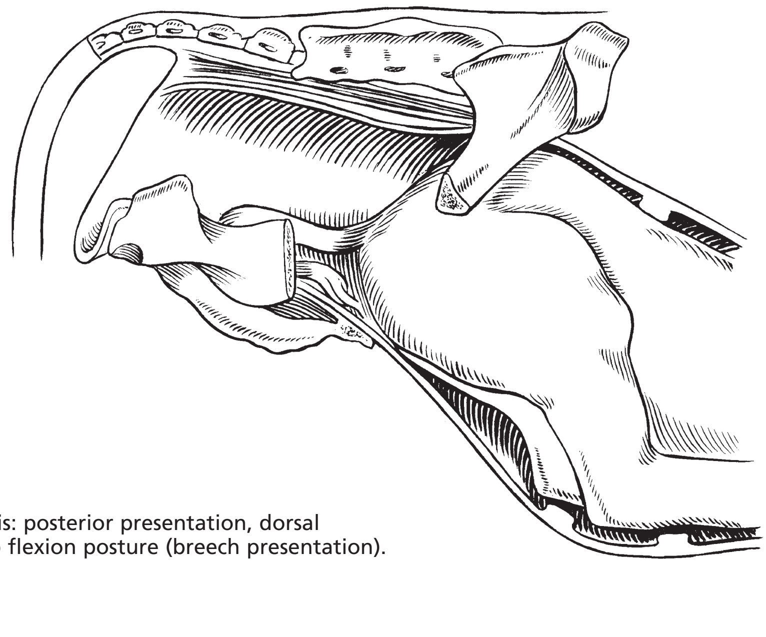

Fig. 15.18 Diagnosis: posterior presentation, dorsal position, bilateral hip flexion posture (breech presentation).

hock flexion, from which point the previously described procedure is carried out.

If it is impossible to bring the hock within reach, and the calf is dead, then fetotomy may be performed. The best method for removing the retained hindlimb is to use the wire-saw fetotome. One tube of the instrument is threaded, and the free end of the wire, attached to an introducer, is passed from above to below around the proximal part of the more accessible limb. The introducer is sought from below and brought out and the other tube of the fetotome is threaded. The fetotome is now passed along the vagina, and the head of the instrument placed against the fetal perineum. At this stage, a most important step in the procedure is to include the fetal tail in the loop of wire and to hold the head of the instrument firmly to the perineum while sawing takes place (Figure 15.19). In this way the femur is sectioned through its articular head. The detached limb is removed. The other hindlimb is similarly removed. An alternative procedure, after the amputation of one limb, is to apply traction – through the medium of an anal hook which is passed into the fetal anus and over the fetal pelvic brim – and to attempt to deliver the calf without extending the other hindlimb. Occasionally, after amputation of one hindlimb, it may be possible to extend the other limb and deliver the fetus by traction on the extended limb.

Although it is somewhat cumbersome procedure, and in most cases is not necessary when epidural anaesthesia is employed, there is little doubt that where difficulty is experienced in extending the legs of a breech presentation, casting the cow and placing her in dorsal recumbency, preferably with the hindquarters raised, can be of tremendous help.

In the case of an impacted breech presentation of a dead fetus, an alternative procedure to percutaneous fetotomy, suggested by Graham (1979), is to cause fracture and collapse of the fetal pelvis by introducing a long-handled hook through an incision in the fetal perineum. The 75 cm blunt hook engages the pelvic brim and the fetal pelvis is fractured by abrupt backward traction. The procedure is repeated once or twice so as to ensure sufficient pelvic collapse. Traction on the unextended, lubricated breech, with the aid of Krey's hooks, may then succeed.

Horses

Occasionally, a mare will foal unaided despite complete retention of the hindlimbs. However, when there is dystocia an attempt should be made to extend the limbs, as described for cattle. Much greater difficulty will be experienced because of the longer limbs of the foal, and there is a very real danger of rupture of the uterus by the fetal foot. Serious consideration should be given to anaesthetising the mare and placing her in dorsal recumbency with the hindquarters elevated (Figure 15.20).

If, after a proper effort, attempts to extend the hindlimbs are unsuccessful and the foal is still alive, no time should be lost before resorting to a caesarean operation. If, as is more likely, the foal

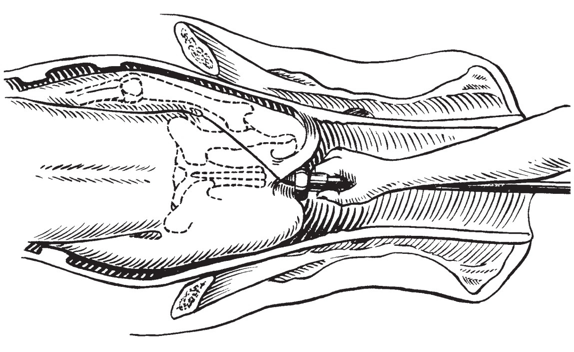

Fig. 15.19 Diagnosis: as in Figure 15.18. Percutaneous amputation of the hindlimb. Note that the fetal tail is within the wire loop.

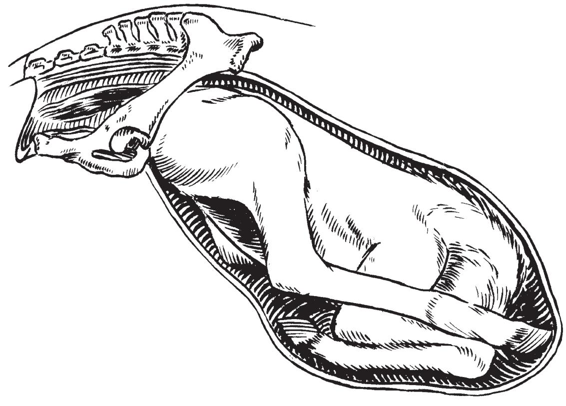

Fig. 15.20 Diagnosis: bilateral hip flexion posture in the mare (breech presentation).

is dead, then under general anaesthesia and following amputation of one hindlimb by means of the wire-saw tubular fetotome, as described for cattle, it should be possible to deliver the foal by traction through the medium of an anal hook or the Krey–Schottler double hook attached to the root of the tail.

Hock flexion posture and hip flexion posture in the ewe

A considerable proportion of twin lambs are presented posteriorly, and because of lack of uterine space, especially where both lambs occupy one uterine horn, one or both hindlegs may fail to extend into the vagina (Figure 15.21). Thus in flocks with a high proportion of twins, hock flexion and hip flexion postures will be common causes of dystocia.

These malpostures may be corrected in the manner described for cattle, but because twin lambs are smaller than singles and since it is a simple matter to raise the ewe's hindquarters, the requisite manoeuvres are much more easily performed than in cattle. In all delayed cases, fetal fluid supplement is indicated. The manipulation of the fetus, including its retropulsion, should be very gently performed.

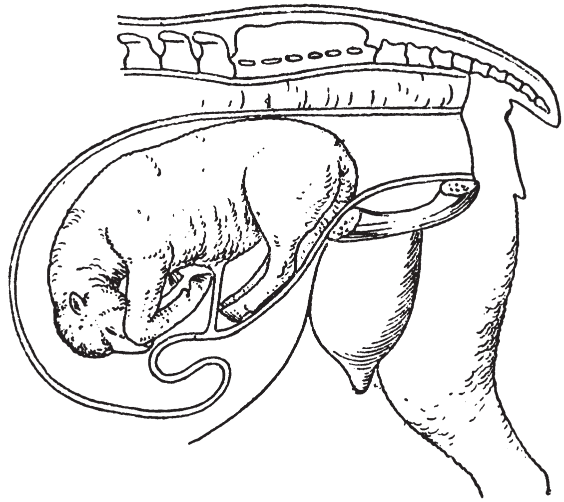

Fig. 15.21 Diagnosis: posterior presentation, dorsal position, bilateral hip flexion posture (breech presentation) (from a paper by H. Leeney in *Transactions of the Highland Agricultural Society, c.* 1890).

In cases of irreducible malposture in dead lambs, appropriate fetotomy or a caesarean operation can be performed.

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16 Dystocia due to defects of position or presentation: treatment

POSITION

Abnormal position of the fetus is encountered more frequently in horses than in cattle. This is considered to be due to the fact that, in late gestation or first-stage labour in horses (but not in cattle), a physiological rotation of the fetus from the ventral to the dorsal position occurs, and that occasionally this fails to occur. The fetus then presents longitudinally – usually anteriorly, but sometimes posteriorly – either with its vertebral column applied to one side of the uterus (right or left lateral position), or facing the floor of the birth canal (ventral position). The process whereby the bovine or ovine fetus sometimes comes to lie in ventral position is not understood. It is hardly likely to be a gestational position; more probably it arises during the first stage of labour when the uterine peristaltic force generates a vigorous reflex response in the fetus that rotates it about its long axis. The mechanism would seem to be similar, or identical, to that which causes torsion of the uterus. Presumably the fetus moves with the amnion, the fetus and amnion revolving within the allantochorion. The greater freedom of the amnion within the allantois of the mare, as compared with the cow, would facilitate this change of position.

In order to make birth possible, fetuses in lateral or ventral position must be rotated into the normal (dorsal) upright position. This can be achieved by first repelling the fetus and then rotating it by appropriate force applied to the presenting extremity. Such rotation is easier to perform with the patient standing. In obstinate cases epidural anaesthesia is extremely useful.

Anterior presentation, lateral position (mare or cow)

In the case of a live calf or foal, the obstetrician passes his or her hand to the fetal head and, by means of the thumb and middle finger, presses on the fetal eyeballs, the latter being protected by the eyelids. Firm pressure causes a convulsive reflex response in the fetus and, by applying a rotational force in the appropriate direction, it is easy to turn the fetus into the dorsal position. The fetal nose and forelimbs are then advanced into the maternal pelvis, and the maternal expulsive efforts assisted by gently pulling on these appendages. Should this method fail, then snares are attached to the limbs and possibly caudal epidural anaesthesia is induced; rotation is performed mechanically, firstly by repelling it as far cranially as possible, crossing the snares in the appropriate direction, and then by applying traction. This will tend to result in the snares becoming more or less parallel, which can only occur if the fetus rotates about its longitudinal axis. It is important to ensure that the snares are crossed in the right direction so that rotation of the fetus is not increased. Unless the degree of faulty disposition is only modest, the procedure will require to be repeated many times before the defect is fully corrected, and birth can be completed by traction. For such a procedure to be effective, it is critical that there should be plenty of fetal fluid supplementation.

Anterior presentation, ventral position (mare or cow)

The same two methods, namely using the hand with eyeball pressure with manual rotation or mechanical rotation by applying traction to crossed snares, as described for the correction of a lateral position defect, can be used, although the procedures will usually need to be repeated several times. Placing the dam in dorsal recumbency with the hindquarters raised will facilitate the procedure.

If the calf or foal should rest on its back with the head and limbs flexed on to its neck and thorax, the fetus must first be repelled so that the head and forelimbs can be extended. Rotation is then carried out.

Posterior presentation, lateral position (mare or cow)

The operator introduces a hand and grasps the stifle region of the upper limb. Simultaneous retropulsion and downward pressure are applied to rotate the fetus through 90°.

Posterior presentation, ventral position (mare or cow)

The operator introduces a hand between the fetal hindlimbs and up to the inguinal region, where one of the thighs is grasped; then, pushing forwards, the operator rotates the fetus through a half-circle. Failing this, traction on crossed limb snares should be used. An alternative procedure is to place a traction bar between the projecting hindfeet and to bind it to them by means of a snare; rotational force is then applied to the traction bar.

There is a grave risk that the hindfeet of a foal in posterior presentation, ventral position, will penetrate the vagina and rectum (Figure 16.1). In such a case a caesarean operation should be performed and the rectovaginal fistula repaired later.

Dystocia due to defects of position in sheep

The methods of treatment are those described for the mare and cow. By raising the ewe into the inclined supine position and infusing fetal fluid supplement, rotation is much easier in this species; instruments are seldom required.

PRESENTATION

Instead of the long axis of the fetus being in line with the birth canal it may be disposed vertically or transversely to the pelvic inlet. Owing to limitation of space in the sagittal plane, absolute vertical presentation is not possible but oblique vertical presentation occurs rarely, in mares rather than cows. According to whether the fetal verte-

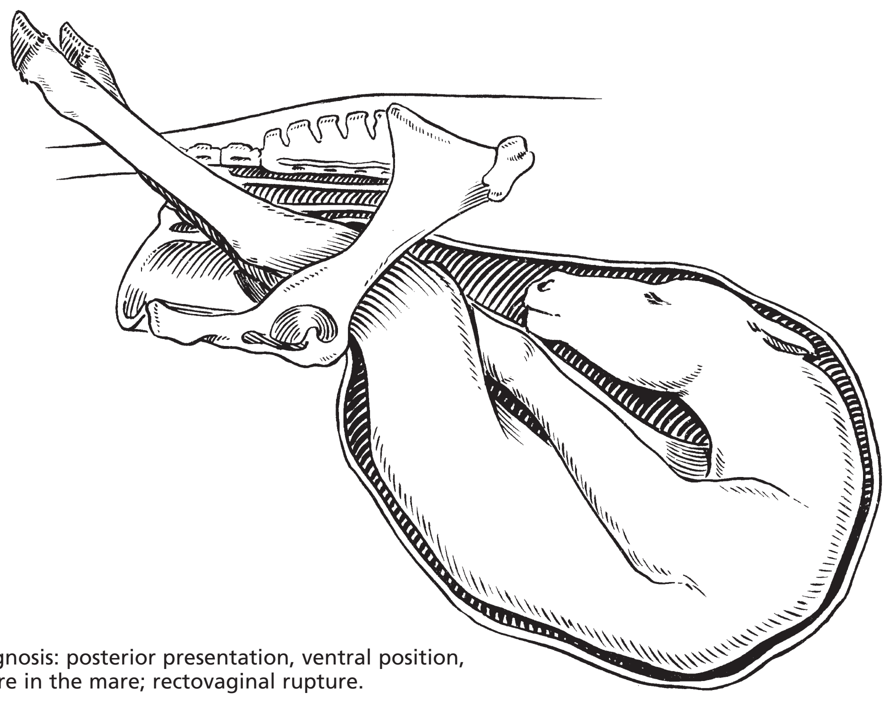

Fig. 16.1 Diagnosis: posterior presentation, ventral position, extended posture in the mare; rectovaginal rupture.

bral column or abdomen is presented at the pelvic inlet, such dystocias are described as dorsovertical or ventrovertical presentations. Transverse presentations are also uncommon and are more likely to be encountered in the mare; they may be ventrotransverse or dorsotransverse and, again, oblique variants are more often seen.

All dystocias that arise from defects of presentation are serious, the special form of bicornual transverse presentation of the mare being notorious. The aim in all cases is to achieve version of the fetus so that a vertical or transverse presentation is converted into a longitudinal one. Obviously the nearer extremity should be moved towards the pelvic inlet, but where both extremities are equally distant it is usually simpler to convert to posterior presentation (two appendages being manipulated rather than three).

Oblique dorsovertical presentation (mare or cow)

According to whether the head or breech is nearer the pelvic inlet, the presentation is converted into anterior or posterior longitudinal. An attempt is made to bring the fetal extremity (head and/or limbs) to the pelvic inlet, and firstly to convert the defect into a ventral longitudinal presentation. The fetus can then be rotated to the dorsal position as described earlier. Retropulsion and the presence of copious fluid (natural or artificial) in the uterus are both essential. A grip is taken on the fetus by means of Krey's hook as near as possible to the more proximal fetal extremity. Then, while retropulsion is applied, the hook is pulled on with a view to bringing the fore or hind end of the fetus to the pelvic inlet. After adjustments of position and posture, the fetus is then delivered by gentle traction.

Should version not be practicable, a caesarean operation should be performed.

Oblique ventrovertical presentation (mare or cow) ('dog-sitting position')

Whereas this abnormality (Figure 16.2) is more frequent than the preceding, it is still rare and is only likely to be encountered in the mare. However, when present it should cause no difficulty in diagnosis; if the veterinary surgeon is called to a foaling mare from which the fetal head and forelimbs protrude, and to which lay traction has been applied without success, it is very probably a case of 'dog-sitting position', oversize being very unlikely in mares. 'Dog-sitting position' aptly describes the dystocia, the foal being disposed with its fore end advanced to a variable degree in the vagina, and its hindparts in the uterus. It differs from normal anterior presentation in that the hindfeet also pass into the birth canal and rest on the pelvic brim. Thus, the more the fetus is pulled, the greater is the impaction. Most cases are severely impacted, but after the induction of epidural anaesthesia and the

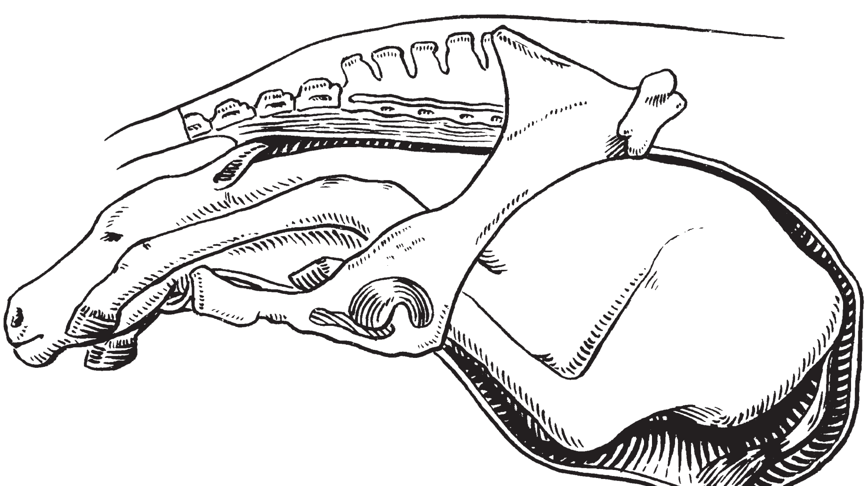

Fig. 16.2 Diagnosis: 'dog-sitting position' in the mare.

infusion of lubricant fluid into the uterus, an attempt should always be made to repel the fetus sufficiently to allow the hindfeet to be pushed off the pelvic brim into the uterus and thus to convert the dystocia into a simple anterior presentation. Traction is then applied. Placing the mare or cow in dorsal recumbency with the hindquarters elevated often helps. Should this attempt fail, then a caesarian operation is the only effective method of treatment. In a case of dog-sitting position where the head, neck and forelimbs protrude from the vulva, retropulsion will not succeed.

Where swelling of the vaginal mucosa prevents vaginal manipulation, a caesarean operation should be performed.

Dorsotransverse presentation (mare or cow)

This is a rare cause of dystocia (Figure 16.3 and 16.4), but oblique variants of it occur in both the mare and cow. The obstetrician should ascertain the polarity of the fetus and decide which extremity is nearer the pelvic inlet. The technique of correction required involves repulsion of the fetus, and the advancement of its nearer extremity to the birth canal. Unless one extremity is within easy reach, uterine version is likely to be an extremely difficult or impossible task in both the cow and mare. If there appears to be a chance of success, the cow should be given an epidural anaesthetic, and in the mare general anaesthesia should be induced, so that she can be placed on her back. Fetal fluid supplement is then instilled and an attempt made by manipulation of the proximal fetal extremity to turn the fetus into ventral position, anterior or posterior presentation. The next step is to rotate the fetus into dorsal position. Finally, it is delivered by traction. If after a short determined effort it is obvious that version cannot be achieved, a caesarean operation should be performed immediately. Fetotomy is very difficult to carry out in this type of dystocia and consequently is not recommended.

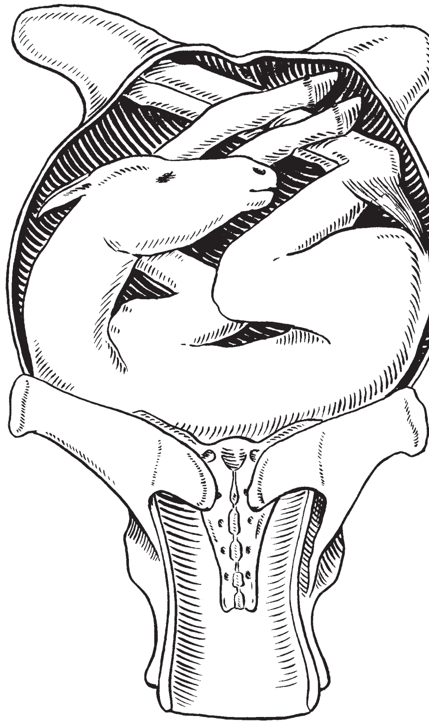

Fig. 16.3 Diagnosis: dorsotransverse presentation in the mare; uterine body gestation.

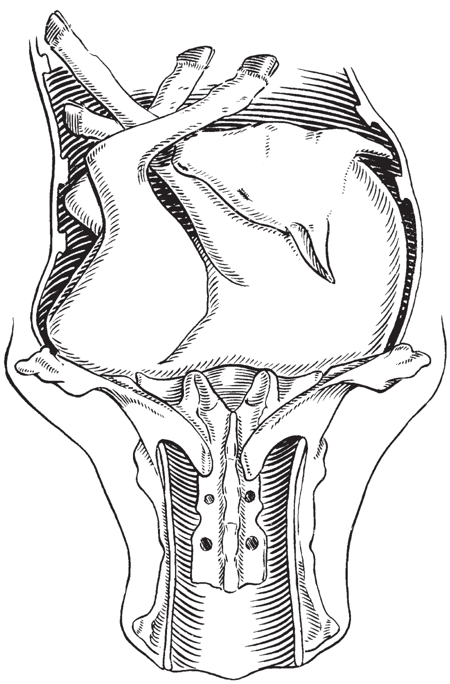

Fig. 16.4 Diagnosis: dorsotransverse presentation in the cow.

Ventrotransverse presentation (mare or cow)

This presentation (Figure 16.5) is more likely to be seen in the mare than in the cow, and oblique vari-

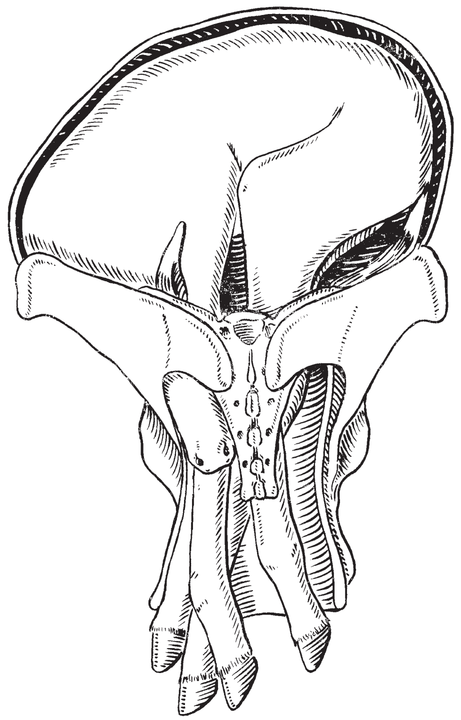

Fig. 16.5 Diagnosis: ventrotransverse presentation in the mare; uterine body gestation.

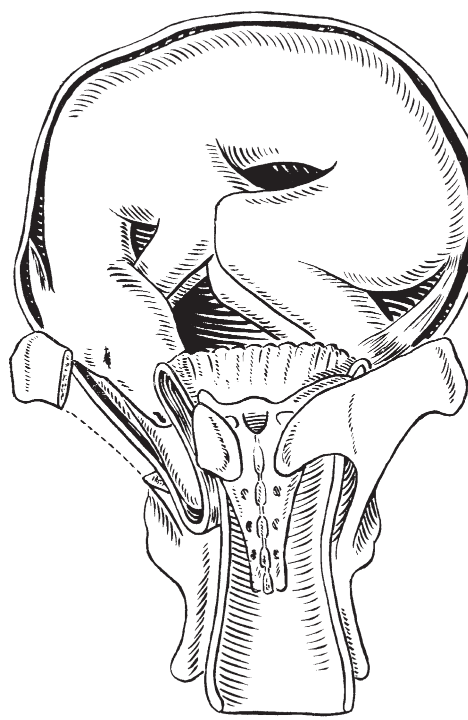

Fig. 16.6 Diagnosis: ventrotransverse presentation with ventral displacement of the uterus in the mare; bicornual gestation.

ants of it are more usual. A variable number of fetal appendages may enter the maternal pelvis. It is possible that the head as well as the forelimbs are in the vagina, but it is usual for two or more legs only to be presented. The condition must be distinguished from twins and double monsters and from *schistosoma reflexus*. The aim of vaginal interference is firstly to convert the abnormality into longitudinal – usually posterior – presentation, ventral position; this means that the posterior extremity must be advanced while the anterior extremity is repelled. General anaesthesia and dorsal recumbency are helpful in the mare. Unless progress with version is soon apparent, the caesarean operation is recommended for both mare and cow.

In the *bicornual type of transverse presentation* peculiar to mares the fetal extremities are disposed in the two horns and its trunk lies across the anterior portion of the uterine body (Figure 16.6). Ventral displacement of the uterus may have occurred, and, if so, it may be impossible to palpate the fetus. As soon as the presentation is recognised a caesarean operation should be performed.

Dystocia due to defects of presentation in sheep

The methods of treatment are those described for the mare and cow. By raising the ewe into the inclined, supine position and by infusing fetal fluid supplement, version is much easier in this species, but in protracted dystocia, caesarean section may provide an easier solution.

17 Dystocia due to twins or monstrosities

DYSTOCIA DUE TO TWINS

Twin gestation in cattle often culminates in dystocia, but in mares abortion is a more likely sequel (see Chapter 26). It is arguable whether twin gestation predisposes to dystocia in sheep, because the increased likelihood of maldisposition and the added risk of simultaneous presentation dystocia are balanced by smaller fetuses and a reduction in fetopelvic disproportion. Twin dystocia is of three types:

- Both fetuses present simultaneously and become impacted in the maternal pelvis (Figure 17.1).
- One fetus only is presented but cannot be born because of defective posture, position or presentation; posture is often most at fault, the lack of extension of limbs or head being due to insufficient uterine space.
- In uterine inertia, defective uterine contractions are caused, either by overstretching of the uterus by the excessive fetal load, or by premature birth. When inertia is present, birth of the first or second fetus does not proceed although presentation is normal.

The smaller size of twin fetuses facilitates manipulative correction and delivery; for the same reason natural or obstetric delivery may be possible despite defective posture.

In the treatment of twin dystocia, the first essential is diagnosis. It is very important, in obstetric practice involving dystocia, that the presenting fetal appendage is identified. If this is made a rule the obstetrician will not blunder into

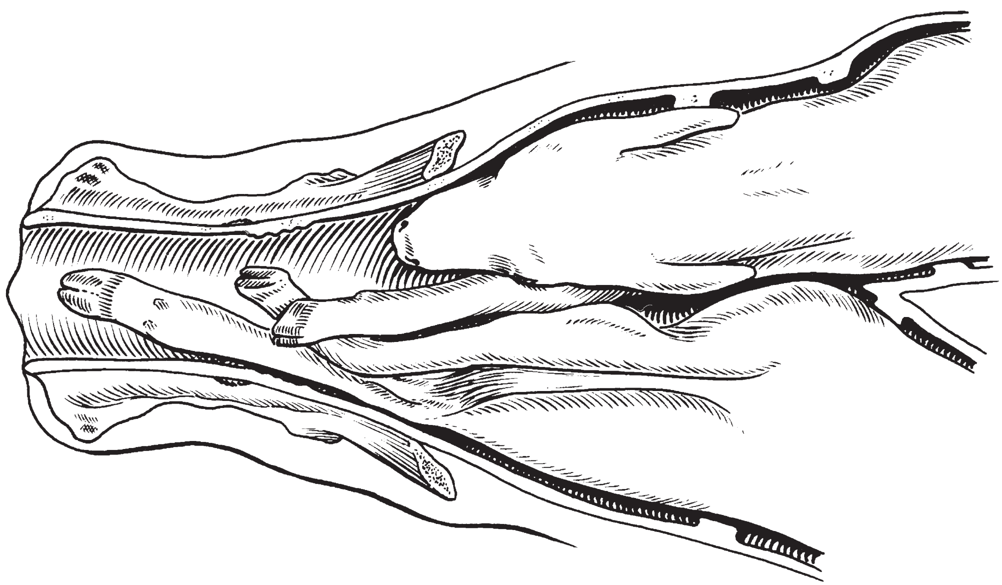

Fig. 17.1 Diagnosis: simultaneous engagement of twins. One twin is in anterior presentation, dorsal position, shoulder flexion posture; the other is in posterior presentation, dorsal position, extended posture.

applying traction simultaneously to two fetuses. Nor should twins be mistaken for a schistosome, double monster or ventrotransverse presentation of a single fetus.

Where a twin is presented with an abnormality of posture, it is treated as if it were a single fetus; in such cases the presence of twins is not known – but may be suspected on account of small fetal size and the history of the dam – until the uterus is searched after delivery and another fetus found. Again, the association of uterine inertia with twins may be known only after delivery of the first fetus.

Little attention has been given by veterinary surgeons to the relationship between the type of dystocia and the disposition of the twins within the uterus. Simultaneous presentation would seem probable when a twin from each horn approached the pelvic inlet; abnormality of posture and inertia would be more likely when both fetuses occupied the same horn. However, Anderson et al. (1978) saw no dystocia in 16 cases of experimentally induced twinning in which a 5-day embryo was placed in each uterine horn. Their observations, and the clinical experience of the author, indicate that dystocia is more likely with unicornual twinning.

When twins are known to be present and retropulsion is required – either of the presenting fetus to correct its posture or of the less advanced fetus to allow delivery of the first twin – it should be performed very carefully. There is a much greater likelihood of causing uterine rupture when twins are present, in both cattle and sheep. Spontaneous rupture has been seen when both fetuses were in the same horn.

There are many stillbirths among cattle twins; the second calf to be born is more likely to survive. Breech presentations are common.

Simultaneous presentation of twins (Figure 17.2) is treated in logical sequence. The polarity of the fetuses is determined, the more advanced fetus recognised and its presenting extremity appropriately snared. Any defect of presentation, position or posture must be diagnosed and treated; correction may be greatly facilitated by means of epidural anaesthesia. Then, with continuing retropulsion on the less advanced fetus, the nearer one is brought into the pelvis and delivered by simple traction. The other fetus, which may be presented in the opposite direction, is then appropriately manipulated. The delivery of ovine twins is more easily achieved if an assistant holds the ewe by its hindlegs in an inclined supine position. When the ewe is delivered of twins the uterus should always be examined for a third fetus.

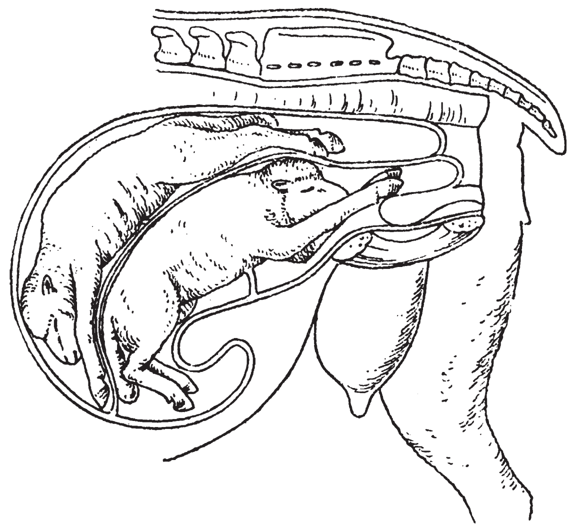

Fig. 17.2 Diagnosis: simultaneous engagement of twin lambs. One twin is in anterior presentation, dorsal position, extended posture; the other is in posterior presentation, dorsal position, extended posture (from a paper by H. Leeney in *Transactions of the Highland Agricultural Society, c.* 1890).

In occasional cases of gross delay, corrective manipulation is impossible and fetotomy of the presenting fetus may be required. Severe pelvic impaction of dead fetuses may be more readily relieved by a caesarean operation.

The afterbirth of bovine twins is likely to be retained.

Vandeplassche et al. (1970) have recorded useful data on 44 cases of equine twin gestation. All pairs were of dizygotic origin (i.e. non-identical). In 33 of 34 twin pregnancies, it was found that one fetus occupied each horn; in the remaining case the twins were in the same horn. Of 44 live foals born, 37 were reared. The study showed that there was a much smaller likelihood of viable twin foals being born to thoroughbred mares than to Belgian draught mares, and this difference might be related to a better uterine capacity in the draught mare (Vandeplassche et al., 1970).

Most cases of equine twin conception are followed by early death of one or both of the conceptuses. About 2% of equine gestations start as normal twin fetal development, but mummification or abortion frequently occur so that fewer than 1% reach term.

DYSTOCIA DUE TO MONSTROSITIES

Monstrosities most often cause dystocia in dairy cattle, the commonest example being *schistosoma reflexus*; next in order of frequency are ankylosed calves including *perosomus elumbis*, double monsters, dropsical fetuses, including anasarcous and hydrocephalic calves, and anchondroplastic monsters (see Chapter 4). The same varieties occur, but to a lesser extent, in sheep. With the notable exception of wryneck, monstrosities are uncommon in mares. Instances of hydrocephalus, double monsters and *perosomus elumbis* occur occasionally in pigs.

With the exception of anasarcous fetuses, gross malformation is often associated with ankylosis of joints and muscular atrophy; consequently many monsters weigh less than normal calves. This, coupled with the fact that they are sometimes associated with abortion or premature birth, means that a monster may be sufficiently small to be passed spontaneously. However, the grossly irregular development, including bending or twisting of the vertebral column and ankylosis or duplication of limbs, means that a wider than normal fetal diameter presents at the pelvic inlet and that severe dystocia results.

Principles of the delivery of monstrosities

Recognition of the exact disposition of the fetal extremities, and an estimate of fetal size, may be very difficult. The obstetrician must then consider whether careful traction – with due regard to lubrication and protection of the birth canal from irregularly disposed appendages – is likely to succeed. Prior to the attempt at vaginal delivery, the diameter of anasarcous, ascitic and hydrocephalic fetuses may be reduced by appropriate multiple or single incisions with a fetotomy knife. If moderate traction does not soon succeed, fetotomy or a caesarean operation must be employed. In view of the worthless nature of monstrosities, fetotomy should be first considered, and in all cases where sufficient reduction of the fetal diameter may be achieved by simple section(s), fetotomy should be practised. Thus, for ankylosed fetuses, including wryneck and *perosomus elumbis*, for cases of anterior duplication and for schistosomes presented viscerally, fetotomy is indicated. The most suitable instrument will be the wire-saw fetotome. The hydrocephalic whose head is too rigid to be reduced by cranial puncture must have the dome sawn off by means of a fetotomy wire.

Where it is obvious, because of excessive fetal size – as in anasarca and extensive duplication – or because of very irregular presentation, that several fetotomy sections will be required, the veterinary surgeon should resort to the caesarean operation. This will be less arduous for the operator and, in general, better for the immediate health and the future breeding potential of the cow.

Occasionally, monstrosities present baffling problems to the obstetrician. This happens when the presenting part of the fetus is normal and the distal extremity is grossly malformed; birth proceeds normally until the malformed portion engages the pelvic inlet. The cause is not apparent and may be impossible to ascertain. Examples are provided by *perosomus elumbis* where the front half of the calf negotiates the birth canal but the ankylosed and distorted hindlimbs become impacted; a hydrocephalic fetus in posterior presentation; and cases of anterior duplication presented posteriorly. In these instances, heavy but unsuccessful traction has usually been applied before the arrival of the veterinary surgeon. This history, together with the normal appearance of the presenting portion, should make the veterinary surgeon suspicious that an abnormality is present in the distal portion. A caesarean operation provides the easiest solution.

Obstetric management of *schistosoma reflexus*

This most familiar bovine monstrosity requires special consideration. The features of the malformation were described in Chapter 4. The weight of the monster calf is usually around 22 kg. It may occur in other ruminants and swine, and may be presented viscerally or by its extremities. It is not uncommon for a fetus in visceral presentation to be naturally born.

With this type of dystocia, fetal viscera may be seen protruding from the vulva (Figure 17.3); if not, they are soon located by vaginal exploration. The viscera may be mistaken for those of the mother and uterine rupture may be suspected, but it should not be difficult by careful examination to dispose of this suspicion, the absence of a uterine tear and the continuity of the viscera with the fetus being soon established. The viscera must be torn away from the fetus whose rigid vertebral angulation may then be felt at the pelvic brim. The fetal diameter is now compared with that of the birth canal; where it seems favourable to birth, Krey's hooks are fastened to the presenting fetus. Reasonable traction, with adequate lubrication, is now applied, the veterinary surgeon paying particular regard to the possibility of damage by bony fetal prominences to the birth canal. In this way, the expulsive efforts of the cow are gently aided, and smooth delivery may be achieved. Where, after a short period of such traction, it is obvious that safe vaginal delivery is not possible, the fetus should be bisected by means of the wire-saw fetotome. One arm of the instrument is loaded and the protruding wire is carried in on an introducer and passed around the spinal flexure of the fetus (Figure 17.4). Passing the introducer around the

Fig. 17.3 Friesian heifer with a *schistosoma reflexus* calf in visceral presentation resulting in the appearance of the fetal viscera at the vulva.

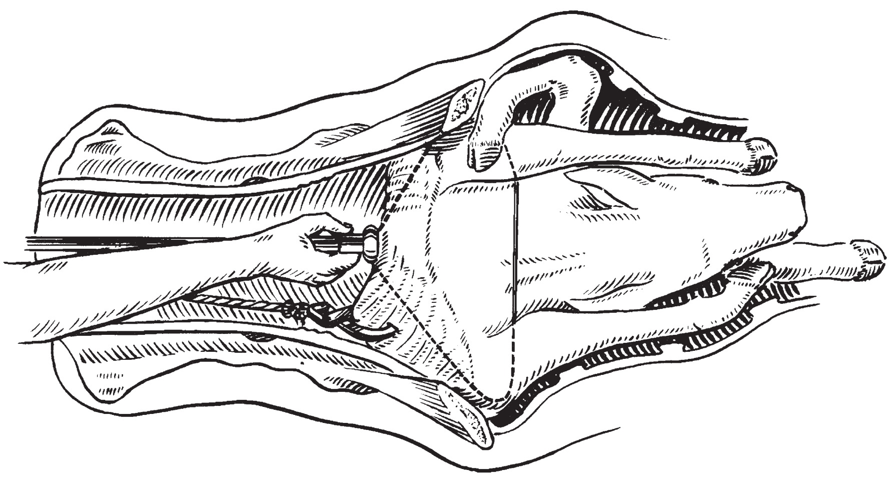

Fig. 17.4 Diagnosis: *schistosoma reflexus* in visceral presentation. The viscera have been removed and the calf is being divided by means of the wire-saw fetotome.

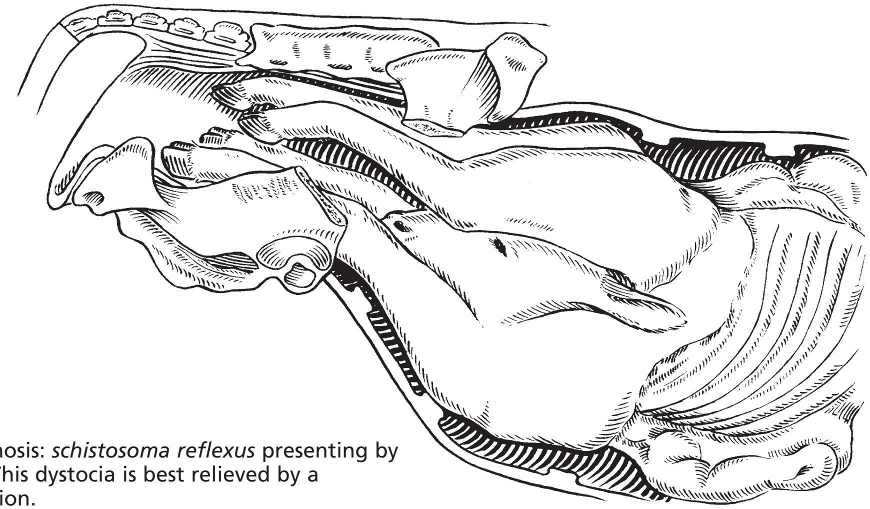

Fig. 17.5 Diagnosis: *schistosoma reflexus* presenting by the extremities. This dystocia is best relieved by a caesarean operation.

fetus may be a tedious task; when accomplished, the other arm of the fetotome is loaded and the head of the instrument is passed into the vagina until it abuts on the fetus. The fetal vertebral column is then sawn through, and the smaller fetal segment withdrawn by means of Krey's hooks. Should difficulty arise over withdrawal of the remaining portion, it too may need to be divided perpendicularly to the first section, again using the wire-saw.

When a schistosome presents by its extremities – three or four legs, with or without the head – the excessive fetal diameter, together with the ankylosis of joints, is likely to prevent natural or manipulative delivery per vaginam (Figure 17.5), and unless the fetus is very small in relation to the maternal pelvis – as might occur in a schistosome twin to a normal calf – time should not be wasted on an attempt at vaginal delivery. Fetotomy or a caesarean operation will be required. In general, it is far easier to deal with such a presentation by the latter method since the fetotomy required will take a long time. Exceptions may be met in the case of small fetuses where the removal of a head or single limb will make birth possible. When performing the caesarean operation for the removal of a schistosome, the veterinary surgeon should always consider the advantage of fetotomy from the laparotomy site; in this way the requisite length of the uterine incision may be kept within reasonable bounds and the risk of uterine rupture during extraction minimised (see Chapter 20 on the caesarean operation).

After successful removal of a schistosome, the uterus should always be searched for injury and to ensure the absence of a second fetus.

The same considerations apply to the treatment of monstrosities in sheep.

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18 Injuries and diseases incidental to parturition

Dystocia is often accompanied by uterine inertia and followed by delay in uterine involution. Because of this interference with normal uterine function, retention of the fetal membranes and puerperal metritis are especially likely to occur. Obstetric trauma to the genital tract also predisposes to infection, and where severe contusion has occurred there is a marked risk of infection particularly by anaerobic bacteria. Prolapse of the uterus is a serious complication of the third stage of labour, but it is more likely to happen after normal birth than after dystocia. These three important conditions that follow delivery of the fetus, namely prolapse of the uterus, retention of the afterbirth and puerperal infection, are given special consideration elsewhere. In addition, there are numerous accidents and diseases that accompany or follow parturition. Traumatic lesions of the soft tissues of the genital tract or bony pelvis may lead to fatal haemorrhage or infection, or to disability due to fractures, dislocations or paralysis. Other complications of parturition comprise displacement, hernia and rupture of the pelvic or abdominal organs. Parturition and the puerperium may also be complicated by metabolic diseases, particularly hypocalcaemia and ketonaemia, and by displacement of the abomasum. A difficult foaling may be followed by laminitis or tetanus, and in all species puerperal animals may incur embolic pneumonia, toxaemia, septicaemia and pyaemia as sequels to uterine infection. Endocarditis, unthriftiness and sterility are possible later sequelae.

While spontaneous trauma, rupture or displacement may occur in unassisted deliveries, the most frequent basic cause of parturient and postparturient disease is delay in giving obstetric aid to dystocia cases. Unskilled and unsympathetic interference is another important cause of genital trauma. If skilled attention were given at the correct time in dystocia there would be relatively few difficult cases, and the amount of postparturient disease would be markedly reduced.

POSTPARTUM HAEMORRHAGE

Bleeding from the maternal side of the placenta in natural separation of the afterbirth is only likely in carnivora where breakdown of the marginal haematoma is accompanied by a green or brown discharge of altered blood. If, however, premature dehiscence occurs when the afterbirth is removed during an elective caesarean operation, severe and even fatal haemorrhage may follow. Because of the histological form of the epitheliochorial and synepithelial chorial placentae of horses, swine and ruminants, significant haemorrhage from the capillary plexuses around the crypts can occur only when excessive force is used in early removal of a cotyledonary-type afterbirth. In veterinary obstetrics, the usual cause of serious haemorrage is laceration of a uterine blood vessel by a fetal appendage, obstetric instrument or hand of the obstetrician. After removal of the fetus much blood may accumulate in the uterus before it begins to escape via the vagina; alternatively blood may drain through a tear in the uterine wall into the abdomen.

When, after delivery of the offspring, there is a profuse haemorrhage from the vulva, the most likely source is the broken ends of the vessels of the umbilical cord which have receded into the vagina. This is likely to occur in uterine inertia where, owing to poor uterine contractions, much of the blood from the fetal side of the placenta (allantochorion) is not expelled into the fetus during second-stage labour. Similar bleeding from mares is seen after the stud-groom has hastened a normal delivery by traction on the fetus and has immediately ligated the umbilical cord (near to the fetal abdomen) and then severed it. Such haemorrhage from the allantochorion does not affect the dam, but the young animal is thereby deprived of a natural blood transfusion and this could be the cause of cerebral anoxia in newborn foals.

If, when the postparturient uterine examination is being conducted, profuse haemorrhage is occurring from a uterine laceration, prompt contraction of the uterus should be promoted by means of an injection of oxytocin. Next day the masses of clotted blood should be manually removed. Haemorrhage associated with uterine rupture is attended to when the uterine tear is repaired. When severe haemorrhage is occurring from a ruptured vaginal vessel an attempt must be made to close the vessel. Ligation is usually not practicable but artery forceps may be applied and left on for 24 hours. Where the vessel cannot be secured, an intravaginal pressure pack can be improvised with a large clean towel, or by the insertion of a large roll of cotton wool. General symptoms of severe haemorrhage and shock can be counteracted by blood transfusion (4–5 litres) from a neighbouring animal.

Fatal haemorrhage from vessels in the broad ligament has been seen in the mare and cow. Rooney (1964) recorded 10 cases of fatal haemorrhage from the ovarian, uterine or external iliac arteries in foaling (eight) or pregnant (two) mares. All were aged mares, and nine of them were thoroughbreds. The ruptures were associated with aneurysms or degenerative changes in the arteries and it was presumed that these lesions were predisposed to by age and that the actual ruptures were caused by stretching during pregnancy or pressure during parturition. Where such haemorrhage is suspected, the only hope of saving the animal would be prompt laparotomy and ligation of the torn vessel.

CONTUSIONS AND LACERATIONS OF THE BIRTH CANAL AND NEIGHBOURING STRUCTURES

Any part of the birth canal may suffer contusion during forcible extraction of the fetus, but the cervix and vulva are more likely to be lacerated than the dilatable vagina. The retroperitoneal fat surrounding the vagina of heifers of the beef breeds makes such animals particularly prone to vaginal contusion when the fetus is oversized. Infection with *Fusiformis necrophorum* is then probable, and a most severe necrotic vaginitis ensues. The condition is very painful and causes continuous, exhausting straining and marked toxaemia. Pyogenic infection is also possible. All vaginal contusions and lacerations should be treated with mild emollient and antibiotic preparations; parenteral antibiotics should also be given. Caudal epidural anaesthesia, particularly when xylazine is used, gives temporary relief from straining (see Chapter 12).

Rupture of the vagina should be repaired, if possible, by suturing although access can be difficult. Infection following rupture may give rise to peritonitis, to severe pelvic cellulitis with marked toxaemia and straining, or to abscess formation with subsequent vaginal constriction. All vaginal injuries should be treated with due regard to the possible sequelae. Lacerations of the cervix may be sutured by applying vulsellum retraction forceps to the organ and withdrawing it to the vulva. Wounds of the vulva and perineum are easily sutured. Mattress sutures of nylon, or other monofilament, non-absorbable suture material, should be used, devitalised tissue, including any loosely attached portions of adipose tissue, being first removed. If lacerations of the vulva and perineum are left unsutured, scar tissue formation and distortion impede the sphincter action of the vulva, with consequent aspiration of air, vaginitis and metritis; a special, and much more difficult, operation is then required.

When Caslick's operation to prevent vaginal aspiration has been performed in the mare, and the vulva has been incised at parturition to allow birth of the foal, the incised tissue should be resutured immediately after delivery. Repair of the vulva, perineum and cervix may be conveniently carried out under caudal epidural anaesthesia.

In cows, previously unsuspected organising haematomata of the vagina may suddenly prolapse from the vulva 4–6 weeks after parturition. These lesions resemble fibromata but are not neoplastic and are easily excised.

Haematoma of the vulva

This is a sequel to contusion of the submucous tissue during delivery. One lip of the vulva is usually affected and an obvious round swelling occupies the vulva orifice. The condition may arise spontaneously in the mare, but in both cows and mares it is likely to follow assisted delivery in which considerable manipulation, or forced traction, was required. Haematoma of the vulva may be confused with prolapse, tumour or cyst of the vagina. If left untreated, natural resolution usually occurs within a few weeks with resorption of fluid and regression of swelling; occasionally, pyogenic infection ensues and may be accompanied by fibrosis and distortion of the vulva, with vaginal aspiration. If left for 3 or 4 days after labour the haematoma may be safely incised and the clot removed without recurrence of haemorrhage. An abscess should be opened and drained.

Perineal injuries at parturition

Serious perineal injuries occur during the second stage of labour in both the cow and the mare, mostly in primiparous animals. These injuries may be classified as first-, second- and third-degree tears and rectovaginal fistulae.

Many heifers sustain slight tearing of the upper commissure because of vulval stretching during normal labour but such lesions heal satisfactorily by first intention without suturing. Tears which extend more deeply into the perineum do not close spontaneously, although epithelial repair is rapid. Such lesions destroy the sphincteric effect of the vulva and lead to aspiration of air into the vagina, even though the integrity of the anus is not impaired. With greater stretching and tearing during the second stage of labour, the wound may extend into and destroy the anal sphincter, thus creating a cloaca through which faeces fall into the terminal vagina (Figures 18.1 and 18.2). Despite rapid epithelialisation, the abnormal communication between the terminal rectum and vagina persists, although its extent may be considerably reduced by wound granulation. Experience suggests that simple rectovaginal fistulae (Figure 18.3) without damage to the anal sphincter are uncommon spontaneous injuries to cattle, although they occur as developmental anomalies in cases of anal atresia.

Fig. 18.1 Third-degree perineal laceration in a cow. Note swelling of the vulva and the tear extending from the dorsal commissure towards the anus.

They may also result from unsuccessful attempted closure of a third-degree perineal tear, as in the mare.

In the mare, the mechanism of perineal tearing is different. In this species, the initial injury is usually perforation of the vaginal roof by a fetal forelimb which may be deflected dorsally during the second stage of labour by a hymeneal rim. As a result of vigorous sustained straining, the limb is then likely to perforate the rectum and be forced, possibly with the fetal head, through the anal orifice, which in turn may be ruptured (Figure 18.4(a)). Early recognition of the injury may allow repositioning of the fetal extremities and normal vaginal delivery, but deliberate incision of the perineum and anal sphincter is usually expedient if the rectum is perforated because a third-degree defect is easier to repair surgically than a recto-vaginal fistula which would otherwise result. Mares in which a Caslick closure of the upper vulval commissure is not reopened before foaling

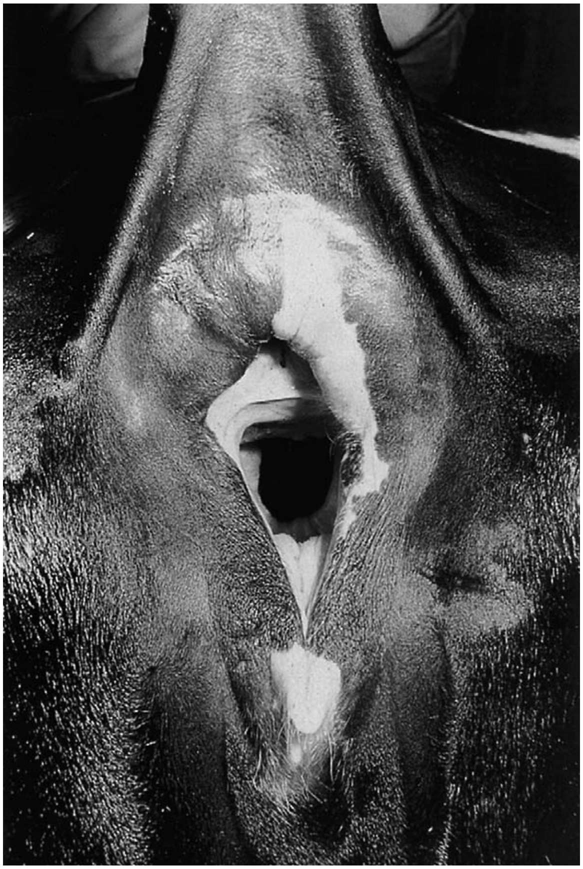

Fig. 18.2 Third-degree perineal laceration in a cow under caudal epidural anaesthesia to cause relaxation of the vulva and perineum. The shelf between the rectum and vagina is just visible.

Fig. 18.3 Acquired rectovaginal fistula in a cow. (a) Vulva dilated to vaginal opening to fistula. (b) With a bandage passed through the fistula. (*Figure 18.3 b, see opposite.)*

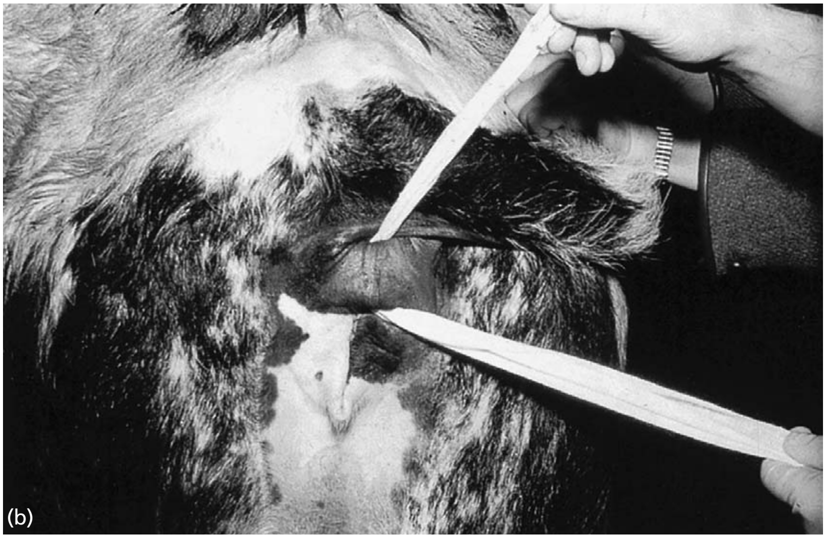

Fig. 18.3 *Continued.*

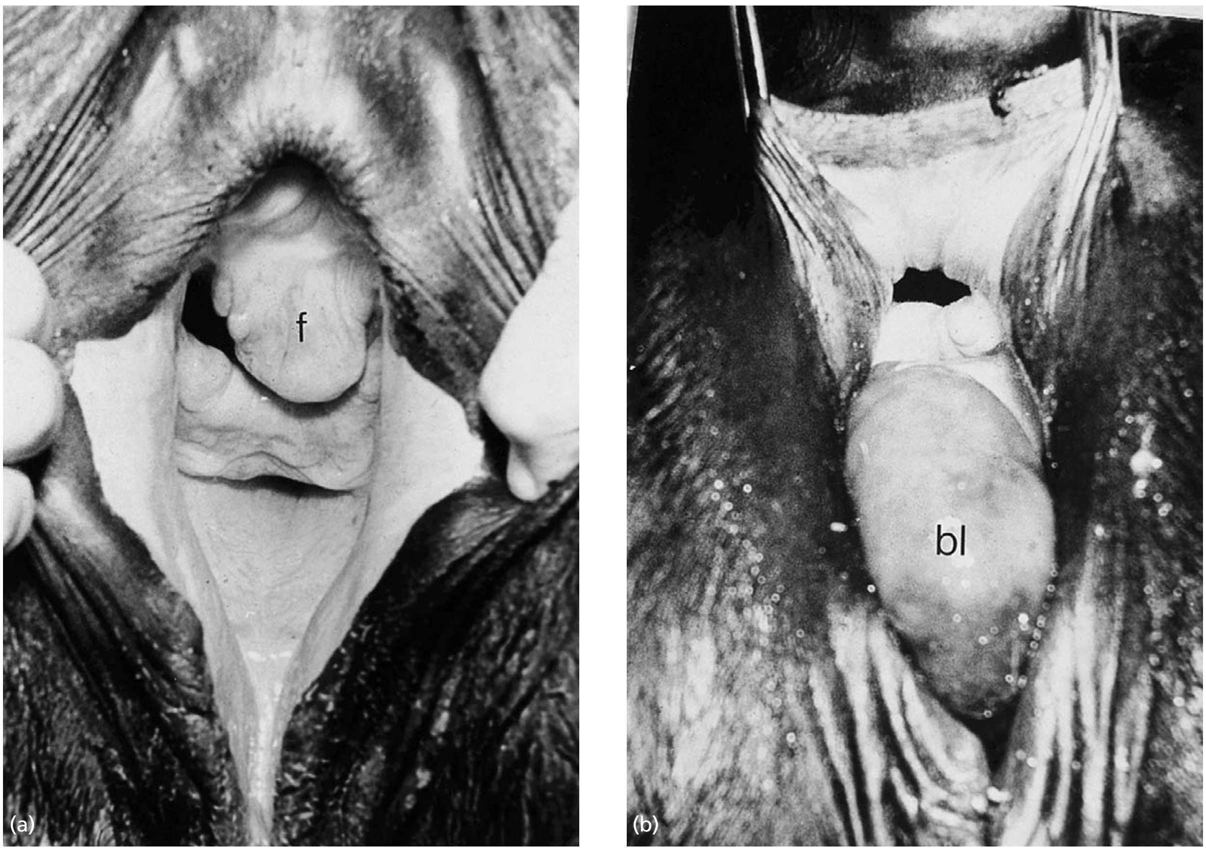

Fig. 18.4 Third-degree perineal defect in the mare. (a) Showing a flap of mucosa (f) attached to the roof of the vagina at the caudal border of the residual shelf. (b) Eversion of the bladder (bl).

may sustain a similar injury in a slightly different way, the tear extending dorsally from the vulva, as in cows. Records of cases presented for repair indicate that third-degree tears are the most common perineal injury in the mare but recto-vaginal fistula formation is still more frequent in the mare than the cow. By contrast, second-degree defects are rare in the mare but not uncommon in cows, simply because of the different mechanisms of tearing in the two species.

Perineal defects granulate and are epithelialised rapidly, but they are lacerated wounds with considerable tissue damage and a degree of superficial sloughing is usual before granulation begins. The extent of inevitable tissue necrosis prejudices the likelihood of first-intention healing after immediate suturing. It is nevertheless advisable to stitch deep perineal wounds that have not perforated the anal sphincter as soon as possible. Third-degree tears with destruction of the sphincter and rectovaginal fistulae should be left to heal by granulation and surgical reconstruction can be undertaken later if necessary. The extent of such defects is considerably reduced by cicatrisation and occasionally small, oblique fistulae in the mare close completely, but in most cases a significant defect remains.

The clinical effects of a third-degree defect are two-fold: continuous aspiration of air into the vagina and contamination of the vaginal lumen with faecal fluids or, worse still, accumulation of faecal boluses in its terminal segment. Pneumovagina in turn, by distorting the lumen, may lead to pooling of urine cranial to the external urethral meatus. Inevitably in both the cow and the mare, these factors result in gross bacterial contamination and ascending infection in the genital tract. In both species, therefore, sizeable cloacal lesions result in infertility and affected mares are also aesthetically unsuited for other uses because of perineal incompetence.

In cases of rectovaginal fistula, the degree of faecal contamination of the vagina depends on the extent of the fistula. The few animals that are able to maintain a normal pregnancy are generally found to have a caudally sited lesion of very limited diameter.

Surgical intervention should be delayed until all tissue surfaces are covered by epithelium and this usually takes 6 weeks or so. In the mare, the urinary bladder is sometimes everted soon after the injury occurs (Figure 18.4b), but it is easily replaced and retained if necessary with sutures. There is no need for other treatment during the intervening period except perhaps for tetanus prophylaxis in the mare.

Second-degree defects are easily obliterated by stripping the vaginal mucosa from the normal level of the upper vulval commissure dorsally on both sides and suturing the submucosal tissues as in a Caslick operation.

For many years, surgical reconstruction of the perineum was based on the technique described by Götze in 1938, in which, after appropriate stripping of the mucosal surfaces, the residual shelf between rectum and vagina was mobilised and fixed as caudally as possible to separate the two cavities. The results were generally good but the operation resulted in considerable postoperative pain and sometimes faecal impaction because of reluctance to defaecate. The method has largely been superseded by the technique described by Aanes (1964), in which the rectum and vagina are separated by the construction of a new shelf from tissues in situ without undue tension on suture lines. Aanes advocated a two-stage operation, but the method to be described for repair of a third-degree defect is a one-stage procedure with other minor modifications of his suturing technique.

In cattle, the operation is ideally performed under caudal epidural analgesia. The same technique can be used in the mare, but the operation can equally well be performed in this species with the animal in dorsal recumbency and the hindquarters raised, under general anaesthesia. Cows require no dietary preparation. In the mare, a laxative diet without roughage is advisable for 3 days beforehand, followed by overnight starvation. After proper cleansing of the site, the rectum is gently packed with towelling; if the mare is anaesthetised, a vesical catheter may be inserted to divert urine from the operation site. In cows, the defect is usually no more than 6 cm deep from the perineum, but in the mare it is considerably longer and sometimes extends almost to the cervix. In both species, tissue forceps are placed on the cutaneous borders of the cloaca down to the normal level of the upper vulval commissure and on the caudal edge of the residual shelf.

Bridges of skin across the defect are removed and it is then possible to see a sharp demarcation between the vaginal and rectal mucosae (Figure 18.5a and plates 3b and c). The first stage of the procedure is to separate the vaginal mucous membrane from the tissues which will subsequently be apposed to create a shelf. The dissection begins at the level of the normal upper commissure and is extended dorsally on the mucocutaneous border and then cranially on both sides along the junction of vaginal and rectal mucous membranes until the incisions meet on the caudal edge of the residual shelf. The final stage of dissection is the separation of vaginal mucosa for 4 cm cranial to the edge of the shelf (Figure 18.5b and c). It is essential that all the vaginal mucous membrane is removed from the tissues which are to be sutured. There is minimal haemorrhage during the procedure and no need for haemostasis. In some cases, cicatrisation results in considerable asymmetry of the cloaca which should be corrected before suturing is begun. The curtain of separated vaginal mucosa is then included in the purse-string-type sutures of polyglycolic acid which are placed and tied serially from the depth of the wound outwards. The method of suturing is illustrated in Figure 18.6 and a stage by state repair in a cow illustrated in plate 3.

It is most important that the stitches tighten properly because dead space predisposes to

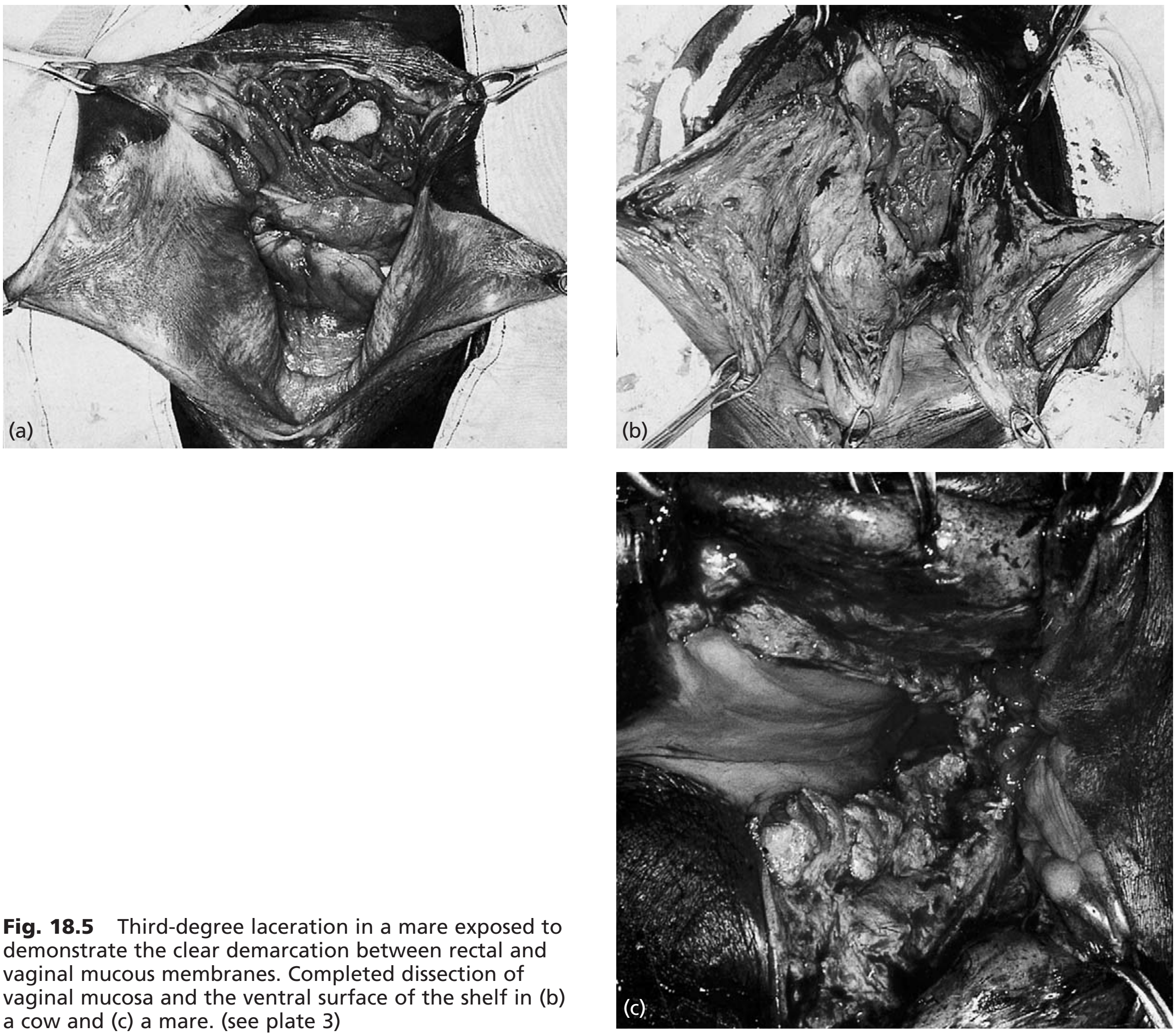

Fig. 18.5 Third-degree laceration in a mare exposed to demonstrate the clear demarcation between rectal and vaginal mucous membranes. Completed dissection of vaginal mucosa and the ventral surface of the shelf in (b) a cow and (c) a mare. (see plate 3)

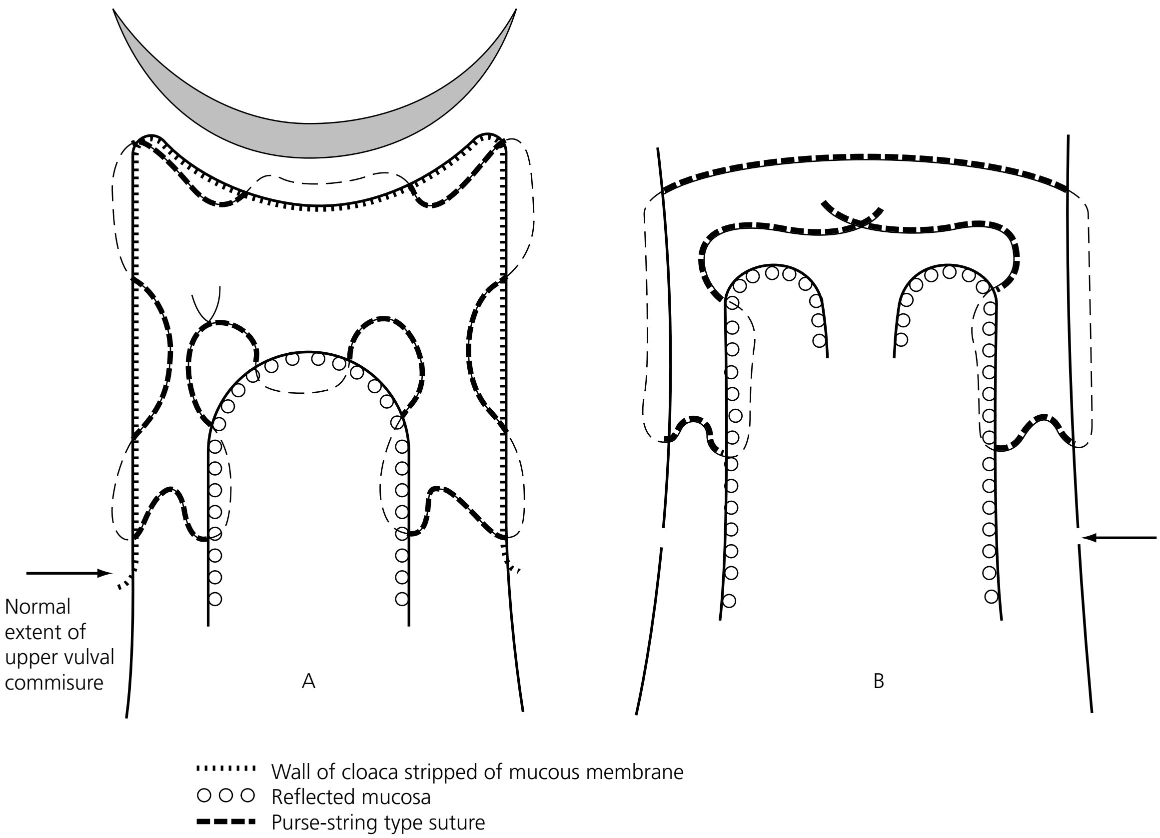

Fig. 18.6 Suturing technique for reconstruction of the perineum. (A) Below the shelf. (B) Caudal to the shelf.

wound breakdown. The operation is completed with mattress sutures in the perineal skin (Figure 18.7). Further minor closure of the upper commissure may be necessary under local anaesthetic infiltration when the integrity of the repair has been properly tested a month or so later. It should be emphasised that, although this operation restores breeding ability, it does not prevent air movement through the incompetent anal sphincter, a consideration which may be important in mares that are to be used for other purposes. In such animals a second operation to strengthen the sphincter can be attempted later by stripping mucocutaneous tissues in the defective segment and suturing what muscle remnants can be identified. The horse's anus is normally somewhat lax, and minor incompetence is no great detriment. If attempted reconstruction is unsuccessful, the operation can be repeated, but the prognosis is then less good because of local fibrosis and reduced vascularity.

Unless the vulval length is inadvertently shortened during reconstruction, subsequent parturition in both the cow and the mare usually occurs normally without the risk of vulval tearing or the need for episiotomy.

Paradoxically, a simple rectovaginal fistula is more difficult to repair than a third-degree defect. Aanes (1964) recommends that such lesions should first be converted into a cloaca and repaired as such after granulation stops. The deliberate destruction of perineum and anal sphincter can be avoided by adopting a different surgical approach to such lesions. Unless the fistula is deeply sited, it can be exposed satisfactorily by a dorsal commissure episiotomy which is extended cranially under the anal sphincter and rectal floor beyond the fistula (Figure 18.8). The

Fig. 18.7 Completed one-stage reconstruction of the perineum in a Friesian cow.

rectal mucous membrane lining the lesion can then be securely inverted with sutures placed in a transverse direction in the submucosal tissues before the episiotomy is repaired in the conventional way.

Perineal defects are usually obvious in mares but are nevertheless sometimes not noticed by unwary purchasers. They are less obvious in cattle, particularly if the anal orifice remains intact.

Damage to the lumbosacral plexus

When a large fetus is forcibly drawn into the maternal pelvis the lumbar nerves which pass over the lumbosacral joint to form the anterior part of the lumbosacral plexus may be damaged; paralysis of the gluteal or obturator nerves is a possible result. This is particularly likely when an oversized fetus becomes impacted in a state of 'hiplock', the nerves being trapped between the lumbosacral promontory of the mother and the iliac bones of the calf. In addition, the obturator nerve, as it passes down the inner surface of the iliac shaft, may be damaged by an oversized fetus.

Gluteal paralysis

Gluteal paralysis is seen in the mare and cow; in the mare it has followed spontaneous birth. It is

Fig. 18.8 A congenital rectovaginal fistula in a donkey exposed by episiotomy.

recognised when the dam is found to have difficulty in rising and when she walks with 'weakness of the hindlimbs'. Later, atrophy of the gluteal muscles is apparent. Prognosis is favourable, the disability usually disappearing in a few weeks, although occasionally complete recovery may take months. In warm weather the affected animal should be placed in a paddock which is free from ditches and obstacles; here a firmer foothold for getting up is more likely than in a barn or loosebox. The animal may be helped to rise by lifting on its tail and then steadying its hindquarters. In order that the mare may suckle the foal and also rest on its feet, slings may be usefully employed. If the mare or cow cannot get up within a few days of parturition the prognosis is grave.

Obturator paralysis

Obturator paralysis is more frequent in cows than mares. The obturator nerve supplies the adductor muscles of the thigh; thus when both nerves are damaged the legs will be splayed and the cow is unable to rise. If the cow is helped to its feet, the legs slide out laterally. When paralysis is one-sided the cow also requires assistance to get up but can stand readily, if the affected leg is prevented from sliding outwards. If the cow falls there is a risk of limb fracture or dislocation of the hip joint. Where there is complete and bilateral paralysis, prognosis should be guarded; where it is unilateral and the animal can walk with assistance, the outlook is favourable. Hobbling together of the hind legs with a strap applied above each fetlock prevents excessive abduction and secondary tearing of the adductor muscles or fracture of the femoral neck during attempts to stand. Most cases show rapid improvement within a few days and progress to a complete recovery. Unless there is marked improvement within a fortnight, recovery is unlikely. Treatment comprises good nursing. The cow should be well bedded with short litter on an earthen floor or on a concrete floor on which sand or grit has first been sprinkled. She must be assisted and maintained on her feet for milking or suckling and as often as possible at other times. The patient should be stimulated to walk but should be prevented from falling awkwardly. Slings are occasionally employed for cattle. Bedsores must be prevented, the animal being turned from side to side, the hindquarters massaged, the bedding frequently changed and the cow's rear and udder kept clean and dry.

RUPTURE OF THE UTERUS OR VAGINA

Rupture of the uterus may occur spontaneously, but faulty obstetric technique is a more frequent cause. Spontaneous rupture is most likely to arise in association with uterine torsion or with cervical non-dilatation but is also possibly due to the gross uterine distension that occurs with twins in one horn, with hydrallantois or with excessive fetal size. The most likely time of spontaneous rupture is in late gestation or during labour. Hopkins and Amor (1964) have remarked on the association of spontaneous uterine rupture and breech presentation; they encountered three cases and cited four other cases from the literature. In their cases (and in another spontaneous rupture with breech presentation seen by the present author) the dorsal aspect of the left uterine horn was torn and the split extended backwards to involve the uterine body and cervix too. They believe that breech presentation predisposes to rupture because the breech of the calf fully occupies the maternal pelvic inlet and allows no egress for the fetal fluid when the uterine and abdominal contractions build up the hydrostatic pressure within the uterus. In a review of 26 cases of uterine rupture, 18 of which were heifers, Pearson and Denny (1975) considered uterine torsion and fetopelvic disproportion to be the major predisposing factors. In this series, 14 of the 26 fetuses were mainly or entirely within the peritoneal cavity; four were still alive at the time of laparotomy. According to the size of the rupture – which may heal without incident or allow escape of the conceptus in the abdomen – and to whether or not infection occurs, there is great variation in the syndrome from cases in which no symptoms are shown to others in which shock and fatal toxaemia soon supervene. Thus in some instances the owner is unaware of the accident and the only evidence of it is the subsequent finding of a uterine adhesion or of a mummified fetus among the abdominal viscera – so-called extrauterine pregnancy. When rupture occurs during labour and the fetus passes into the abdomen, labour pains and straining cease and uterine inertia may be suspected until a uterine exploration proves otherwise. Alternatively, the dam's intestines may prolapse into the uterus and even protrude from the vulva; the condition may then be confused with dystocia due to *schistosoma reflexus* in visceral presentation. Accidental rupture of the uterus is likely to occur in the most difficult dystocia cases: those in which the initial disposition of the fetus is markedly irregular and difficult to rectify and those in which there has been much delay in treatment with the development of unfavourable complications. Insufficient uterine space for the extension of a limb or head, inordinate traction on a wrongly disposed or oversized fetus and excessively vigorous retropulsion are the immediate causes of uterine rupture. When the cervix is incompletely dilated, traction on the fetus may cause rupture of that organ. Careless use of the obstetric forceps in the bitch is a cause of uterine rupture. Lastly, rupture of the uterus may be due to external violence as, for example, when the parturient dam falls heavily or receives a severe kick or horn-gore on its abdomen.

When making the initial examination of a dystocia case, the veterinary surgeon must always explore the genital tract for traumatic lesions that may have been caused by unskilled lay interference or which, rarely, may have arisen spontaneously. If uterine rupture is found then, or occurs during subsequent manipulations, the obstetrician must decide – largely on considerations of size and site of the lesion and the amount of manipulation, or traction, still required to effect delivery – whether to proceed with the delivery per vaginam or whether to perform laparotomy, extract the fetus and repair the uterine rupture from the laparotomy site. Except where a small dorsal rupture is discovered and the amount of obstetric interference still required is small, laparotomy is indicated. The procedure then adopted is almost identical to that described for caesarean section, the only complication being the possibly unfavourable site of uterine rupture in relation to the abdominal incision. The accidental rupture may be extended and the fetus extracted or, if the rent is unfavourably placed, another surgical incision must be made for delivery and then both it and the rupture must be repaired. The tear in the uterus is much more accessible for suturing after the fetus has been removed.

Spontaneous rupture of the vaginal wall in late pregnant ewes was first described by White (1961); since then it has been a relatively common finding. Small intestine passes into the vagina and protrudes from the vulva; frequently the ewe will be found dead, presumably from shock. The precise aetiology of the disorder is still unknown; it is generally believed to be associated with cervical vaginal prolapse (CVP) and is discussed in Chapter 5. In one case, which was considered to have a similar aetiology, Fox (1962) noted complete prolapse of the intact pregnant uterus through a tear in the vaginal roof. O'Neill (1961) observed several parturient ewes that were unable to lamb in which rupture of the uterus was present. Prompt adoption of the caesarean operation and repair of the uterine tear gave good results.

PROLAPSE OF THE BLADDER

Prolapse of the bladder may follow a rupture in the floor of the vagina or eversion through the dilated urethra (Brunsdon, 1961) and may occur during or after parturition (see Chapter 10). The rounded organ protrudes from the vulva. The kink that forms in the urethra prevents micturition; thus the organ progressively distends with urine. The condition must be distinguished from prolapse of the vagina, cyst or tumour of the vagina, haematoma of the vulva and prolapse of perivaginal fat. The surface of the bladder is cleaned and the organ is punctured with a hypodermic needle to allow drainage of urine. It is then dressed with an antibiotic powder and gently pushed back into place through the vaginal rupture. The latter is then repaired. Epidural anaesthesia will greatly facilitate return of the prolapsed organ.

EVERSION OF THE BLADDER

Eversion of the bladder is most likely in the mare (see Chapter 10). In this species the urethral opening is wide and parturient straining very forceful. The organ becomes everted during labour and may be injured during fetal expulsion. It should not be difficult to identify the everted bladder. It is pear-shaped and attached to the vaginal floor; urine drips from the two openings of the ureters and the congested mucosa is apparent. Epidural anaesthesia should be induced. The bladder is first cleaned, and any lacerations are repaired by suture. The organ is then compressed between both hands and gradually forced back into the urethra. Further manipulation is then applied to the vaginal floor until the bladder is properly replaced. Antibiotic therapy lasting several days should be prescribed. Tetanus antitoxin should be given. Eversion of the bladder is rare in cattle. Brundson (1961) described a case which occurred during second-stage labour and which he successfully replaced.

PROLAPSE OF PERIVAGINAL FAT

Prolapse of perivaginal fat is most likely in fat heifers of beef breeds and is a sequel to a rupture of the vagina, often a small one. The fat should be carefully removed with scissors, and if possible the vaginal tear should be sutured.

PROLAPSE OF THE RECTUM

Slight eversion of the rectum is a common accompaniment of powerful expulsive efforts. It recedes after delivery. Severe prolapse is likely only in the mare; if it is already present in a dystocia case when the veterinary surgeon arrives, an attempt should be made to reduce the prolapse and an assistant should be instructed to maintain the organ in position by pressing a towel against the mare's anus. Epidural anaesthesia may be needed to replace the rectum. When the prolapse has been present for some hours before veterinary assistance is available and the organ has become markedly oedematous and contused or torn, it may be difficult or impossible to replace it and maintain it in position. Submucous resection under epidural anaesthesia, or under a general anaesthetic, must then be carried out. In the mare, parturient prolapse of the rectum, no matter how transient, may prove fatal because stretching or tearing of the colic mesentery can result in infarction of the terminal colon (Figure 18.9). The affected segment of bowel becomes atonic, defaecation stops and the mare's condition deteriorates insidiously during the next few days.

PUERPERAL LAMINITIS

Puerperal laminitis is a troublesome complication of puerperal metritis. It is essentially an equine condition, but the other farm animals are occasionally affected. In the mare the condition is a likely sequel to retention of the placenta. Two to four days after foaling the typical stance of laminitis is seen, the hind legs being placed well forward to ease the weight on the more severely affected forefeet. It is a most painful affection and causes rapid loss of weight. Owing to the prolonged periods of recumbency and diminution in milk secretion the foal may require artificial feeding.

Avoidance of puerperal laminitis lies in preventing metritis by treating cases of dystocia promptly and carefully, and by the appropriate treatment of retained fetal membranes (see Chapter 26).

PARTURIENT RECUMBENCY

Recumbency, as a complication of parturition, is occasionally seen in all species but is essentially a bovine condition. Under this heading, cows which become recumbent in late gestation should first be considered; the cause here is nutritional, and two separate entities are seen. In one type, recumbency is associated with starvation. Cases occur towards the end of the winter when fodder is scarce or poorly saved. Cattle on hill farms are chiefly affected. Premature induction of calving with corticosteroids (see Chapter 6), or an elective caesarean operation, can be used provided the animal is not too severely affected. Otherwise, in the interest of the animal's welfare, euthanasia should be performed and measures taken to ensure that similar cases do not recur. A prompt caesarean operation and dietary supplementation are indicated. The other entity is a syndrome that appears to be identical to pregnancy toxaemia of ewes. Affected animals are in good bodily condition and are usually pregnant with twins. The general behaviour becomes sluggish, appetite is poor and ketosis, sometimes accompanied by icterus, is present. Premature induction of calving or termination of the pregnancy is normally followed by rapid recovery. Cases which have been

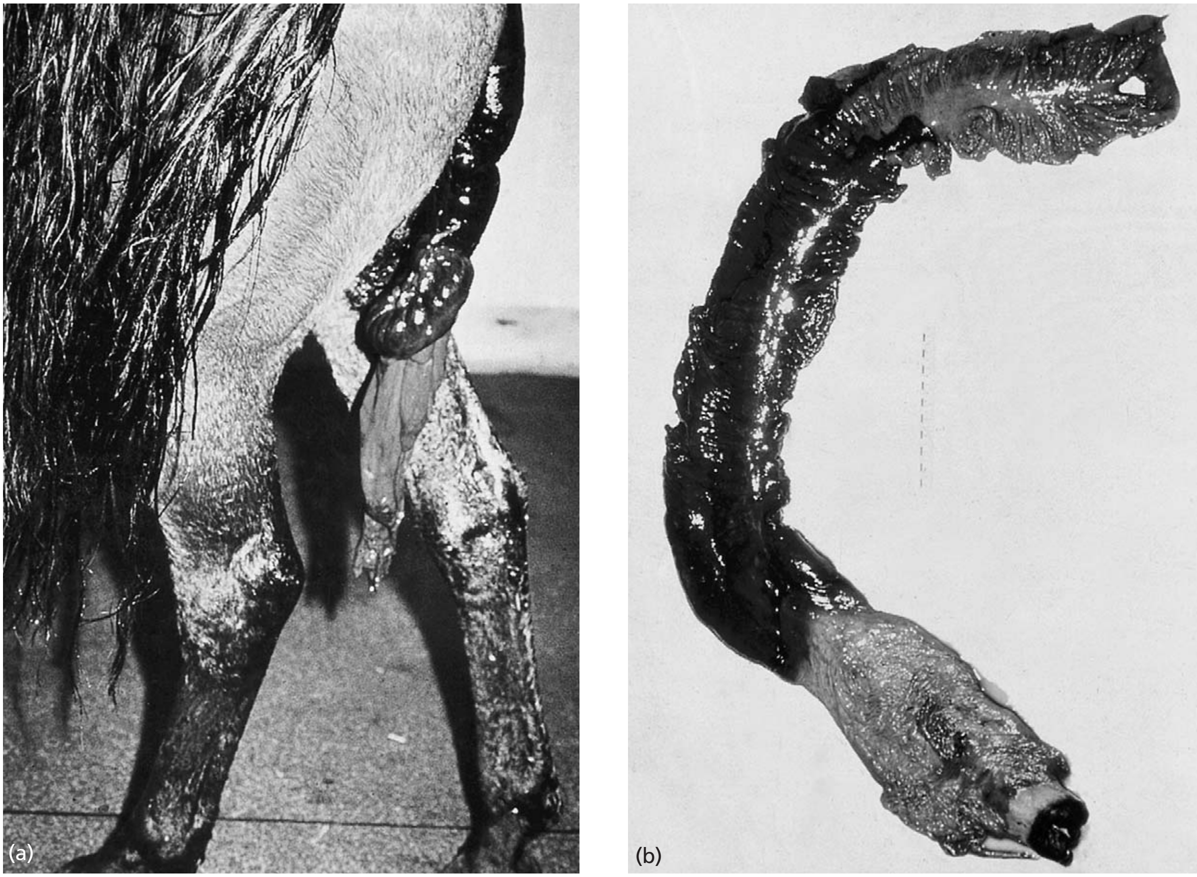

Fig. 18.9 Complications of second-stage rectal prolapse in a mare. (a) Infarction of prolapsed colon. (b) Infarcation after reduction of the prolapse.

unsuccessfully treated therapeutically have shown marked fatty infiltration of the liver. The cause may be due to an excess of concentrated food in early pregnancy and to a deficient diet in late gestation.

Recumbency due to parturient hypocalcaemia, or puerperal metritis

Hypocalcaemia is the chief cause of recumbency in parturient and puerperal cows, although it might be confused with the final stage of severe puerperal toxaemia resulting from uterine infection. A proper consideration of the history and due regard to the symptoms should differentiate the conditions. Puerperal metritis usually follows dystocia and is often accompanied by retention of the afterbirth. There is a fetid vulval discharge and diarrhoea; straining is frequent and there is an expiratory grunt; the pulse is frequent but the temperature, although at first raised, may be falling in a case of advanced toxaemia and is therefore unreliable. A vaginal and uterine examination should verify the suspicion of metritis as a cause of recumbency. Other severe toxaemias that may cause parturient recumbency are acute mastitis, traumatic pericarditis and peritonitis associated with uterine rupture.

True hypocalcaemia occurs occasionally in sows, but the most likely cause of postparturient recumbency is toxaemia due to metritis or mastitis. Incomplete parturition with retention of a fetus or a portion of the afterbirth should always be suspected. Failure of milk secretion is one of the symptoms of toxaemia and hypocalcaemia; it sometimes results from lack of the ‘letdown stimulus’. So-called agalactia of sows is thus not a specific syndrome but merely a symptom common to several quite different affections.

Physical inability to rise

Physical inability to rise may be due to muscular weakness or to lesions of the locomotor system. Inanition due to a variety of diseases may coincide with parturition. Locomotor lesions that may occur during labour and cause recumbency include dislocations of the hip and of the sacroiliac joints, fracture of the pelvis, femur or vertebral column, rupture of the gastrocnemius muscle and paralysis of the obturator or gluteal nerves. A diagnosis of disease of the locomotor system depends on a methodical clinical examination with a view to eliminating the several possibilities. The degree and form of the disability and the manner of the unsuccessful attempt to rise often give a strong indication of the cause. The examination includes the humane manipulation of the hindlimbs with the help of an assistant to determine the presence of excessive mobility or crepitus; it is combined with a rectal examination of the pelvic bones. Regional absence of peripheral sensation may verify nerve paralysis, including paraplegia associated with vertebral fracture. In cases of recumbency due to physical inability, or pain associated with attempts to rise, the affected animal is usually bright, its appetite is good and, when undisturbed, its temperature and pulse are unaffected. Each case must be treated on its merits, and the reader is referred to other texts for further information. It is not unusual in cattle practice to fail to discover the cause of recumbency despite a meticulous and complete examination; apart from recumbency such cases appear normal in every way. In these instances a brief application of the electric goad causes a determined attempt to rise. This is sometimes successful and in any case the extent of the disability may then be more clearly seen. The repeated application of the electric goad must be thoroughly deprecated.

Where no cause of recumbency can be found in an animal that appears normal in other respects, tissue swelling, oedema or haemorrhage in the vicinity of nerves is possible. If such were the case, the normal recovery processes would diminish pressure on the nerves, and this would be reflected in progressively better attempts to rise. Experience in cattle practice shows that if a cow is still unable to rise after being recumbent for a week, the prognosis is grave. Slings, hoists and other devices are sometimes used to encourage the patient to stand, but in general they are of little use. The best contribution that can be made to a recovery is the provision of first-class nursing. This comprises placing the recumbent animal on ample, soft, clean bedding which overlies a dry floor and which is frequently changed. The patient is turned from side to side as often as possible, with concurrent massage of the limb muscles. Meanwhile close veterinary attention is paid to the health of the cow's uterus and udder.

PUERPERAL TETANUS

Puerperal tetanus is a possible sequel to uterine manipulation for dystocia, retention of the afterbirth or prolapse of the uterus. It is most likely to be seen in mares 1–4 weeks after foaling.

All equine obstetric interference should be accompanied by prophylactic injections of tetanus antitoxin.

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19 Postparturient prolapse of the uterus

Prolapse of the uterus is a common complication of the third stage of labour in the cow and the ewe. It occurs less frequently in the sow and is rare in the mare and bitch. In the ruminant species the prolapse is generally a complete inversion of the gravid cornu, while in the sow and the bitch inversion is generally partial and comprises one horn only. Cases are on record in which the bitch has everted one horn before she has completely delivered the fetuses from the other. In the mare the rare cases of prolapse are generally partial only.

THE COW

The incidence varies from 2 per 1000 calvings in range beef cattle in America (Patterson et al., 1979) to 3 per 1000 cows per year in Scandinavian dairy cattle (Rasbech et al., 1967; Ellerby et al., 1969; Odegaard, 1977; Roine and Soloniemi, 1978).

The occurrence seems to be affected by seasonal as well as regional factors, the condition being commoner in some years and in some localities.

Multigravida (of the dairy breeds) are more often involved than are heifers. In the majority of instances the prolapse occurs within a few hours of an otherwise normal second-stage labour, although in some it may be delayed several days. In the latter group the condition is generally associated with a grossly protracted and assisted labour. Rarely, where delivery is achieved by heavy traction, the uterus prolapses immediately after the calf is withdrawn.

Aetiology

The cause of prolapse of the uterus is not clear, but there is no doubt that it occurs during the third stage of labour, within a few hours of the expulsion of the calf, and at a time when some of the fetal cotyledons have separated from the maternal caruncles. The only conceivable force that could lift the heavy uterus out of the abdomen into the pelvis and thence propel it to the exterior is abdominal straining. Gravity, through the medium of a sloping stand, bank or hillside, and traction by a variable weight of freed dependent afterbirth – containing variable loculi of retained uterine fluid and urine – are probable additional forces. Straining occurs normally during the third stage and is synchronous with the continuing peristaltic contractions of the uterus which occur every $3\frac{1}{2}$–4 minutes (Benesch and Steinmetzer, 1931, 1932). One can imagine the uterus being more affected by abdominal straining when it is relatively flaccid, and it is a particularly apt clinical observation that many cases of uterine prolapse show a simultaneous hypocalcaemia (milk fever) which is known to be conducive to uterine inertia. The authors believe, therefore, that uterine inversion and prolapse are associated with the onset of uterine inertia during the third stage when a portion of detached afterbirth occupies the birth canal and protrudes from the vulva. This concept of an association with inertia corresponds with the greater frequency of prolapse in cows than heifers, in dairy rather than beef cows and in closely confined and highly fed cows rather than those at range. Vandeplassche and Spincemaille (1963) are of the opinion that the pregnant horn does not undergo a progressive inversion from its anterior extremity; only the posterior two-thirds invert. The actual protrusion of this portion can occur very quickly in one bout of straining.

Some cattle with extreme laxity of the perineum and vulva may prolapse immediately after every calving. In Australia, uterine prolapse is a feature of the disease seen in sheep grazed on clover pastures containing oestrogenic substances.

The signs of this condition are obvious. As a rule the affected cow is recumbent, and if in lateral recumbency rumenal tympany will be prominent, but occasionally the cow is standing with the everted organ hanging down almost to its hocks (Figure 19.1).

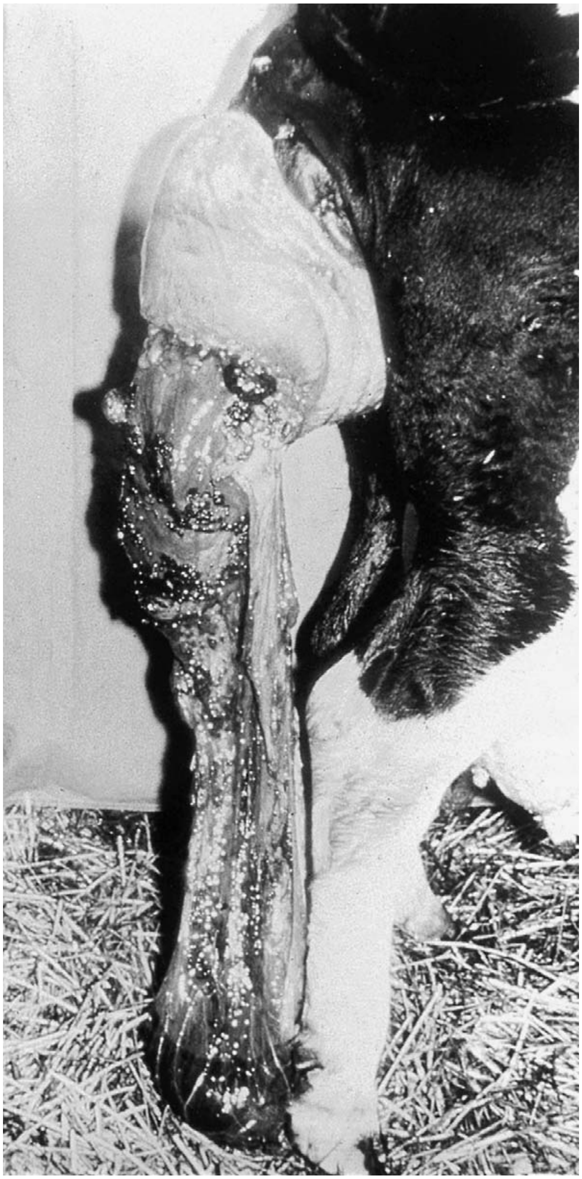

Fig. 19.1 Uterine prolapse in a cow. Note that in the placenta, which is still attached, fetal fluids have accumulated.

Prognosis

The prognosis will depend firstly on the type of case, secondly on the duration of the condition before treatment is forthcoming, and thirdly on whether the organ has sustained severe injury. Nevertheless, as the condition is generally encountered, that is, as a sequel to a normal parturition, and professional assistance is forthcoming within an hour or two of its occurrence, the prognosis is good. Replacement of the organ does not offer insurmountable difficulties and recurrence after replacement is uncommon. Moreover, such animals generally conceive again. Patterson et al. (1979) reported that 40% of cows became pregnant after uterine prolapse. Not infrequently, an animal which has everted her uterus at one parturition calves subsequently without trouble; in fact, repetition of the condition is the exception rather than the rule.

Occasionally prolapse of the uterus is followed in a matter of an hour or so by the animal's death. On post-mortem examination in such cases it is found that death was due to internal haemorrhage consequent on the weight of the everted organ having torn the mesovarium and the ovarian artery. Even in those cases in which there has been delay and in which the endometrium is grossly contaminated and deeply congested, the prognosis is not hopeless, for the recuperative powers of the organ are quite astonishing, and thus when dealing with dairy cattle amputation of the everted organ should be considered only when injury is gross and when resolution is clearly impossible.

Treatment

Replacement of the everted organ. On notification of the case, the farmer should be instructed to wrap the prolapsed viscus in a large towel or other suitable material to prevent further contamination if, as is likely, the cow is recumbent; if she is standing, the organ should be supported by a large towel or sheet held by people on either side, until professional assistance is forthcoming. It is good practice to give a preliminary injection of calcium borogluconate (as for milk fever) and to relieve rumenal tympany, if present, by passing a stomach tube. In the past, the chief difficulties in replacement of the organ have been associated with the almost continuous straining which manipulation of the uterus provokes and with the fact that pressure had to be applied in an uphill direction. Numerous methods of overcoming these difficulties have been introduced: the tension of a rope around the posterior abdomen, raising the animal's hindparts on boards or on a truss of straw, or even casting her and raising her hind parts by means of a block and tackle hooked to a figure-of-eight rope around the hocks. Plenderleith (1980) described a method which is now in common usage amongst practitioners. The cow is placed in sternal recumbency with both hind legs pulled out behind her (weight therefore being taken on her stifles). The assistant sits astride the cow, facing the rear, and holds the cow's tail up vertically. This manoeuvre causes the slope of the vulva to be upwards. The veterinary surgeon kneels between the cow's hocks and supports the weight of the prolapsed organ on his or her thighs, prior to replacement.

Whether the cow is standing or recumbent, an epidural anaesthetic should be given. This will prevent straining, and also has the advantage that defaecation is in abeyance during the operation. The everted organ should be thoroughly washed with warm normal saline solution. If the fetal membranes are already partially detached and their complete removal can be carried out easily and without injury to the caruncles, this should be done. But when attachment is complete or when attempts at detachment are associated with haemorrhage, it is better that the organ be replaced with the membranes still adherent. The subsequent management of the retained fetal membranes should be on the principles outlined on p. 413.

The prolapsed organ should be palpated in order to detect the possible presence within it of a distended urinary bladder; if such is the case, it should be relieved by the use of a catheter. The uterus should be supported by assistants holding the corners of a towel beneath the mass or upon a piece of board about 1 m long covered by a clean cloth or towel.

Smyth (1948) describes the operation of replacement as follows: 'Having well soaked the hands, the operator commences to replace the uterus little by little, starting with those portions nearest the vulval lips. By gentle pressure, the nearest cotyledons are pushed into the vagina, taking care that the lips of the vulva remain well apart and do not become turned inwards. It is generally best to replace portions of the upper and lower surfaces alternately. When the last portions only remain to be replaced, an assistant should press against these, using the palms of both hands, while the operator endeavours to draw the lips of the vulva over the prolapse. As the mass disappears through the lips of the vulva the operator, using a clenched fist, should then continue to press it forward to the full length of the arm. It is important that the uterus should be pressed forwards beyond the cervical ring; to ensure this the operator locates the margins of the dilated cervix, draws them towards him- or herself and, if possible, at the same time pushes the uterus in a forward direction with the other hand. In some cases it may be found helpful to grasp the cervical ring at several points in succession and with a piston-like movement of the hand and arm insinuate the uterine mass through it.'

When this has been accomplished, the cervix should lie unoccupied at the level of the pelvic brim, and if the whole uterus has passed the cervix it will promptly regain its normal position. To ensure complete replacement of the uterus, 9–14 litres of clean warm water are delivered into the uterus by gravity feed and immediately removed by siphonage, the weight of water effacing any remaining inversion of the horn. To help restore uterine tone, and thus to prevent recurrence of the prolapse, oxytocin should be given. Preoperative treatment with oxytocin, although reducing the size of the prolapsed organ, increases the turgidity of the everted organ and makes replacement more difficult. Even if the animal shows no clinical signs of hypocalcaemia, calcium borogluconate therapy should be given, together with parenteral antibiotics.

A final advantage of caudal epidural anaesthesia is that for an hour or so after replacement of the organ straining will be prevented; the duration will be extended if xylazine is used as well. It has been customary to insert vulva sutures to prevent the possibility of re-prolapse. This practice is controversial; many consider that it serves no useful purpose since, if the prolapse has been replaced correctly, it should not recur. It may even stimulate the cow to strain, allowing the prolapse to recur within the animal and thus not be detected. Others consider that, provided the cow is re-examined 24 hours later and the sutures are removed, it can prevent the recurrence of a complete prolapse which will be much more difficult to replace a second time. In uncomplicated cases it is generally found that within 24 hours of replacement the degree of cervical contraction present is such that recurrence is very unlikely.

Amputation of the everted organ. This operation can be adopted as a last resort in those cases in which the uterus has undergone such severe changes that replacement of the organ must inevitably result in death and in occasional long-standing cases where it is found physically impossible to replace it because of the unfavourable texture of the organ. The prognosis is grave, and it is doubtful if it can be justified on welfare grounds.

OTHER SPECIES

Ewe (Figure 19.2)

The method of replacement is similar to that described for the cow, except that it is easier to perform because of the facility with which the hindquarters of the ewe can be kept raised by an assistant; caudal epidural anaesthesia should always be used except in those situations where a long delay may occur before it could be treated by a veterinarian. However, because of their different physical relationship to the caruncles, the fetal cotyledons cannot readily be detached and rather than damage the uterus by persistent attempts to separate them, it is preferable to leave them attached and return them with the uterus; failure to detach them at this stage will not significantly affect the prognosis. Anaerobic infection should be anticipated and prophylactic antibiotic used.

Fig. 19.2 Uterine prolapse in a ewe.

Mare

Aetiology

The disorder is relatively uncommon in this species. It is likely to be related to the expulsion of the fetal membranes which tend to separate from the endometrium much more readily in the uterine body but seem to be more firmly attached at the horns, particularly the tip. The consequence of this is that, as the uterine contractions during the third stage of labour persist to assist in the separation and expulsion of the fetal membranes, the pull on the attached membranes at these points cause the eversion of the tip or tips of the horns. The continual uterine contractions, and the subsequent straining as the mass of the fetal membranes enter the pelvis, cause the whole of the uterus to be inverted and prolapsed. Some interesting observations regarding this hypothesis for the aetiology were made at the Royal Veterinary College in mares which were subjected to elective caesarean operation at about 320 days of gestation. In three such cases, uterine prolapse occurred during expulsion of the fetal membranes, and gynaecological examination of them revealed that the fetal membranes had completely separated from the endometrium, except at the site of the hysterectomy wound, where it had become accidently attached to the uterus, or at the tip of the non-pregnant horn. It seemed that the weight of the separated and dependent portion had caused sufficient traction on the uterus to evert part of it and then, presumably, the mare strained to cause the prolapse. This is the reason why, before repairing the uterine incision during a caesarian operation, the allantochorion should be separated from the endometrium for some distance (see Chapter 20). Similar observations have been made in three cases of retained fetal membranes in which uterine prolapse occurred while the membranes were being removed, and was undoubtedly due to the traction applied to the allantochorion by the veterinarian. The eversion of the uterus caused at the point of attachment of the allantochorion was quickly converted into a prolapse when the mare strained. Therefore, it is suggested that in spontaneous cases of uterine prolapse an important causative factor is the weight of those portions of fetal membranes that are dependent from the vulva and the traction which it exerts on the uterus during the passage of a peristaltic wave along that organ. In view of these observations, it is important that undue traction on the detached portion of allantochorion should not be applied while the more anterior retained portion is being freed. This is also why the use of an oxytocin drip is the preferred method of treating retained fetal membranes (see Chapter 26).

Treatment

The approach is very similar to that described above for the cow. It is important to ensure that the mare is adequately restrained to prevent trauma to the prolapsed organ and to prevent injury to the veterinarian; sedation may be required. Caudal epidural anaesthesia is a requirement to prevent straining (see Chapter 12); in some cases where the mare is very fractious, general anaesthesia may be required, in which case the added advantage of elevating her hindquarters can greatly assist replacement. Before replacement, an attempt should be made to remove the fetal membrane but only if the allantochorion can be readily separated from the endometrium without causing haemorrhage; if this is excessive then as much as possible should be cut away before replacement. If the replacement is made under caudal epidural anaesthesia with the mare standing it is helpful to have assistants support the everted organ; as well as providing physical assistance, it also counteracts the effects of passive venous congestion.

The uterus should be replaced starting at the part adjacent to the vulva as described in the cow. The technique is easier than in the cow because of the absence of the caruncles; this tends to reduce the amount of haemorrhage. After replacement it is important to ensure that the organ is completely inverted; an intrauterine infusion of saline, with subsequent removal by siphoning, can be used, followed by the use of oxytocin to hasten involution. Systemic antibiotics as well as anti-inflammatory agents should be used and there is a relatively high probability of laminitis occurring. Vulval sutures should never be used.

Sow

The consensus of veterinary opinion is that pigs are unable to tolerate uterine prolapse, unless the uterus is replaced easily and quickly; frequently by the time help is summoned the sow will have died, due most likely to a fatal haemorrhage following rupture of the uterine vessels, or possibly shock. The degree of prolapse will vary from part of one horn at its simplest, to both horns at its most extreme.

The sow should be deeply sedated or preferably given a general anaesthetic (the methods are described under the caesarian operation in Chapter 20), and placed in an incline with the head facing downwards or suspended by her hind legs. If the uterus is traumatised, then euthanasia is preferable, particularly in commercial pig units because there will be a delay before she can be served by the boar and become pregnant. An alternative procedure which merits a trial is to ‘float’ the uterus back into the abdomen with the aid of water pressure. The sow is placed on her side, head downwards, on a slope and the end of a soft tube of rubber or plastic, of 2 cm diameter and 1.5 m long, is gently passed into the stoma of the prolapsed viscus and eased along as far as possible. Clean water is then allowed to flow into the prolapsed viscus. The weight of the introduced water gradually draws the prolapsed organ back into the abdomen; the tube is introduced further and more fluid infused. By this means the whole uterus is not only returned but completely replaced without manipulation. In the case of non-commercial pet sows, replacement can be attempted under general anaesthesia via a laparotomy as described below for the bitch.

Penny and Arthur (1954) have described post-oestral prolapse of the uterus in a gilt which was irreducible, despite abdominal taxis by means of a laparotomy.

Bitch and queen cat

A laparotomy can be performed, and with simultaneous external manipulation and abdominal taxis replacement can be attempted. It is more usual, however, to carry out 'external' hysterectomy on the prolapsed organ. The prognosis is favourable after amputation.

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20 The caesarean operation and the surgical preparation of teaser males

THE CAESAREAN OPERATION

The cow

The caesarean operation is a routine obstetric procedure in cattle practice which has high maternal and fetal survival rates and is less exhausting, speedier and safer than fetotomy (Parkinson, 1974; Cattel and Dobson, 1990). A prompt decision to perform a caesarean operation is important for optimum success (Dawson and Murray, 1992). The need for urgent intervention is indicated if there is evidence of fetal hypoxia as shown by hyperactive movements of the fetus and expulsion of the meconium, identifiable in the amniotic fluid. A successful prognosis depends on several factors:

- skill and speed of the surgeon
- duration of dystocia
- availability of skilled assistance
- surgical environment
- concurrent disease
- presence of a live calf.

Indications

The reasons for surgery include most causes of dystocia but analysis of published cases shows that the following six major indications account cumulatively for 90% of all caesarean operations:

1. fetomaternal disproportion
2. incomplete dilatation of the cervix
3. irreducible uterine torsion
4. fetal monsters
5. faulty fetal disposition (presentation, position or posture)
6. fetal emphysema.

In individual series, their relative frequency varies considerably depending primarily on the breed of cattle predominantly at risk, and to a lesser extent on whether fetotomy is routinely practised. If the birth canal is fully dilated, fetal causes of dystocia may be amenable to relief by fetotomy, but failure of cervical dilatation and irreducible uterine torsion are absolute indications for surgery. Non-surgical delivery may seem advisable if the fetus is grossly infected, but laparohysterotomy is often obligatory in such cases because of premature uterine involution, emphysema of the fetus or constriction of the birth canal.

The indications for a caesarean operation and the reasoning behind an appropriate decision have been extensively discussed (Cox, 1987; Pearson, 1996; Green, et al., 1999). The prognosis and cost should be discussed with the owner prior to surgery and preferably written, informed consent should be obtained.

Fetomaternal disproportion. Fetomaternal disproportion is consistently the most frequent overall indication for a caesarean operation in cattle. Four particular forms may be encountered.

Physical immaturity of the dam. In herds in which bull and heifer calves are kept together or where a bull runs with suckling cows, calves may conceive at an unexpectedly early age. It is not uncommon for heifers to be parturient at term at only 14 months of age and, in exceptional cases, at only 1 year of age. Even at 18 months of age, the maternal pelvis is still immature and usually too small for vaginal delivery.

Fetal oversize. The majority of cases of disproportion are animals that are mature and at normal term with a normally developed fetus. Among dairy breeds, the Holstein–Friesian is more susceptible to this form of dystocia during the first pregnancy than the Ayrshire or Jersey. Certain beef breeds are also frequently affected with fetomaternal disproportion and not only during the first pregnancy. Double muscling or muscular hypertrophy is well recognised in certain breeds such as the Belgium Blue (see Chapter 14).

The management of dystocia caused by feto-maternal disproportion depends largely on experienced clinical assessment of how much traction can safely be exerted without risk of serious birth canal trauma or, worse still, impaction of the fetus after only partial delivery. This is the most worrying of all obstetric problems to be encountered in cattle practice, with ample scope for errors of judgement, which may lead to death of the fetus and the dam. In many cases of oversize, the fetal head cannot be drawn into the maternal pelvic cavity and the decision to perform a caesarean operation is obvious. In others, traction is more effective and the decision is less clear. The difficulty lies in knowing when to abandon traction in favour of surgery. Excessive traction in such animals may merely exacerbate the degree of dystocia and compromise the success of an eventual caesarean operation. One practical guideline is that a caesarean operation is indicated if the head and both elbows, or both stifles in posterior presentation, cannot be pulled into the pelvic canal by traction by one person. However, even then, if the calf has a large chest or pelvis, subsequent obstruction can occur. The frequent finding in hospital referrals of fractured limb bones in oversized calves suggests either an unreasonable degree of traction or traction in a wrong direction. In heifers particularly, precipitate traction early in second-stage labour is to be avoided unless there is obvious dystocia, because the vestibule and vulva will not have relaxed sufficiently, and perineal damage is more likely to occur. If in doubt about the decision, it is probably better for the welfare of the cow and calf to perform a caesarean operation (Green et al., 1999). The deliberate adoption of breeding policies, which require caesarean delivery, is not justifiable in ethical terms.

It is not uncommon for several heifers in a group to require more than normal assistance or have to be delivered by caesarean operation. If the time interval permits, the premature induction of parturition in the later calving animals within 10 days of anticipated term may be of considerable benefit (see Chapter 6). Where an elective operation is required, it should be performed during the first stage of labour (see Chapter 6).

Fetal monsters and infection. The most extreme form of disproportion is sometimes encountered in fetal anasarca and achondroplasia, which greatly increases the cross-operational diameter of the fetus. Conjoined twins are also usually too large for vaginal delivery. Commoner than all of these, however, is emphysema due to secondary putrefaction, which frequently develops in protracted dystocia.

Postmaturity. A moderate prolongation of pregnancy up to 290 days, or thereabouts, is a normal feature of certain breeds, but in occasional animals of any breed, gestation may last for considerably longer, even beyond 400 days. Postmaturity results in continued fetal growth in utero, particularly of the skeleton. In such cases, dystocia at term is due not simply to fetal oversize, but also to inadequate relaxation of the birth canal.

Incomplete cervical dilatation. Incomplete dilatation of the cervix is a common cause of dystocia in cattle, but it should be diagnosed only after careful assessment of the findings on vaginal exploration. Cervical dilatation during the first stage of labour is a gradual process and the presence of a cervical rim is not in itself an indication of dystocia, provided that the fetal membranes are still intact. Care should be taken in such cases not to perforate the membranes unless the cervix remains undilated 2 hours or so later. Slow, or arrested, dilatation in multiparous cows may be associated with uterine inertia caused by hypocalcaemia; in these animals, the response to calcium therapy is rapid.

If, on initial or subsequent examination, the cervix is incompletely dilated and the membranes are already ruptured, with a fetal extremity presented against or through the cervix, or if the fetus is already dead, then further cervical dilatation is unlikely. If the cervical rim is shallow and membranous, or if it stretches sufficiently for the head to be drawn into the vagina, normal safe delivery may be possible. In these cases, irrespective of the degree of dilatation, the cervix is usually too thickened and indurated for vaginal delivery to be safely attempted, and further delay results only in fetal death and a greater risk of intrauterine infection.

The presence of an incompletely dilated cervix after the birth of one twin with the other fetus still in utero, often in a breech presentation, clearly indicates that the cervix constricts soon after becoming fully dilated. The frequent finding of faulty fetal disposition in cases of apparent failure to dilate may indicate that, in these cases at least, the cervix in fact is constricting and the dystocia is fetal rather than maternal in nature. Failure of the cervix to dilate or remain dilated is not uncommon in premature calvings and can result in the fetal head becoming trapped in the anterior vagina. Incomplete dilatation of the cervix is an important complication of uterine torsion. After manipulative correction of the torsion, the cervix is often only partially dilated and seldom dilates further (Pearson, 1971). In such cases, the cervical rim may be deep, but it is usually thin and stretches in response to traction on the fetus. Operation of the cervical rim in the midline dorsally during traction may allow safe vaginal delivery, but it should be remembered that the fetus (in cases of uterine torsion) is usually larger than normal and that a cervical incision may tear causing severe haemorrhage or uterine rupture.

Irreducible uterine torsion. Torsion of the uterus in cattle constitutes a major indication for a caesarean operation, either because the torsion is irreducible or because the cervix fails to dilate after correction. In most cases of postcervical torsion, the degree of cervical dilatation and vaginal twisting permits the introduction of a hand into the uterus for manipulation of the fetus, but if the torsion affects the cervical canal or uterine body, the fetus is totally inaccessible. Such torsions are an absolute indication for a caesarean operation. Uterine torsion differs from all other causes of dystocia in cattle in that one or both of the fetal membranes usually remain intact even if the placenta separates, unless they are deliberately perforated. The presence of fetal fluids thus protects the fetus and the uterus from infection; in this respect the condition carries a favourable prognosis. Torsion, however, may still have seriously detrimental effects on the uterus. Rotation through 360° is common, and two or three complete revolutions of the uterus sometimes occur. The greater the degree of uterine rotation, the greater the interference with venous circulation within the uterus and its mesometrial and mesovarian attachments. The combination of uterine displacement and oedematous swelling of its wall may well result in perforation of the uterine body, especially by the fetal head. In exceptionally protracted cases, a fetal extremity may impinge, through a uterine tear, on the urethra or segments of large intestine and cause rupture of the urinary bladder or gut.

In most cases of uterine torsion, the prognosis is excellent, but paradoxically the operation may be technically difficult, firstly because small intestine is usually displaced and impedes access to the uterus, and secondly because the presence of fetal fluids may make the uterus difficult to handle and impossible to exteriorise for suturing.

Fetal monsters. *Schistosoma reflexus* is by far the commonest gross structural defect in cattle (see Chapters 4 and 17). Occasional cases are born normally without assistance and others may be extracted with moderate traction. Most affected fetuses, however, cause dystocia because the characteristic angulation of the spine greatly increases the cross-operational diameter, although the body weight may be less than normal for the breed. The fetus is presented in one of two ways; its exposed viscera may protrude from the vulva or the limbs and head may lie in the vagina and can be felt to be attached to the misshapen trunk. The latter presentation may be confusing in cases in which the appendages are enclosed in an inverted pouch of the skin which is all that can be palpated (Figure 20.1).

Cases of *schistosoma reflexus* occur sporadically in several breeds, sometimes as twin to a normal fetus (Figure 20.2), and are often still alive at delivery. It is noticeable that they are seldom associated with protracted parturition, presumably because they cause obvious manifestations of dystocia. The dystocia can be relieved by either fetotomy or a caesarean operation. If hysterotomy is performed, longer-than-normal abdominal and uterine incisions may be necessary and care is essential in manipulating the fetus from the uterus in order to avoid uterine tearing, which easily follows excessive traction. This manoeuvre is usually facilitated by the lubricant effect of residual amniotic fluid. The prognosis after a caesarean operation is excellent, but the dam should not be rebred to the same sire.

Achondroplasia or bulldog calf deformity and anasarca or fetal dropsy cause dystocia due to the

Fig. 20.1 *Schistosoma reflexus*. Incised skin pouch enveloping the trunk, head and limbs.

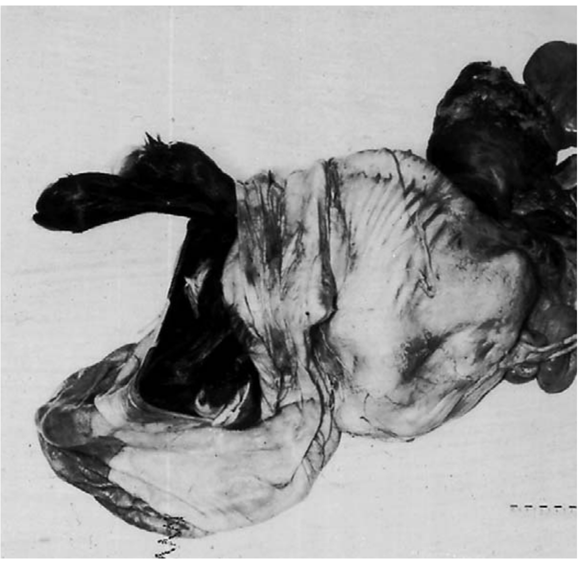

Fig. 20.2 *Schistosoma reflexus* twinned to a normal calf.

extensive subcutaneous accumulation of tissue fluids, which greatly increases the cross-operational diameter of the fetus and causes gross disproportion irrespective of the fetal body weight; the latter may also be considerably increased (see Chapter 4). Both defects may also be associated with severe fetal ascites, placental oedema and hydroallantois.

Lesions of the fetal central nervous system may cause muscle contracture of the limbs, which prevents normal extension in preparation for birth. Arthrogryposis, sometimes associated with torticollis and kyphosis, has been shown to result from viral infection of the dam during pregnancy and is also recognised as a genetic abnormality in the Charolais breed (Figure 20.3). Because the muscle contracture fixes limb joints in either flexion or extension, depending on the joint, the condition is sometimes called ankylosis, but the bones are not fused. Spina bifida is less common in cattle but causes similar contractures, usually of the hindlimbs only because the lesion is thoracolumbar in position. Fetal anencephaly and the deformity described as *perosomus elumbis* may also cause limb abnormalities. In most cases of muscle contracture, the musculature of affected limbs is palpably underdeveloped. The degree of contracture may be too severe for attempted delivery, but if the forelimbs in an anterior presentation can be brought into the vagina, traction may cause the flexed hindlegs to perforate the uterus below the pubic brim. Conjoined fetuses occur occasionally with varying degrees of fusion and generally require caesarean delivery unless only the head is duplicated.

Faulty fetal disposition. Provided that the cervix is fully dilated and remains so, most early cases of faulty fetal disposition can be corrected

Fig. 20.3 Fetuses with varying severity of torticollis and muscle contracture of the limbs.

manually or relieved by relatively simple fetotomy. However, the loss of fetal fluids followed by uterine contraction often makes these manipulations difficult and time-consuming and more likely to result in rupture of the uterus. In protracted cases, constriction of the cervix may prevent vaginal correction of the dystocia and the fetus is then likely to become emphysematous.

Fetal emphysema. Fetal emphysema is a frequent complication of protracted parturition in cattle and, irrespective of the primary cause of dystocia, it is often the immediate indication for a caesarean operation. Such cases should be assessed realistically before the operation is undertaken because fetal putrefaction can seriously influence maternal survival. Bacterial culture of such fetuses usually yields heavy growths of coliform, or coliform and clostridial organisms. The latter infection is associated with a high maternal mortality rate in the immediate postoperative period, probably because of endotoxaemic shock. On cursory examination, the clinical status of these cows may seem reasonable despite gross uterine distension; the pulse rate, however, is usually significantly raised and the animal noticeably quiet on handling. Such premonitory signs are likely to be followed, as soon as the uterus is incised, by the onset of rapidly deteriorating shock, which is sometimes fatal within 24 hours, despite intensive supportive therapy. Experience suggests that coliform infection alone is less serious than clostridial putrefaction, but preoperative differentiation is not possible. Despite the significant mortality rate in this group of cases, surgery is nevertheless worthwhile because there is usually no alternative, except for slaughter.

Miscellaneous indications. Occasionally, animals are encountered with full cervical dilatation and a normal-sized fetus, in which the caudal part of the birth canal is too constricted for delivery even after episiotomy (see Chapter 10). The condition is associated particularly with Friesian heifers which are sometimes older than is usual at the time of first calving. The natural termination of pathologically prolonged pregnancy may also be associated with absence of normal parturient changes in the vagina and vulva and a consequent need for a caesarean operation.

Abortion in late pregnancy sometimes requires treatment by a caesarean operation for several contributory reasons, such as incomplete birth canal dilatation, cervical constriction, fetal deformity and faulty fetal disposition. Such cases are uncommon, but they are nevertheless important because they may be associated with specific zoonotic infections.

Fetal mummification and *hydrops uteri* may now be treated initially by inducing parturition, but a caesarean operation may still be necessary if induction fails or the birth canal is insufficiently dilated for vaginal delivery. Neal (1956) described a two-stage caesarean operation for cases of hydroallantois. A left flank incision is made as for a normal caesarean operation. Once the uterus has been identified, large-bore sterile tubing is used to drain the allantoic fluid through a stab incision in the uterine wall and the tube is retained by a purse-string suture. The allantoic fluid is drained slowly, monitoring the pulse continuously; if the pulse accelerates, drainage is suspended for 10–15 minutes. When as much fluid as possible has been drained from the uterus, the tube is withdrawn and the purse-string suture closed. A routine caesarean operation is then performed, rather than waiting 24 hours as originally described (Cox, 1987).

Laparotomy is essential in cases of uterine rupture. If this disorder occurs as a preparturient complication, the fetus usually lies totally within the peritoneal cavity and may survive, if the cord is not twisted, until the placenta separates at term. More frequently, rupture occurs as a complication of dystocia, particularly of uterine torsion, or as a result of manipulation of a fetus which is oversized or has faulty disposition. Uterine rupture during parturition may result in considerable uterine haemorrhage and hypovolaemic shock.

Repeated dislocation of the sacrococcygeal articulation during assisted delivery in successive parturitions, or a healed pelvic fracture, can result in massive bony obstruction at the site and constitutes an uncommon indication for surgery.

Restraint, anaesthesia and preparation for surgery

A caesarean operation may be performed with the dam standing, or in sternal, lateral or dorsal recumbency. The choice depends on the surgeon's preference, demeanour of the animal and available facilities.

For standing surgery, the animal should be restrained using a halter, preferably in a calving pen, tied such that the animal's right flank is against a wall and the head is in the corner, in order to limit movement during surgery. The halter should be tied with a quick-release knot in case of recumbency. Nose bulldogs are often required for additional restraint. Sedation should be avoided if possible because it can cause recumbency during surgery and may be detrimental to fetal survival. If sedation is necessary, xylazine is commonly used (0.05–0.1 mg/kg intramuscular or a reduced dose intravenously; however, the latter is not a licensed route of administration in the UK). Unfortunately, xylazine is an ecbolic, making surgery more difficult, and can cause ruminal bloat, which can obstruct the surgical wound. A rope can be attached to the right hind leg above the fetlock and laid underneath the animal's body so that if the cow becomes recumbent during surgery, the rope can be pulled to enable the animal to lie in right lateral recumbency. The tail is tied out of reach of the operative site, usually to the halter or to the right hock.

Alternatively, surgery may be performed on the recumbent animal; this is particularly indicated in fractious animals. If the cow is not already recumbent, a sedative can be administered (xylazine 0.2 mg/kg intramuscular) or the animal cast using a rope. The animal should be placed in right lateral or semi-sternal recumbency with the body slightly tilted to the right. Bales of straw may be used to prop the cow in a stable position for surgery. In addition, the legs may be hobbled and some surgeons prefer the left hind leg to be extended caudally and fixed by a rope.

Two or more assistants are usually required for successful surgery: as a minimum, one to restrain the cow and one to deliver the calf. Communication with the assistants by the surgeon is important. Briefly describe how the surgery will be performed, and outline the role of each assistant and how to proceed in the case of a crisis such as recumbency of the cow during surgery.

The location for surgery should be selected carefully with the objectives of ensuring good hygiene, lighting, facilities for restraint and a suitable floor surface. Avoid performing surgery in buildings occupied by large numbers of other cattle. Ideally, use a clean calving pen or other unoccupied building. Clean bedding should be provided, although vigorous shaking of straw will cause unwanted clouds of dust. Lighting should be provided that illuminates the desired surgical site. The surgeon should ensure that the light is not placed such that the surgical site is in the shadow cast by the surgeon; equally, the light must not shine in the eyes and distract the surgeon. Many veterinarians carry a portable halogen lamp and stand, for use on the farm; alternatively, one solution is for the surgeon to wear a head-torch.

Facilities for restraint should be appropriate for the size of animal and designed to avoid injury to animals or humans. A ring fixed in the wall of a calving box and offset 50 cm from the corner is ideal; if the animal goes down the offset ring encourages the animal to lie on the right-hand side. The floor surface should provide adequate friction for animal and surgeon. Slippery concrete floors can lead to accidental falls during surgery. A 20–30 cm-thick base of sand with clean straw on top provides an ideal surface. Facilities for the calf should also be prepared at this stage; a warm, straw pen complete with resuscitation equipment would be ideal.

Anaesthesia. The choice of anaesthetic method varies between surgeons and the selected surgical site. For flank incisions, paravertebral anaesthesia of the nerves associated with the transverse processes of T13, L1, L2 and L3 is indicated. Each site is infused using 20 ml of 2–3% lignocaine with adrenaline; 12–14 ml to block the ventral nerve branches, 6–8 ml for the dorsal branches (Cox, 1987). Signs of successful anaesthesia are a warm, hyperaemic and flaccid flank with no response to pain when tested with an 18 gauge × 1.5 inch needle. The advantage of paravertebral anaesthesia is that the whole flank musculature is desensitised and flaccid, which facilitates exploration of the abdomen during surgery and closure of the wound. Also the flank incision can be extended readily if necessary during surgery. One disadvantage is that the technique is more difficult to perform than other methods. In addition, the cow may be unsteady after surgery due to loss of lumbar muscle tone and paresis of the ipsilateral hindlimb. Finally, the vasodilatation in the muscle layers causes a greater degree of haemorrhage that requires careful haemostasis.

A local anaesthetic line block or inverted-L block of the flank is an alternative to paravertebral anaesthesia. An 18 gauge × 1.5 inch needle is used to administer 2% lignocaine with adrenaline at several sites; the number of sites is dependent on the length of the proposed incision. At each point, 5 ml of local anaesthetic is injected subcutaneously in each direction of the incision line, and a further 10 ml into the musculature. The technique is quick and reliable, and requires minimal training. However, the parietal peritoneum may not be effectively anaesthetised, causing reaction by the patient when it is incised. Sloss and Dufty (1977) reported particular problems of inadequate analgesia with an inverted-L block in fat animals. A similar reaction will occur if the incision has to be extended during surgery to extract the calf. Furthermore, because the flank is not flaccid, apposition and suturing of the muscle layers can be difficult, and there may be an adverse effect on wound healing.

Epidural anaesthesia using lignocaine can provide adequate anaesthesia of the flank, although such anaesthesia also tends to cause recumbency, which may be prolonged in cattle. Caulkett et al. (1993) reported that epidural anaesthesia using 0.07 mg/kg xylazine produced good analgesia for caesarean in 45% of cases without severe ataxia. However, there is prolonged time to onset of anaesthesia and it was not effective in 17% of cases.

Preoperative preparation. Preoperative antibiosis is strongly recommended (Cox, 1987). Commonly, 10 mg/kg each of an antibiotic mixture of procaine penicillin and dihydrostreptomycin is administered intramuscularly. Tocolytic agents, such as the β-adrenergic agonist clenbuterol hydrochloride (30 g) administered by intramuscular or slow intravenous injection, are widely used and can facilitate exteriorisation of the uterus during surgery; furthermore, they counter the effect of xylazine on the uterus. A caudal epidural injection may be administered to reduce straining (see Chapter 9). Unfortunately, severe tenesmus occasionally fails to respond adequately to epidural anaesthesia.

A wide surgical field should be prepared. Initially, dirt and dust should be brushed from the flank and back of the animal before the operative field is clipped or shaved. In the case of a flank incision, the entire flank should be clipped from the transverse processes dorsally to the milk vein ventrally, and from the caudal ribs to the hind leg, level with the tuber coxae. The skin should be prepared using a surgical scrub (7.5% povidone-iodine or 4% chlorhexidene gluconate solution) followed by surgical spirit. Sterile drapes should be applied; in the standing animal a large single drape with a suitable window can be placed over the back of the cow and down the flank. A useful alternative in the field is to use a wide roll of plastic film wrapped around the cow's body leaving only the surgical site exposed.

Surgeons and assistants should wear protective surgical scrub suits, even in the field situation. Furthermore, consideration should be given to wearing sterile surgical gowns with long-sleeved plastic gloves (with the finger tips cut off, and the gloves held in place with elastic armbands) and surgical gloves. Surgical gloves are particularly important for those veterinarians who do not wear protective gloves for foot trimming and other work in cattle practice that causes gross contamination of the hands.

Operative technique

The adequacy of anaesthesia should be carefully tested prior to surgery because the muscle and peritoneum may remain sensitive despite skin desensitisation. Left flank incision is the most common technique and is most appropriate for the standing animal. The surgeon has to judge whether the animal will remain standing during the procedure; if not, recumbency prior to surgery should be induced. One advantage of the left flank incision is that the rumen can be used to prevent exposure of the intestines. However, in individual cases a large rumen, particularly if the animal is straining, can interfere with surgical access to the abdomen. Another advantage of the flank incision in the standing animal is easier correction of uterine torsion. Finally, wound dehiscence is more manageable in the flank, compared with lower abdominal incisions.

A vertical skin incision is made in the middle of the left flank starting 10 cm ventral to the transverse processes and extending approximately 30–40 cm long. Alternatively, a slightly oblique incision from caudo-dorsal to cranio-ventral, about 30° from vertical can be used, starting 10 cm from the tuber coxae. The advantage of oblique incision is that the internal abdominal oblique muscle can be split along its fibres and there is improved access to the genital tract (Cox, 1987). Potential disadvantages are incision of the circumflex iliac artery if the incision is extended too far caudo-dorsally and lack of anaesthesia if too far cranio-ventrally, when using a paravertebral anaesthetic. If the breed of dam or other indication for surgery suggests that future elective caesarean operations may be necessary, the first incision should be made at the cranial border of the flank, thus allowing for subsequent incisions more caudally (Figure 20.4).

A ventrolateral incision is particularly indicated for the removal of an emphysematous fetus. The cow should be in right lateral recumbency. An oblique incision, starting from the flank fold dorsal to the attachment of the udder, is continued cranially, parallel to the ventral border of the ribs. The advantage of this approach is that it gives good exposure of the uterus, even when it is friable, and it minimises the risk of uterine contents contaminating the abdominal cavity. However, repair of the abdominal muscle layers can be more difficult if the muscles are under tension and sutures may tear through the tissues. A surgical drain may be inserted during repair of the wound, particularly if a dead fetus was delivered (Figure 20.4).

A midline or paramedian incision is not commonly used in the field because general anaesthesia or heavy sedation is required and respiratory function of the dam is compromised. However, the technique gives excellent access to the uterus.

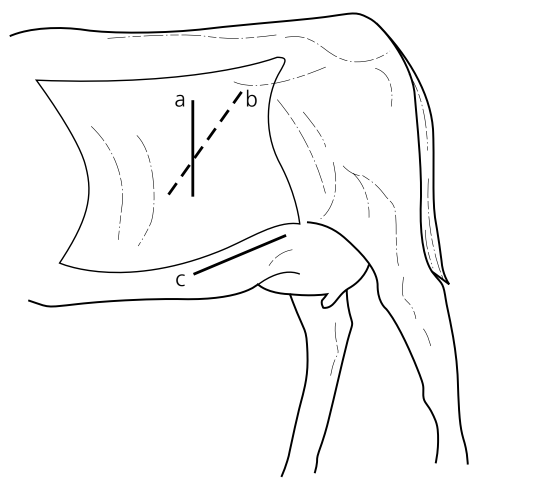

Fig. 20.4 Incision sites for caesarean operation: left flank – standard, vertical incision used in the standing or recumbent cow (a); left flank – alternative, oblique incision used in the standing or recumbent cow (b); low left flank – used in the recumbent cow, and particularly suitable for extraction of an emphysematous fetus (c).

A non-absorbable suture should be used for repair of all muscle layers of the incision because post-operative wound dehiscence has severe implications, including herniation (Figure 20.4).

A right flank incision is uncommon; however, it is indicated if the left flank approach is obstructed by adhesions as a result of previous surgery. Access to the uterus is good, but the small intestines are difficult to retain within the abdomen and they interfere with the surgery.

With left flank approach the following muscle layers are incised: cutaneous, external abdominal oblique, internal abdominal oblique and the transverse abdominal muscle. They are incised using a scalpel, unless the fibres can be split parallel to the skin incision. Haemorrhage from the muscle layers is usually minimal; however, when large vessels are involved, haemostats should be applied and the vessel ligatured if necessary. The peritoneum is incised using a scalpel, taking care not to puncture the rumen which lies immediately beneath the peritoneum (Figure 20.5). Entry into the peritoneal cavity is usually signalled by the sound of air entering the potential space. The incision can be extended using scissors, rather than a scalpel, to reduce the risk of cutting abdominal organs. Often, a variable amount of peritoneal fluid, sometimes blood-tinged, is immediately apparent in the abdominal cavity. Greater volumes are present in cases of prolonged dystocia, uterine infection, torsion or rupture. Additionally, in the case of uterine torsion or uterine infection, there may be large fibrin clots present in the abdomen. In cases of uterine torsion, the small intestines may also be displaced to a position immediately caudal to the rumen to such an extent that loops may spill through the abdominal incision.

The surgeon should explore the abdomen and note the disposition of the calf. The uterus should be exteriorised by grasping and applying traction to a distal extremity of the calf, usually the hindleg. To aid exteriorisation of a hindlimb the calf's foot can be held using the surgeon's right hand and the hock with the left hand, so levering the foot up through the incision. Often, it is then possible to lock the hock into the ventral aspect of the skin incision, whilst the foot is retained by the flank above the dorsal aspect of the incision, so relieving the tension on the surgeon's arms. Exteriorisation

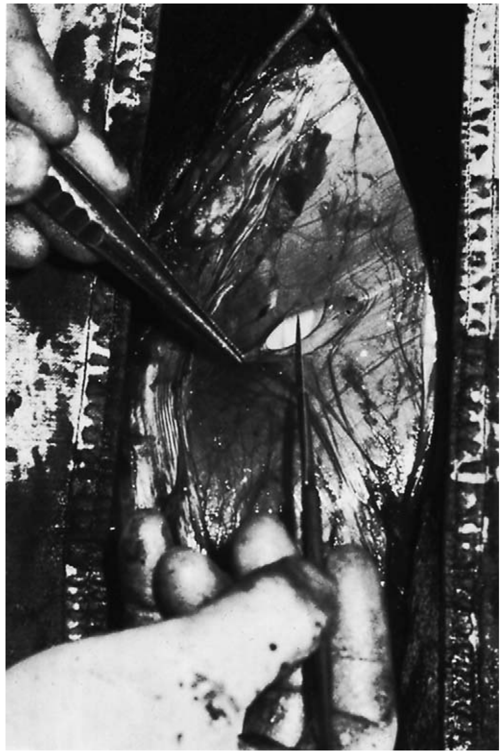

Fig. 20.5 Incision of the peritoneum.

of the uterus prior to incision is a critical step in the subsequent success of the surgery (Figure 20.6). However, traction on the uterus may require considerable strength and tenacity on the part of the surgeon. Manipulation of the uterus also causes stretching of the mesometrium and can cause pain manifested by grunting and the cow displaying other signs of discomfort. Furthermore, once the uterus has been handled the myometrium often contracts making exteriorisation more difficult, unless a tocolytic has been administered prior to surgery.

If the calf is in the right uterine horn, it will be necessary for the surgeon to rotate the uterus along its longitudinal axis to bring the calf's limbs to the flank wound. Rotation can be achieved by traction on the leg with the left hand, whilst pushing the dorsal aspect of the uterus away from the surgeon with the flat of the right hand. A similar technique can be attempted, if the indication for caesarean operation was irreducible uterine torsion, to correct the torsion before incision of the uterus. However,

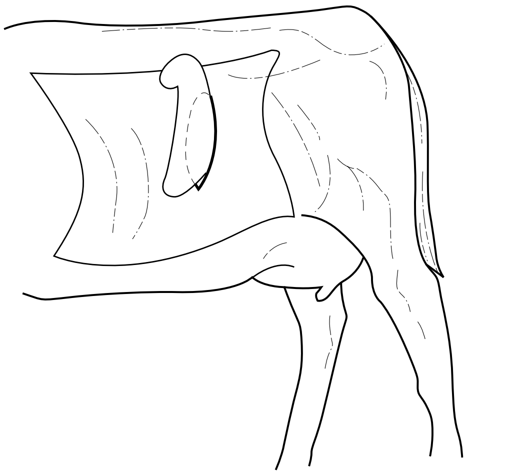

Fig. 20.6 Exteriorisation of the fetal hock through the cow's abdominal wound, prior to incision of the uterus.

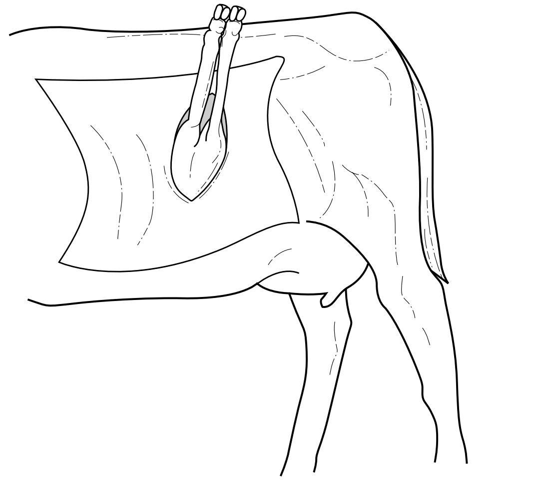

Fig. 20.7 Removal of the calf in anterior presentation through the cow's flank incision. Initial traction is dorsal and lateral.

the uterine wall in these cases is often oedematous and friable; care must be taken in order to avoid digital penetration of the wall.

The uterine wall is incised over the calf's leg from toe to hock along the greater curvature and parallel to the longitudinal muscle layers of the myometrium. The incision can be made using a scalpel or scissors. If the incision in the uterus is too short, the uterus may tear uncontrollably during extraction of the calf. If the incision extends too close to the cervix, suture repair may be difficult. Care should be taken to avoid incising the calf, particularly if fetal fluids are sparse. In addition, the surgeon should avoid incising cotyledons, which can lead to profuse haemorrhage. If an incision of the uterus has to be made within the abdominal cavity, often because the uterus has become friable and liable to damage by further handling, then a Roberts' embryotomy knife can be used (see p. 268). However, such an incision leads to gross contamination of the abdomen with fetal fluids which are unlikely to be sterile, particularly if dystocia is the indication for surgery. Furthermore, incision within the abdomen is often made more difficult by bouts of straining by the dam.

The allantochorion and amnion are ruptured manually, and the calf's fetlocks grasped by the surgeon, exteriorised and passed to an assistant (Figure 20.7). Alternatively, sterile calving ropes or chains may be attached. Initially, in the case of forelegs, both the legs and the head should be exteriorised by the surgeon. Then the calf is extracted by assistants whilst the uterus is held by the surgeon. Initial exteriorisation of the hindlimbs is done dorsolaterally and then caudally, once the calf's pelvis emerges, such that the calf is rotated and removed in a similar way to per vaginam delivery of a calf in posterior longitudinal presentation (Figures 20.8 and 20.9). Delivery of a calf in anterior presentation through the abdominal incision is similar to that for a normal anterior longitudinal presentation. A finger and thumb grip in each orbit is often helpful in bringing the head through the uterine and abdominal incision. On occasions, the fetus may lie so far within the vagina such that retropulsion per vaginum by an assistant is sometimes necessary, after careful washing and lubrication of exteriorised parts of the calf. The fact that many cows urinate immediately after removal of a presented fetus, suggests that urine has been retained because of urethral compression.

The emphysematous fetus presents unavoidable risks of peritoneal contamination, not least because its hair and hooves may already have been shed. In such cases, incision of the uterus is often followed immediately by the escape of gas and fetid fluid; parts of the fetus may be grossly swollen and crepitate on handling. The uterine wall is often

Fig. 20.8 Anticlockwise rotation of the fetus before delivery.

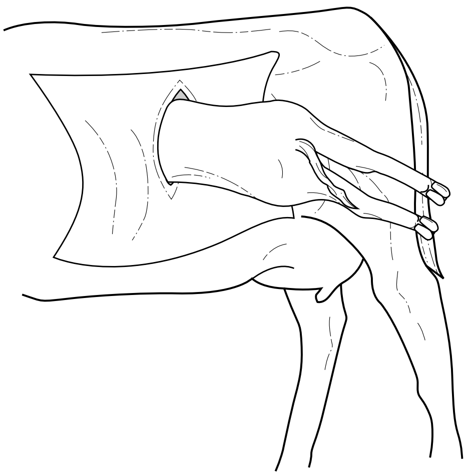

Fig. 20.9 During delivery of the calf's hips the body is rotated and traction is directed caudally and laterally.

tightly stretched, and intrauterine manipulation can be difficult. Flank and uterine incisions of adequate length are therefore essential. Such a fetus often requires considerable traction, not only on limb snares but also with sharp or blunt hooks applied in the orbits and at appropriate points on the trunk or upper limbs to secure additional purchase. It may be necessary to incise deeply at several sites over the thorax and abdomen to release gas, and sometimes partially to eviscerate the fetus, before removal is possible. Incision of the fetal abdominal wall may also be necessary where there is ascites. In rare cases, the fetus simply cannot be removed from the uterus because it is impossible to make a uterine incision of adequate length; in such animals, fetotomy may be attempted. After the removal of a severely emphysematous fetus, the uterus is often noticeably ischaemic, of cardboard-like consistency and totally atonic.

A live calf should be immediately attended to by an assistant, whilst the surgeon examines the uterus, initially for the presence of a second fetus. In addition, any lacerations of the uterine wall should be noted and repaired. The fetal membranes are removed if they can be readily detached, which is uncommon. Otherwise, they are returned to the uterine lumen and any protruding tissue trimmed so that it is not incorporated in the suture line of the uterine incision. This approach is justified on two grounds. Firstly, it should be assumed that if the fetal membranes can physically be separated, they will be expelled naturally and more completely by uterine contractions. Secondly, if deliberate detachment of the fetal membranes is attempted before they would normally separate and be expelled, then there may be haemorrhage or incomplete removal either of microvilli or of larger masses of placental tissue. It is common practice to place antimicrobial pessaries in the uterine lumen before repair of the hysterotomy wound, but the value of these is questionable. If the fetal membranes are subsequently expelled naturally, so too are the pessaries. If they are retained, then the antimicrobials can have no more than a minimal local action in the lumen and are probably ineffective in controlling deep infection.

The edges of the uterine incision are inspected for haemorrhage, particularly from the cotyledonary vessels. It is advisable to exteriorise both uterine horns before the genital tract begins to involute, which will facilitate inspection and repair of the wound. Large vessels that are haemorrhaging should be ligatured. The uterus is supported by an assistant or held using uterine forceps (Figure 20.10) and the incision is sutured using 6–8 Metric catgut or polyglactin 910. Catgut has advantages over synthetic suture materials particularly when the uterus is friable because the latter have a 'cheese wire' effect. However, catgut causes greater tissue reaction and thus is more likely to produce adhesions. Preferably, a large round-bodied needle and suture material for the uterus should be prepared prior to surgery. Suturing should start at the cervical end of the uterine incision because if the uterus starts to involute the cervix retracts into the abdomen before the ovarian extremity. A variety of suture patterns have been employed; all are continuous inverting patterns with the objective of creating a water-tight seal by apposing serosal surfaces, whilst causing the minimum of subsequent adhesions and uterine scarring. The Utrecht method (Figure 20.11), a modified Cushing pattern, is started using a buried knot and then a continuous interlocking, inverting pattern. The advantage of this pattern is minimal adhesion formation following surgery. A single layer is usually sufficient, and this pattern is particularly efficient if the uterine wall is flaccid during repair of the wound. Alternatively, a Lembert suture pattern can be used with the needle passing at right angles to the incision, or a Cushing pattern, where the needle passes parallel to the incision. Many surgeons oversew the first suture with a second continuous pattern, particularly if the uterus is friable where the suture material can tear through the tissues. Great care should be taken to avoid the fetal membranes being incorporated in the uterine repair.

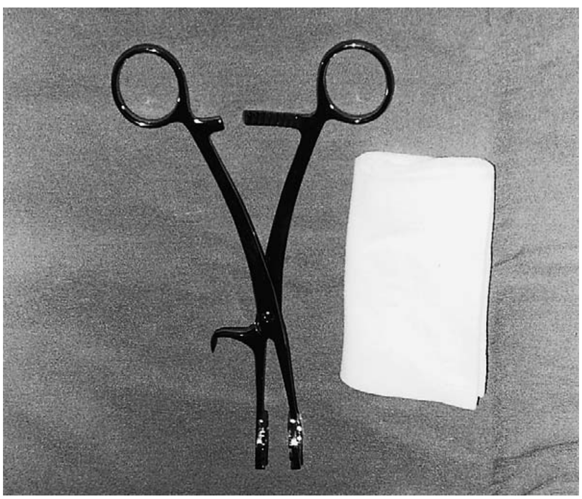

Fig. 20.10 Uterine clamps, or forceps, for an assistant to hold the uterus whilst the caesarean incision is closed by the surgeon.

Once the uterine incision has been repaired, the surface should be cleaned with sterile gauze and/or Hartman's solution to remove blood clots and other debris and returned to its correct location within the abdomen, ensuring that there is no torsion of the genital tract (see Figure 20.12). Oxytocin (20–40 i.u.) may be administered intramuscularly to hasten uterine involution at this point. The administration of water-soluble antibiotic, such as crystalline penicillin, within the abdominal cavity is recommended by some surgeons, but not others (Cox, 1987). However, metronidazole should not be used because it is prohibited in food-producing animals in Europe, despite being recommended by some surgeons (Dawson and Murray, 1992). The peritoneal cavity should be closed as quickly as possible to reduce the chance of bacterial contamination. The abdominal flank incision should be repaired in three layers: peritoneum and transverse abdominal muscle, internal oblique muscle and external oblique muscle. A continuous suture pattern is used, starting at the ventral commisure of the incision for the first layer. Care is taken to appose the peritoneum and transverse

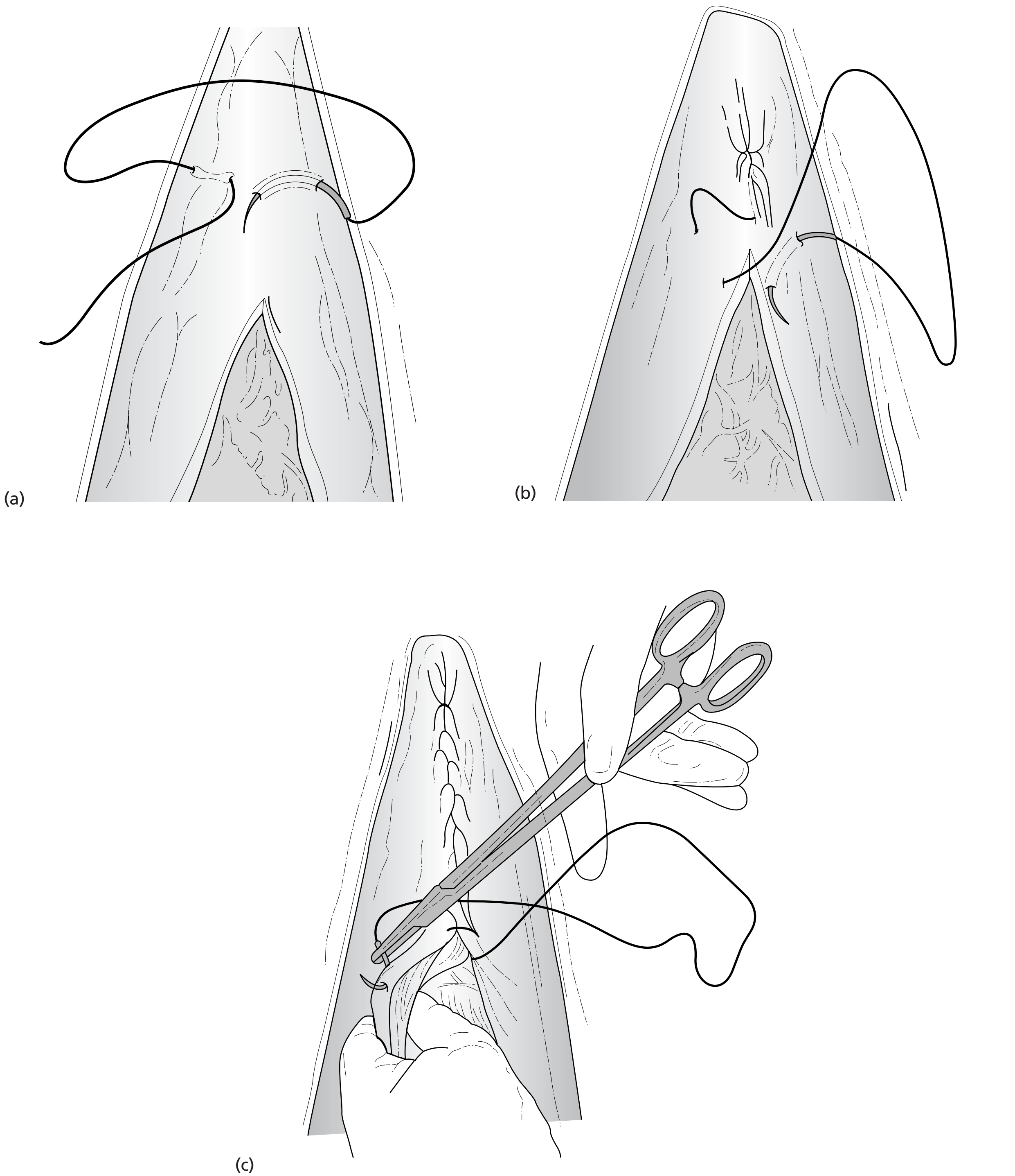

Fig. 20.11 Utrecht uterine suture technique. (a) The initial knot is buried in a fold of uterine wall. (b) The round-bodied needle is directed at an angle oblique to the incision. (c) This suture pattern produces an interlocking water-tight repair. (Adapted with permission from *Techniques in Large Animal Surgery*. AS Turner and CW McIlwraith. Williams and Wilkins)

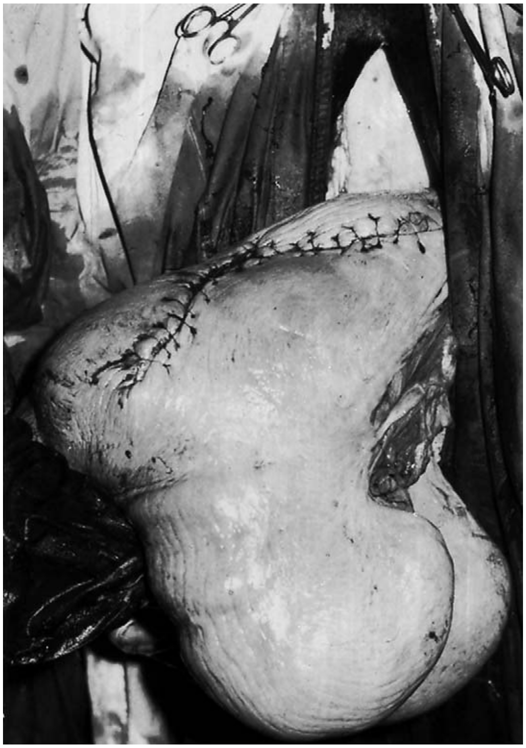

Fig. 20.12 The hysterotomy repaired with a continuous inversion suture of polyglycolic acid.

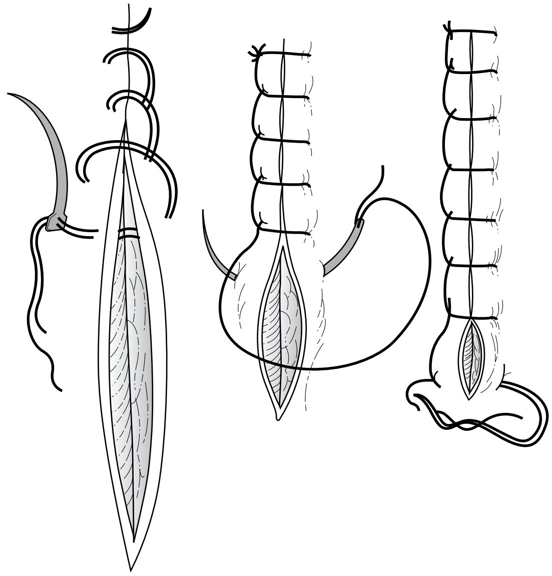

Fig. 20.13 The skin is sutured using a Ford interlocking pattern. (Adapted from *Techniques in Large Animal surgery*. AS Turner and CW McIlwraith. Williams and Wilkins)

abdominal muscle to avoid leakage of air from the abdominal cavity into the muscle layers following surgery (Sloss and Dufty, 1977). Air leakage is less likely following surgery on a recumbent animal, because less air is sucked into the abdomen during surgery. The amount of air within the abdomen can be reduced by assistants compressing the ventral abdomen and flank immediately prior to closure of the dorsal aspect of the peritoneal incision. Sutures should be placed approximately 1 cm apart using 6–8 Metric catgut. To reduce potential dead space between the muscle layers of the flank, deeper bites with the suture can be made periodically into the deeper muscle layers. Antibiotics may be infused between each muscle layer; approximately 250 mg/ml each of procaine penicillin G and dihydrostreptomycin as a mixture is commonly used. The skin is repaired using 5–7 Metric sheathed multifilament nylon in a Ford interlocking pattern (Figure 20.13). A single simple suture may be included at the dorsal and ventral aspects of the wound to allow drainage and/or flushing in the case of wound infection. Alternative suture patterns include a horizontal mattress or cruciate suture.

Postoperative care

Calf. The calf should be dried and the navel dressed with antiseptic immediately after delivery. Once surgery is completed, 2–3 litres of colostrum from the dam should be administered to the calf using an oesophageal feeding tube, if necessary. The dam should be introduced to the calf promptly, particularly in the case of a suckler cow and calf, to form a maternal bond.

Dam. The wound should be cleaned following surgery and the teats and udder examined. Oxytocin (20–40 i.u.) should be administered intramuscularly to stimulate further uterine involution. In addition, calcium borogluconate should be administered intravenously to mature dairy cows to prevent hypocalcaemia and facilitate uterine involution. A non-steroidal anti-inflammatory agent should be considered, at least in cases of animals that have had severe dystocia, uterine torsion or uterine infection prior to surgery. If there is evidence of surgical shock, intravenous fluid therapy is indicated; 2–3 litres of hypertonic

(7.2%) sodium chloride are particularly effective. Antibiotic should be administered for an appropriate period, usually 3–5 days, or until the fetal membranes are expelled.

The dam is often re-examined 24–48 hours after surgery and particular note of the rectal temperature, demeanour, appetite and faecal consistency should be noted. The faeces are often dry and the cow mildly constipated following surgery. Pyrexia, depression, inappetence and diarrhoea may indicate peritonitis. If the fetal membranes have been retained, appropriate treatment should be instituted.

Skin sutures are removed 3 weeks after surgery. In addition, a postnatal examination of the genital tract can be performed at this time because endometritis is more common following caesarean operation. Insemination should be delayed until >60 days postpartum.

Success rates and complications of surgery

Fetal survival following caesarean operation partially depends on the indication for surgery. However, Barkema et al. (1992) reported a calf mortality rate of 12% following casesarean operations, compared with 5% for control calvings.

Maternal survival rates following caesarean operation are high; most surveys report 90–98% dam survival (Dehghani and Ferguson, 1982; Cattel and Dobson, 1990; Dawson and Murray, 1992). In a series of 1134 operations performed principally for dystocia, Pearson (1996) reported a 88% maternal survival rate, despite the fact that 37% of calves were dead at the time of surgery. Furthermore, 80% of cows survived even when an emphysematous fetus was present (Vandeplassche, 1963). However, there are several complications reported following a caesarean operation.

Subcutaneous emphysema. Air often leaks from the abdominal cavity into the subcutaneous tissues and muscle layers following surgery if the peritoneum is not closely apposed, causing emphysema (Sloss and Dufty, 1977). The condition is more common in animals that have tenesmus after surgery, usually as a consequence of dystocia, and can extend as far as the shoulders in some cases. Although unsightly, it has no significant detrimental effect on the animal and treatment is not required. Dependent on the volume of air, the tissues return to normal in 1–8 weeks.

Metritis and retained fetal membranes. Dystocia, twins, uterine torsion and fetal monsters are common indications for a caesarean operation; the procedure itself predisposes to retained fetal membranes. Removal of the membranes during surgery is rarely possible. However, if they are retained more than 24 hours after surgery, gentle attempts at removal can be made daily by exploration of the vagina only. Intrauterine and intramuscular antibiotic can be administered, and once the membranes have been expelled, gentle lavage of the uterine lumen with 5 litres of warm, normal saline can be administered using a sterile widebore tube.

Peritonitis. Diarrhoea, pyrexia, inappetence and abdominal pain are the common presenting signs of peritonitis following a caesarean operation. Fortunately, the omentum and/or the use of antimicrobial therapy often limit the peritonitis. However, in many instances there are recurrent cycles of peritonitis and healing leading to formation of extensive adhesions and chronic weight loss.

Inadequate repair of the uterine incision, particularly in the presence of a metritis, is the principal cause of postoperative peritonitis. However, in some cases, the peritonitis may already exist at the time of surgery. The incidence is increased in the case of a dead or emphysematous fetus, after severe dystocia, rupture of the uterus or presence of a fetal monster, and after spillage of uterine fluids into the abdomen during surgery.

A variety of treatments have been suggested including parenteral antibiosis, intra-abdominal administration of antibiotic through the right flank, surgical lavage of the peritoneal cavity and intravenous fluid therapy.

Wound dehiscence. As many as 6% of animals may have complications related to dehiscence, abscess or seroma formation around the abdominal incision (Dehghani and Ferguson, 1982). Predisposing factors for wound dehiscence include inadequate asepsis, low abdominal incisions, trauma to tissues during surgery, environmental contamination, tenesmus and a poor temperament of the animal after surgery. In addition, removal of skin sutures too early after surgery can lead to the incision line opening up; 3 weeks is a minimum period.

Serum-like fluid occasionally accumulates at the ventral aspect of the wound between the muscle layers if the dead space is not occluded and will resolve spontaneously or can be drained surgically.

In other cases, there may be formation of an abscess. In most instances, this can be lanced, drained and irrigated as a granulating wound and second intention healing will follow. Antibiosis may be necessary in some cases if there is pyrexia.

Nerve paralysis. Animals that are recumbent during surgery have the risk of temporary or permanent peroneal nerve injury. In addition, a number of cows may have sustained trauma to the obturator nerve during dystocia prior to caesarean operation.

Fractures. The dam may sustain a fracture whilst attempting to rise after surgery. However, more common is a long-bone fracture or growth-plate separation of the calf during attempts to correct dystocia prior to caesarean operation.

Postpartum haemorrhage. Haemorrhage from the abdominal incision is usually limited, although dependent on the haemostatic concern of the surgeon. However, haemorrhage from the uterine incision can be considerable and in some cases fatal, if the cotyledonary vessels are disrupted. Occasionally the haemorrhage may be minimal at surgery, but may progress in the 24 hours following operation. Furthermore, in sporadic cases the large vessels in the broad ligament may be damaged causing considerable blood loss. Prevention is by careful incision of the uterus, supporting the genital tract adequately during surgery, and attention to haemostasis. Treatment of severe haemorrhage is by a blood transfusion. In addition, 20–40 i.u. oxytocin may be administered repeatedly to stimulate uterine contraction in an attempt to reduce uterine haemorrhage.

Blackleg. Dehghani and Ferguson (1982) reported that 0.5% of cases died suddenly within 24 hours of surgery as a result of blackleg with lesions located distant from the operative site.

Postoperative fertility

Postoperative productivity implies not only the maintenance of bodily condition and an acceptable level of lactation, but also the ability to conceive again and sustain a developing fetus to term. Numerous data are published on fertility rates after a caesarean operation but their significance is qualified by the fact that many animals are culled without being inseminated or served again. In 10 such series, the percentage of cows that subsequently conceived postoperatively ranged from 48 to 80%, with a mean value of 72% for 2368 animals, compared with 89% after normal calvings (data cited by Boucoumont et al., 1978). Vandeplassche et al. (1968) reported that 60% of 1857 cows and heifers that had a caesarean operation were subsequently inseminated, and 74% of these eventually conceived with an average of 1.8 inseminations per conception. However, there was an increased incidence of abortion, hydrallantois and failure of the cervix to dilate at the next parturition, probably due to scar tissue in the uterine wall. Although the calving interval is increased in cows following a caesarean operation compared with normal calvings, the principal cause of economic loss is the higher culling rates (Barkema et al., 1992). Interestingly, in the latter study the calving-to-first insemination interval was similar between caesarean and control cows, but the calving-to-conception interval was 18 days longer. Reduced fertility may occur as a consequence of increased incidence of retained fetal membranes and endometritis, uterine adhesions that hinder involution and adhesions that affect the ovary or uterine tube, and reduced endometrial tissue competence. In addition, there is an increased frequency of abortions during subsequent pregnancies, possibly as a result of scar tissue formation within the uterine wall limiting expansion of the uterus and/or nutrition of the fetus.

The mare

Because it is not often necessary, the caesarean operation in the mare is still widely regarded as a serious and, by inference, dangerous. In fact, the mare tolerates this surgical interference as well as most other species and the generally good recovery rate after a caesarean operation has largely disproved the myth that the horse's peritoneal cavity is exceptionally vulnerable to infection or the development of dangerous postoperative adhesions.

There is, however, little doubt that increasing familiarity with modern techniques for inducing and maintaining general anaesthesia has greatly improved the chances of maternal recovery. Even in specialist equine hospitals in areas of high stud density, the caesarean operation is not a common procedure. In recording the results of a series of 71 operations performed at Ghent, Vandeplassche et al. (1977) also comment that they carry out 15 fetotomies for every caesarean operation. Nevertheless, there are now a substantial number of reports of successful operations, many performed in the field under less than optimum conditions.

If the foal is alive, the operation should be performed with minimum delay. If the fetus lies in the maternal pelvic canal, it suffers fatal anoxia because of dehiscence of the allantochorion within 1 or 2 hours of the beginning of second-stage labour. This observation is corroborated by the fact that 70% of foals born by hysterotomy at Ghent were stillborn or died soon after birth (Vandeplassche, 1980). Intrepid surgery in the field may therefore be more expedient than referral to a specialist hospital.

Indications

The range of indications is more limited than in cattle. Cervical dystocia is not recognised in the mare, and disproportion and fetal monsters are less common than in other species. The major indication in the Ghent series, accounting for 39 of 71 cases, was bicornual pregnancy or transverse presentation, followed by other faulty dispositions complicated by injury, contraction or infection (13 cases) and uterine torsion (10 cases). In a much smaller series of 34 cases at the University of Bristol veterinary school, uterine torsion was the most frequent indication.

With considerable experience of equine dystocia, Vandeplassche et al. (1977) regard the following indications as absolute:

- faulty fetal disposition that cannot be corrected by other means (e.g. tranverse presentation)
- vulvovaginal or uterine trauma
- vaginal oedema
- irreducible uterine torsion
- severe congenital deformities (wryneck, ankylosed limbs, hydrocephalus).

In these forms of dystocia, the caesarean is the primary method of delivery rather than a last resort.

Significantly, these authors also specify forms of dystocia which they regard as contraindications for surgery; these include lateral deviation of the neck, hydrocephalus, breech presentation of a dead fetus, twin dystocia and prolapse of the maternal bladder.

Anaesthesia

Reposition of preparturient uterine torsions can be carried out by laparotomy in the standing animal under local analgesia or nerve block (Vandeplassche, 1980). However, new and improved anaesthetic agents and better patient monitoring have significantly reduced the risk to the mare during a caesarean operation. For details of anaesthesia in this species the reader is advised to consult a specialist textbook. It is important to stress that there may well be a conflict between the obstetrician and the anaesthetist because dorsal recumbency may induce ‘supine hypotension’ if the gravid uterus compresses the posterior vena cava and thus impedes venous return and reduces cardiac output. The mare should therefore be placed in lateral or dorsolateral recumbency during preoperative preparation and retained in dorsal recumbency for as short a period of time as is commensurate with effectively performing the operative procedure.

Operative technique

The operation can be performed through a midline, paramedian or ventral flank laparotomy. The midline approach is now widely adopted for gastrointestinal surgery and is even more satisfactory for caesarean section because this operation considerably reduces intra-abdominal pressure, and the wound can therefore be repaired easily without excessive tension on the sutures. All other approaches necessitate muscle division, which results in greater operative haemorrhage and postoperative oedema. Provided that the midline incision is properly repaired, the risk of incisional hernia is negligible.

The mare's uterus is seldom so tightly contracted that a fetal limb cannot be grasped through the uterine wall and brought through the abdominal wound. For this reason a uterine incision of adequate length is easily made on the greater curvature of the gravid horn with little risk of tearing during manipulation of the fetus. In many cases, hysterotomy is followed immediately by profuse haemorrhage from the submucosal plexus of arteries and veins which are too numerous to be ligated individually. As a means of controlling such haemorrhage, Vandeplassche (1973) recommends the insertion of a continuous suture through all layers of uterine wall along the edges of the incision, after the placenta has first been detached from this area. The fetus is then extracted making maximum use of joint flexibility and gently supported outside the abdomen with its umbilical cord intact. The equine fetus is less sensitive than the fetal calf to 'pinching' stimuli in utero and, unless the placenta is separated, fetal viability should be assumed until cord or heart palpation proves otherwise. If the foal is alive, the cord is left intact for several minutes until breathing begins. The cord is then ligated or preferably divided by stretching. If the foal is dead, the placenta may already have separated and is then easily removed through the hysterotomy. If the placenta remains attached to the endometrium, it is better not to attempt manual separation because this procedure results not only in diffuse endometrial bleeding, but also in retention of microvilli which predisposes to subsequent endometritis. The uterine incision is repaired with polyglycolic acid inversion sutures in one or two rows, depending on whether the first row of stitches tears through the uterine wall, which is sometimes noticeably fragile. After the removal of clotted blood and other debris, a soluble antibiotic preparation may be sprinkled on the uterine incision.

After laparohysterotomy, the abdominal incision is easy to suture because of flaccidity of the stretched abdominal musculature. It is important to insert closely spaced sutures of appropriate material in a continuous or interrupted pattern. The peritoneum and subperitoneal fat need not be stitched. The laparotomy repair is completed with a continuous subcutaneous suture and appropriate stitches in the skin.

Postoperative management

After all caesarean operations, oxytocin should be administered to induce uterine contraction even when the placenta has been removed at surgery. Vandeplassche et al. (1971) recommend immediate oxytocin therapy followed by a supplementary slow intravenous infusion of 50 i.u. in saline if the placenta is not expelled within 4 hours. The latter method of administration probably has a more physiological effect. Experience suggests that oxytocin therapy in the mare is sometimes followed by excessively vigorous uterine contraction and eversion of the cornua into the vagina and threatened eversion through the vulva even after the placenta has been expelled. After oxytocin therapy, the placenta is usually expelled within 12 hours, but in occasional cases it may separate but remain within the uterus and anterior vagina and is then easily removed per vaginam. Retention for longer than 24 hours is no longer regarded as an indication for immediate manual separation provided that antibiotic therapy is maintained, but removal may still be justified in draught-type mares which are particularly susceptible to systemic reactions. After removal of the placenta, intrauterine antibiotic preparations may have a beneficial effect, but more important by far is siphonage of any uterine fluids that accumulate, especially in mares which show signs of anaphylactic response or which are not recovering satisfactorily. Vandeplassche et al. (1977) have commented on the 2–3-day delay in contraction after caesarean section and recommend division of perimetrial adhesions per rectum at the end of this period.

Antibiotic therapy is generally considered advisable pre- and postoperatively, especially if the foal has been dead for some time, resulting in putrefaction.

Abdominal incisions in the horse are usually followed by local oedema of varying severity. After midline laparohysterotomy, diffuse subcutaneous oedema may extend along the ventral abdomen to the presternal region, but the swelling slowly subsides over a period of 7–10 days. The administration of diuretic agents appears to disperse the oedema more quickly, but the necessity for such treatment is questionable. Wound infection is treated by removal of appropriate skin sutures to provide drainage.

Maternal recovery rate and causes of death

If the dystocia is of short duration, the prognosis for maternal recovery is good. In the Ghent series of 77 operations, 62 mares (81%) recovered (Vandeplassche et al., 1977). The Ghent data suggest that most deaths occur during, or very soon after, surgery and are attributable to shock caused by uterine haemorrhage or gross uterine infection. Haemorrhage can be largely prevented by haemostatic suturing of the uterine incision, and the effects of fetal emphysema and other forms of shock can be countered by intensive fluid therapy during and after the operation.

Because most deaths occur during the immediate postoperative period, the clinician is more likely to be worried by two particular complications which may develop within the next few days. The onset of diarrhoea should be viewed with the greatest concern because body fluid loss is rapid and severe and fluid reserves are soon depleted even if the mare continues to drink. The role of antibiotics in the pathogenesis of this disorder and their value in its treatment are unclear, but there is no argument about the necessity for immediate fluid replacement therapy to maintain hydration and normal electrolyte status. The other complication is laminitis, which has long been recognised as a sequel of placental retention in animals of the heavy draught breeds. The earliest sign of this supposedly anaphylactic reaction may be severe pulmonary oedema with dyspnoea and nasal regurgitation of fluids. Pedal pain is then manifested by reluctance to move or even to stand and, without careful clinical examination, the resultant recumbency during the early postoperative period may easily be mistaken for terminal illness justifying euthanasia. In such cases, accumulated uterine fluids should be removed immediately by siphonage. Diuretics are indicated for severe oedema, and the laminitis is treated by dietary restriction and the control of pain.

Postoperative fertility

Stashak and Vandeplassche (1993) found that in a series involving 82 mares that had a caesarean operation, 34 (41.5%) were not bred again; of the 48 (58.5%) that were bred 28 (58.3%) became pregnant. In the same study, of the 48 mares that became pregnant, 33 (82.5%) foaled at term and of those that did not, most aborted at various stages of gestation. Thus the caesarean operation in the mare should not have too great an effect on subsequent fertility provided it is performed quickly after the onset of dystocia, and before there if heavy bacterial contamination of the uterus either from manual interference or from putrefaction of the foal. The report by Arthur (1975) of two mares which each conceived after two elective operations suggests that the hysterotomy per se is less important in this respect than the state of the fetus and the uterus at the time of surgery.

The sow

The sow, like the bitch, is a difficult obstetrical patient because although the need for surgery may be clear, it is not always possible to identify a particular cause of dystocia even after the operation has been performed.

Indications

In a series of 57 operations reported by Renard et al. (1980) the major indications were irreducible vaginal prolapse (32%), fetopelvic disproportion including fetal emphysema (32%), secondary uterine inertia (23%) and, surprisingly, nondilatation of the cervix (10%). Preparturient vaginal prolapse may be complicated by rectal prolapse and retroversion of the urinary bladder and even of the gravid uterus, and often undergoes considerable trauma and marked oedematous swelling. Fresh prolapses at term need not interfere with parturition but, if manual delivery is necessary, oedema rapidly develops, and the tissues then tear readily. Inertia of a primary or secondary nature is an important indication for surgery and, because of delay, the fetuses in such cases are often emphysematous. In secondary inertia, particularly, it is not always easy to be certain that fetuses remain in the uterus. If they cannot be palpated or ballotted through the uterine or abdominal wall, and if fetal heart sounds cannot be detected, radiography is advisable before surgery is undertaken. Less frequent clinical indications for a caesarean operation are maternal immaturity and pelvic deformity, uterine torsion of one or both cornua and fetal deformities such as hydrocephalus or conjoined piglets. Preparturient elective hysterotomy is also performed as an alternative to gravid hysterectomy to obtain disease-free piglets which are then fostered or reared artificially.

Anaesthesia

Because of difficulties in restraint, the operation is usually performed under deep sedation and local analgesia, or general anaesthesia. The best method under field conditions is the use of a combination of azaperone (1.0 mg/kg) and ketamine (2.5 mg/kg) intramuscularly as a sedative, followed by intravenous ketamine (2 mg/kg) and midazolam (100 μg/kg) about 15 minutes later (Clutton et al., 1997). The sow can then be intubated and anaesthesia maintained with oxygen/nitrous oxide/halothane. Alternatively it is possible to perform the caesarean operation using local infiltration and the ability to 'top up' with further doses of the latter combination intravenously. Brodbelt and Taylor (1999) have reported the use of two combinations of substances injected intramuscularly which is a much easier technique than the intravenous route. The combinations were azaperone (2 mg/kg) outorphanol (0.2 mg/kg) and ketamine (5 mg/kg), or detomidine (100 μg/kg), butorphanol (0.2 mg/kg) and ketamine (5 mg/kg). These combinations allowed endotracheal intubation, but it is likely that a caesarean operation could be performed with local infiltration at the surgical site.

Renard et al. (1980) recommend the use of anterior epidural analgesia but also reported a very high incidence of postoperative hindlimb paresis which they attributed to lateral recumbency on a hard surface. Provided that the animal is adequately restrained under sedation, local analgesia or paravertebral nerve block may also be successfully employed.

Operative technique

The operation is performed through a vertical sublumbar or ventral flank incision on either side (Figure 20.14). Each gravid horn should be exteriorised in turn for incision outside the peritoneal cavity in order to minimise peritoneal contamination. If the fetuses are not emphysematous, it is usually possible to evacuate both horns through a single incision in the centre of each horn, with the piglets at the ovarian poles and the base of the cornua being squeezed down the horn and grasped through the incision. If the fetuses are emphysematous multiple incisions sited directly over or between them may be necessary. The piglet's umbilical cord is long and, even without placental separation, forceps clamping or ligation is possible before division. Fetal membranes which have not separated should be left in situ and not forcibly removed by traction. Because the cornua are long, it is important to palpate the genital tract in its entirety to ensure that all piglets have been removed. The uterine incision(s) is repaired with inversion sutures of polyglactin 910. The sow's uterus, like the mare's, is apt to tear if the suture is pulled too tight but this is of no consequence if rapid contraction is induced by postoperative oxytocin therapy.

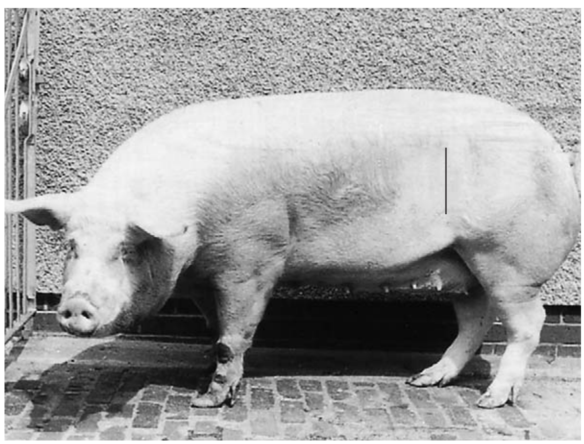

Fig. 20.14 Position of skin incision in the left sublumbar fossa for a caesarean operation in a sow.

Maternal recovery rate and causes of death

Unless the fetus and uterus are grossly infected, the maternal recovery rate after a caesarean operation in the sow is excellent. In a series of 78 unselected cases, Renard et al. (1980) reported a maternal recovery rate of 72%. Deaths are usually due to the combined effects of toxaemia and surgical shock and occur during the immediate post-operative period. Animals which are likely to die can often be identified before surgery because of a characteristic blotchy cyanosis of the limbs, ears and udder. The adverse effects of peritoneal contamination are more easily avoided in the sow than in larger animals because the uterus can be totally exteriorised during the operation. Other frequent complications recorded by Renard et al. (1980) include constipation, locomotory problems exacerbated by the sow's tendency to remain recumbent, and the mastitis-metritis-agalactia syndrome. Severe preoperative vaginal prolapse may recur after surgery and require the insertion of a temporary retaining perivaginal suture.

The ewe

Indications

The main indications for the caesarean operation in the ewe are:

- failure of the cervix to dilate
- irreducible or severely traumatised vaginal prolapse
- fetopelvic disproportion, particularly in primiparous animals with a single fetus
- fetal emphysema after protracted dystocia.

Less frequent indications are uterine torsion, vulvovestibular stricture and faulty fetal disposition which cannot be corrected because of maternal immaturity or uterine contraction. Vaginal prolapse should initially be treated conservatively by reposition and the insertion of vulval retention sutures, in the hope that pregnancy will continue to term (see Chapter 5), but many cases undergo early labour with incomplete dilatation of the cervix. Unfortunately, lambs from such animals frequently die of prematurity, after showing characteristic convulsive limb movements and respiratory embarrassment.

Anaesthesia

Hysterotomy is usually performed through a left flank incision under paravertebral, inverted-L nerve block or local infiltration analgesia with the animal in right lateral recumbency, using 2–3% lignocaine hydrochloride with adrenaline. Care is essential in inducing local analgesia in sheep because accidental intravenous administration or the injection of an excessive quantity of anaesthetic agent may rapidly result in convulsions. In addition, the body wall is much thinner than in the cow, and thus care must be taken not to penetrate the abdominal viscera.

Operative technique

The left sublumbar region is close-clipped and the skin prepared for aseptic surgery. The skin is incised in the mid-sublumbar fossa, and the underlying muscles are incised in the same way as described above for the cow. However, it is important to stress that the body wall is very much thinner, and great care must be taken not to incise into the rumen accidently. It is also important in a high sublumbar incision to recognise the highly vascular mesometrial attachment to parietal peritoneum. A fetal extremity, preferably the hock, is grasped through the uterine wall so that an incision can be made in the same way as that described for the cow (Figure 20.15); however, it is important to stress that more than a single fetus is likely to be the norm. Often, this can make identification of which extremity belongs to which fetus difficult. It is even more important to remember to explore the uterus, particularly the opposite horn to that incised, to ensure that *all* lambs have been removed before suturing the uterine incision. It is always possible to remove all lambs through a single incision.

The fetal membranes should be removed if they can be readily detached; if not, then that which cannot be returned to the uterine lumen, thus interfering with the closure of the uterine incision, should be excised. The uterus should be closed using a single inversion suture pattern such as Lembert's or Cushing's, using an adsorbable material (Figure 20.15). The sheep, more than any other species, is highly susceptible to the toxaemic effects of intrauterine clostridial infection, and most deaths are due to this complication.

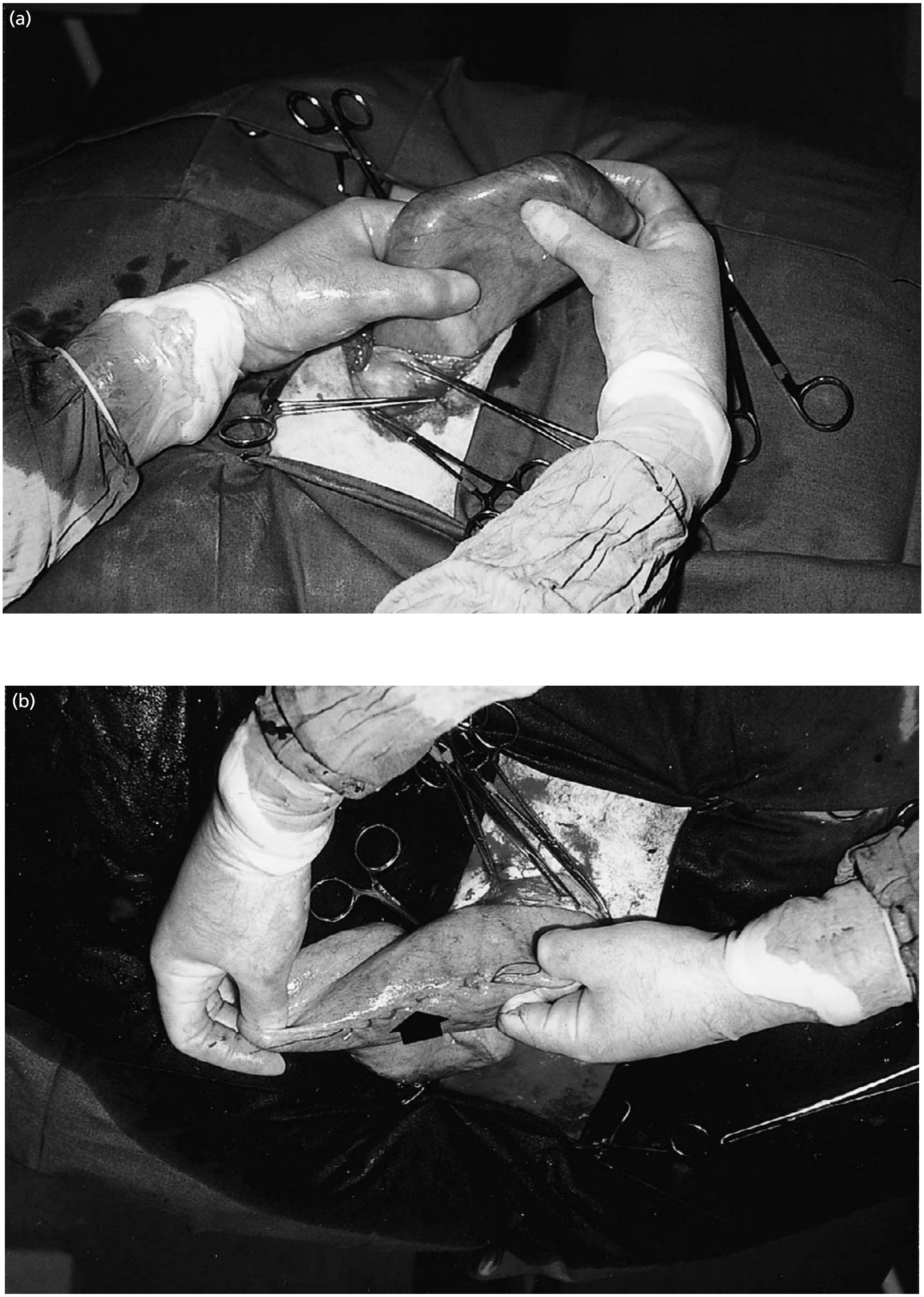

Fig. 20.15 Caesarean operation in the ewe for the relief of incomplete cervical dilatation. (a) The exteriorised uterus over a fetal hindlimb. (b) Hysterotomy repair with inversion sutures.

Postoperative fertility

There are no data on the fertility of ewes after a caesarean operation. Quite often ewes will be culled because they have had the procedure performed, or because of the cause of the dystocia. However, in the author's experience with experimental ewes, fertility was not impaired and the ewe, because of the seasonal pattern of reproduction, invariably had a long anoestrus which allowed for recuperation.

SURGICAL PREPARATION OF TEASER BULLS AND RAMS

In intensively managed dairy herds, teaser bulls fitted with marking devices are sometimes used to assist in oestrus detection (see Chapter 22). Teaser rams are used for slightly different purposes: firstly, to concentrate the lambing period by ensuring that all ewes in the flock are undergoing cyclical activity before the stud ram is introduced, and secondly, to hasten the onset of cyclical activity in anoestrus ewes and to some extent synchronise cyclical activity.

Teaser bulls are prepared by surgical manipulations of the penis or prepuce to prevent intromission or by vasectomy or by the occlusion of other genital ducts induced by the injection of chemical irritant agents. Surgical procedures include penectomy (Straub and Kendrick, 1965), fixation of the penis to the ventral abdominal wall (Belling, 1961) and partial occlusion of the preputial orifice (Bieberly and Bieberly, 1973), but these techniques prevent protrusion and ejaculation and are thought, for these reasons, to lead to frustration and rapid loss of libido. Moreover, in the UK these procedures are considered to be unacceptable mutilations. A more sophisticated technique for surgically deviating the prepuce from the ventral midline has been described by Rommel (1961) and Jöchle et al. (1973), but would not be acceptable in the UK.

Vasectomy is still the generally accepted method of preparing teaser bulls. With the reservations that coitus may transmit venereal diseases, and that vasectomised animals retain a normal masculine aggressiveness and can lose libido because of overwork, teasers prepared in this way perform satisfactorily for an indefinite period of time. The technique is reviewed by Pearson (1978), with particular emphasis on the possible legal implications of improper surgery. In ruminants, which have a pendulous scrotum, the spermatic cord is exposed through an incision in the scrotal neck and, after splitting of the tunica vaginalis reflexa, the vas deferens is identified as a distinctively dense tubular structure lying in its own separate fold of mesorchium (Figure 20.16). At least 3 cm of the vas is resected between

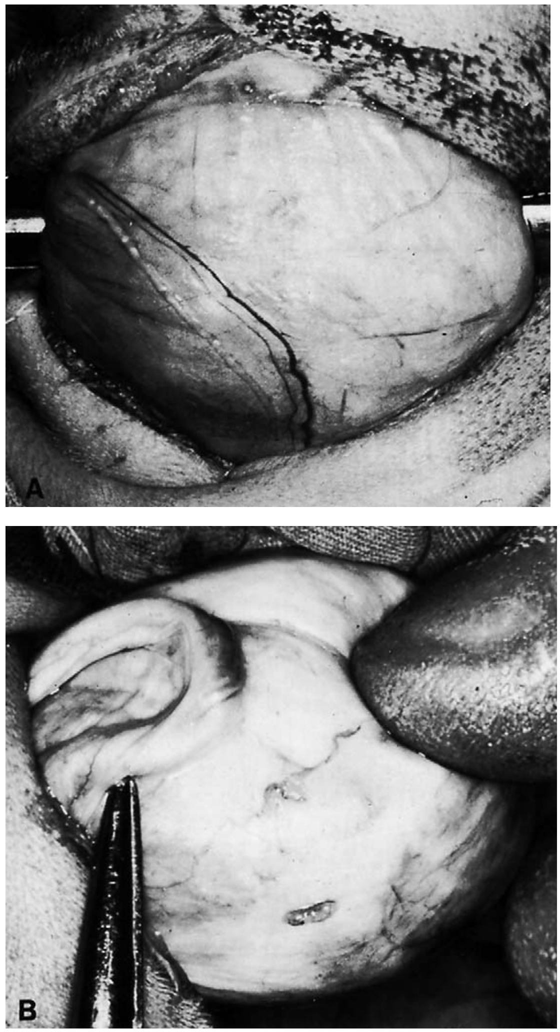

Fig. 20.16 Vasectomy in the bull. (a) The spermatic cord is elevated through the skin incision in the neck of the scrotum. (b) The vas deferens is exposed after incision of the tunica vaginalis reflexa.

Table 20.1 Effect of bilateral vasectomy (at day 0) on semen quality of daily ejaculates in a bull

Semen	*Days before and after vasectomy*						
	–2	*–1*	*+1*	*+2*	*+4*	*+6*	*+8*
Motility	5+	4+	–1	0	0	0	0
Density (× 10^6)	3605	2200	1465	70	10	5	0
Volume (ml)	3.5	1.5	1.5	3.0	1.0	1.5	1.0
Sperm count (%)							
Normal live	86	70	8	0	0	0	
Normal dead	14	20	84	78	92	80	All
Abnormal live	NR	NR	NR	1	4		dead
Abnormal dead	NR	NR	NR	20	4	20	

NR = not recorded

non-adsorbable ligatures, and the scrotal skin is sutured after the testis has first been pressed into the scrotum to draw the cord back within the tunica which need not be closed. The prudent clinician will always keep the excised tissue in a preservative in case there is a subsequent history of cows or heifers conceiving to the bull. It is doubtful if the cost of routine histological examination can be justified. This is certainly true for rams. The effect of vasectomy on sperm quality is immediate (Table 20.1); in the bull, viable extragonadal sperm reserves can probably be completely exhausted by one or two natural or artificial services, but the ram may continue to ejaculate immotile sperm from ampullary reserves for a considerable period afterwards. Vasectomy is not often requested in the boar, but in this species the vas is approached by an inguinal or scrotal incision.

A non-invasive method of chemical sterilisation without loss of libido was described by Pineda et al. (1977), who found that the injection of chlorhexidine in dimethyl sulfoxide into the epididymides of dogs induced long-lasting and probably irreversible azoospermia. The effect of this technique was tested by Pearson et al. (1980) in bulls and rams. Four bulls became aspermic within 2 weeks of the injection into each cauda epididymis of 5 ml of a preparation containing 3% chlorhexidine gluconate in 50% dimethyl sulfoxide in aqueous solution and remained aspermic throughout a trial period of at least 54 weeks. Experimental and clinical trials of the same technique in rams are equally encouraging.

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21 Genital surgery in the bitch and queen cat

THE CAESAREAN OPERATION

The bitch

A common concern of many owners is that when pregnancy length exceeds 65 or 66 days parturition is 'overdue'. However, there is often a misunderstanding of the normal reproductive physiology, since whilst the 'endocrinological' length of pregnancy is 65 days, there is a large variation in the 'apparent' length of pregnancy. The latter, which is the interval from the day of mating to the day of parturition, can vary from 58 to 72 days in normal bitches of all breeds (Krzyzanowski et al., 1975). True causes for prolonged gestation include the bitch that has had unnoticed primary uterine inertia or dystocia, whilst in some cases a non-pregnant bitch is mistakenly thought to be pregnant.

Bitches that are within their physiological pregnancy length and those that are non-pregnant do not have abnormal clinical signs. Those bitches that have had primary uterine inertia may have previously had a small-volume vulval discharge, and may have exhibited uterine and possibly abdominal contractions that were unnoticed by the owner. Subsequently, there is placental separation and the onset of a green-coloured vulval discharge. Dams then become systemically ill as the fetuses die and decompose; a large-volume vulval discharge may be present. Initially, rectal temperature may be normal, but this may subsequently increase, and terminally it may become subnormal.

There are several methods that may be used to predict the time of expected parturition in the bitch. Had the bitch been monitored during oestrus (using measurement of plasma progesterone concentrations to detect the optimal mating time), it would have been seen that the time from ovulation to parturition is tightly regulated (63 ± 1 days). Similarly, the study of vaginal cytology during oestrus may be useful since the onset of the metoestrus vaginal smear is related to parturition (58 ± 4 days), although not as precisely as the time of ovulation. A third useful assessment is to instruct the owner to record the rectal temperature twice daily during the last third of pregnancy, since a decline in rectal temperature precedes parturition by approximately 12–36 hours. In many bitches, however, none of these procedures has been undertaken, and therefore it is important to perform a full clinical examination to ensure that the dam is clinically well and that she is pregnant. Measurement of plasma progesterone concentration can then be used to assess whether parturition is imminent. Progesterone concentrations decrease approximately 24–36 hours before whelping. Detection of high plasma progesterone concentration therefore indicates that parturition is not imminent, whilst a low progesterone concentration indicates that parturition is imminent, or should already have occurred. Plasma progesterone can be easily measured in the practice laboratory by the use of enzyme-linked immunosorbent assay (ELISA) test kits.

The major obstacle to rational assessment of apparent dystocia is the physical impossibility of carrying out a proper internal examination of more than just the caudal reproductive tract. Except in the smallest breeds, even the cervix is beyond reach on digital vaginal exploration, and evaluation of the cervix can only be made by endoscopy. The clinician must therefore rely greatly on behavioural signs and the nature of the vulval discharges, and interpret these observations on the basis of experience of normal parturition.

Failure of cervical dilatation is not recognised in the bitch or queen. In normal whelpings, the onset of voluntary abdominal straining signifies cervical relaxation and stimulation of the pelvic reflex by some part of the conceptus in the cranial vagina. As the second stage of parturition progresses, the nature of abdominal contractions changes. Initially, bouts of straining are brief and perfunctory, but as the fetus passes into the vagina the duration and intensity of straining increase. As the fetus distends the perineum, straining becomes forcefully sustained. The pattern of straining, in cases of apparent dystocia, may therefore indicate the likely site of the fetus in the birth canal.

Primary or secondary uterine inertia is a common cause of dystocia in the bitch. Abdominal and uterine contractions are roughly synchronous but are not necessarily of equal intensity. The continuation of forceful involuntary straining cannot therefore be taken as evidence of continuing uterine contractions. This consideration is important, because uterine contraction is by far the more effective of these expulsive forces and is essential for delivery, irrespective of the degree of abdominal straining. Uterine contraction is involuntary, but straining can be inhibited consciously, usually in anticipation of pain immediately before the birth of the first fetus. It is important to realise that during normal whelpings there are periods of rest, indeed of sleep, when abdominal and presumably uterine contractions stop. Such behaviour does not necessarily indicate the onset of inertia. In this respect, it is interesting to consider the intervals between births in normal parturition. In a series of 50 normal whelpings, the shortest interval was 10 minutes and the longest 360 minutes (England, unpublished observations). In polytocous species, it is unrealistic to expect all the fetuses to be born alive. Most commonly, the last pups to be delivered are stillborn. In many normal bitches, the period of straining before the birth of the first puppy may be considerably longer than the intervals between births, and 2 hours may frequently elapse between rupture of the allantochorion and birth of the presented fetus. In general, the incidence of dystocia is lowest in young, primiparous animals. Many bitches that are affected with primary inertia later in life have had a normal first parturition.

Indications

In larger animals the cause of dystocia can usually be identified, but this is often not possible in the bitch. Frequently the decision to operate is therefore based largely on a subjective assessment of the circumstances of the case including:

- the duration and progress of parturition
- the number and viability of fetuses born and unborn
- the nature of vulval discharges
- changes in the pattern of straining
- the often uninformative findings on vaginal examination.

It is sometimes difficult to be sure that dystocia has supervened; the correct management of these cases, without resorting always to caesarean operation, requires experience and sound clinical judgment. It is therefore more realistic to indicate when surgical interference may justifiably be considered than to catalogue the various maternal and fetal causes of dystocia, all of which may, on occasion, constitute a valid reason for caesarean operation.

In discussing dystocia in the bitch, Freak (1975) described three forms of delay to parturition: delay in the initiation of parturition; delay in propulsion; and delay in delivery despite vigorous straining. Most cases of dystocia present in exactly these ways.

Delay in the initiation of parturition. Delay in the initiation of parturition may be due to several causes. There may, for example, be psychological inhibition in bitches suddenly transferred to a strange environment not conducive to the normal progress of parturition. There may, in individual animals, simply be a long but normal first stage of parturition. In such cases, it is helpful to know if allantoic fluid has been lost, but it is more important to appreciate the significance of the dark greenish-black discharge that arises from marginal areas of the placenta. This fluid is not released until at least one placenta has separated, and its appearance before straining or the birth of a pup signifies primary uterine inertia. In many such cases, it is the only sign of cervical dilatation, and justifies immediate surgery if more than one or two fetuses are present. After the birth of one pup, dead or alive, this discharge has less significance unless the bitch shows other signs of inertia.

Delay in propulsion during parturition. In bitches which have undergone a normal first stage of parturition, vigorous unproductive straining for more than approximately 3 hours may indicate dystocia; such cases should be carefully assessed by diagnostic ultrasound or possibly abdominal auscultation to confirm fetal viability. Detecting fetal heartbeats can reveal the viability of fetuses; the normal fetal heart rate is greater than twice that of the maternal rate (Verstegen et al., 1993). Careful vaginal examination is essential to detect obstructive dystocia. These cases may be difficult to assess and offer ample scope for errors of judgement; a live fetus may well be born during preparation for surgery. Without positive signs of dystocia, such animals should be left a little longer unless straining abates or a placental discharge appears.

Delay in delivery despite vigorous straining. An excessively long interval since the birth of the last fetus may also be difficult to interpret. In bitches pregnant with only one or two fetuses, a delay at this stage may be normal, but if it exceeds 3 hours and is associated with vigorous straining, there is probably obstructive dystocia, the cause of which may be obvious on vaginal examination, abdominal palpation or even radiography and ultrasonography. An alternative explanation for continued straining without birth is the onset of inertia of a primary or secondary nature. The management of delay during the second stage of parturition is difficult because of problems in recognising the signs of inertia, largely because abdominal straining may continue after inertia develops. A tentative diagnosis of inertia is more convincing if abdominal straining stops or is reduced in frequency and intensity, but this does not always occur. The assessment of these cases should be based on the assumption that, in primary inertia, the longer the delay, the more likely are the fetuses to die. The clinician learns by experience that it is better to perform an occasional hysterotomy unnecessarily than to delay until all the fetuses are dead. Primary inertia is occasionally due to hypocalcaemia or hypoglycaemia and responds spectacularly to appropriate therapy (Freak, 1975). Apparent inertia towards the end of the second stage of parturition is likely to be secondary in nature and may respond quickly to the intramuscular administration of oxytocin.

The non-surgical relief of dystocia was admirably reviewed by Freak (1975). Certain forms of fetal dystocia may be corrected easily by finger, forceps or vectis manipulation per vaginam. Vaginal forceps delivery, under general anaesthesia if necessary, is particularly indicated in bitches in which the last one or two fetuses, usually dead, cannot be expelled naturally. In fact, in such cases, it is sometimes possible to milk the fetus into the birth canal by manipulation through the abdominal wall.

In some brachycephalic breeds, caesarean operation is performed as a routine, largely on account of the exhaustive length of parturition and the high incidence of dystocia and stillbirths. Elective surgery may also be indicated for other reasons such as pelvic deformity or gravid inguinal metrocele. Whatever the reason, surgery should normally be delayed until the onset of first-stage parturition in order to avoid the risk of fetal prematurity. Prolongation of pregnancy beyond its ‘expected’ length is not an indication for immediate caesarean. Provided fetal movements and heart sounds are detectable, and the bitch remains healthy with no abnormal vulval discharge, the case should be observed carefully but left until other signs develop. Alternatively, surgery can be planned upon the detection of a decline in plasma progesterone concentration, measured using an ELISA method, or following the detection of a decline in rectal temperature.

Prolongation of pregnancy, sometimes up to 70 days or even more, in bitches carrying only one or two fetuses is a particular cause for concern. In the ‘single-pup syndrome’ fetal endocrine secretion may be inadequate to initiate the process of parturition, and the fetus may be larger than normal and therefore less likely to pass easily through the birth canal when parturition begins. These cases are best managed by performing a caesarean operation, to avoid the risk of fetal death following primary uterine inertia.

Anaesthesia

When considering anaesthesia for caesarean operation, it should be remembered that:

- The dam may be ‘normal’, or she may be debilitated and require careful anaesthetic management.

- There is often no time for pre-anaesthetic preparation.
- The dam may have recently been fed.

The general aims of the procedure are to ensure adequate oxygenation (by intubation and provision of inspired oxygen), to maintain blood volume and prevent hypotension (by the administration of intravenous fluid therapy), and to minimise maternal and fetal depression during surgery and after delivery (by reducing the dose of anaesthetic agents used). A number of factors are important when considering the most appropriate fluid for intravenous administration; for example, there may be increased alveolar ventilation (an effect of progesterone) causing respiratory alkalosis, although the enlarged abdomen may produce a decreased tidal volume causing respiratory acidosis, there may be loss of acid because of vomiting, and there may be loss of blood as a result of the surgery. The best-choice agent is probably lactated Ringer's solution administered at a rate of 10–20 ml/kg/hour.

It is not possible to discuss all of the anaesthetic options for caesarean operation in this text; however, there are a few points worth considering. For premedication, atropine is best not given routinely since it blocks the normal bradycardic response of the fetus to hypoxia, and it relaxes the lower oesophageal sphincter, making aspiration more likely. Phenothiazine tranquillisers are very useful agents since they smoothe anaesthetic induction and reduce the subsequent dose of induction and maintenance agents; they are, however, rapidly transported across the placenta. α_2-Adrenoceptor agonists such as medetomidine and xylazine are contraindicated because of their severe cardiorespiratory depressant effects. Similarly, the respiratory depressant effect of opioids makes them unpopular. Metoclopramide may be administered intravenously prior to induction to reduce the risk of vomiting during the procedure. For the induction of anaesthesia, dissociative agents such as ketamine are best avoided because they produce profound depression of the fetuses. The ultra-short-acting barbiturates and propofol appear to be most useful, since they are either rapidly redistributed or are metabolised, and therefore have limited effect upon the fetuses after delivery.

For maintenance of anaesthesia, the volatile agents are preferable, especially those with low partition coefficients such as isoflurane. This agent has a rapid uptake and elimination by the animal, and it may have a better cardiovascular margin of safety than the more soluble agents such as halothane.

Whilst nitrous oxide may be used to reduce the dose of other anaesthetic agents, it is rapidly transferred across the placenta, and although it has minimal effects upon the fetus in utero, it may result in a significant diffusion hypoxia after delivery. In certain cases, inhalational agents are used for anaesthetic induction, and in this case nitrous oxide is useful for speeding the induction of anaesthesia via the second gas effect.

Small animals should be protected throughout surgery from the risk of hypothermia (Waterman, 1975).

Surgical technique

The uterus is conventionally approached by a ventral midline coeliotomy, although some use a flank incision. Large mammary veins will normally hamper the midline incision, but once these are ligated there is no haemorrhage from deeper tissues. Care should be taken to ensure that the mammary tissue itself is not damaged. The ventral approach allows the incision to be made as cranially as necessary, and allows equal exposure of the two uterine horns. The length of incision depends upon the expected size of the fetuses; ideally it should be sufficiently large to enable the uterus to be exteriorised.

Speed of the surgery is important for two reasons: to ensure minimal fetal hypoxia, and to prevent hypotension of the dam caused by compression of the caudal vena cava by the gravid uterus. In large-breed dogs, tilting laterally on the operating table may reduce the risk of compression of this vessel.

Once the linea alba is incised, care should be taken not to damage the uterus which may be lying in close apposition to this structure. Once the uterus has been identified, it should ideally be exteriorised and packed off using swabs to prevent contamination of the abdomen with fetal fluid. However, care must be taken when manipulating the gravid uterus, which has a thin wall and is liable to tearing. On some occasions, it is only possible to exteriorise one horn at a time. The uterus should be incised in a relatively avascular area of the dorsal surface of the uterine body, although in some cases a ventral incision may be made. The latter is usually necessary when there is impaction of a fetus that prevents exteriorisation; a ventral incision leads to peritoneal contamination with fetal fluid. When making the uterine incision, care must be taken not to lacerate the underlying fetuses and it is best to extend the incision with scissors. It is conventional to remove the fetuses within the uterine body first, and then to milk down remaining fetuses to the same incision (Figure 21.1). During this procedure, the membranes of proximally positioned fetuses normally rupture and fetal extremities (either the head or the pelvis) can be grasped through the incision to apply traction. Once at the incision, the amniotic sac may be ruptured and fetal fluids should ideally be removed by suction. The umbilical vessels should be clamped approximately 2 cm from the ventral abdominal wall of each pup and the umbilical cord can then be severed distally. In some cases – for example, primary inertia with two fetuses, or secondary inertia when most of the litter has been delivered naturally – the fetuses may be positioned within the tips of opposite uterine horns. In these cases, bilateral cornual incisions are indicated, rather than a single uterine body incision.

Once fetuses have been delivered, they should be passed to an assistant for resuscitation. At this time, the pups should be inspected for congenital abnormalities such as cleft palate, and if necessary

Fig. 21.1 Removal of fetus through an incision in the uterine body.

the cord can be ligated with suture material. After each pup is delivered, the associated placenta should be removed by gentle traction or by gentle squeezing of the uterine wall and twisting of the cord; those that are firmly adherent should be left in position, since forceful removal will result in haemorrhage. Such haemorrhage may be significant, especially in toy breeds. Attached placentae will be expelled by uterine involution, supplemented by exogenous oxytocin administration after the termination of the procedure. It is important to ensure that all fetuses are removed, and careful inspection of both uterine horns up to the ovaries and the uterine body is essential.

The uterus and the broad ligament should be assessed after delivery of all pups; small traumatic lesions should be identified for subsequent repair. The uterus should rapidly begin to contract and involute. If the uterus is compromised, an ovariohysterectomy may be considered, although some suggest that this should be avoided because of the increased fluid loss and surgical time.

The uterine incision is usually closed using a two-layer inverting continuous pattern such as Cushing or Lembert with an absorbable suture material (polyglactin 910, polyglicaprone 25, polydioxanone or glycolic acid). There should have been minimal peritoneal contamination, but if this has occurred the peritoneum should be lavaged with several litres of warmed physiological saline prior to closure of the coeliotomy. Omentum may be placed on to the region of the uterine incision to reduce the likelihood of adhesion formation. If there is no evidence of uterine involution at the time of closure of the abdominal incision, then oxytocin should be administered, although care should be taken in the hypovolaemic animal since it may produce peripheral vasodilatation and hypotension. Oxytocin may be required especially if halothane anaesthesia has been used, since this agent is known to delay uterine involution. The coeliotomy should be closed in the normal manner, although some use buried subcuticular sutures since these are less frequently interfered with by the sucking pups.

Occasionally, a caesarean operation reveals unexpected findings such as uterine torsion (which is more common in the cat than in the dog) or uterine rupture. The latter may cause serious haemorrhage and hypovolaemic shock, but if the uterus involutes the bleeding may stop spontaneously. Uterine rupture probably accounts for most recorded cases of so-called ‘extra-uterine’ or pseudoectopic pregnancy in the bitch. Such fetuses are encapsulated by the omentum and peritoneum and subsequently become heavily calcified, without apparent detriment to the dam.

After protracted, neglected dystocia, particularly with fetal putrefaction, the uterus may be irreversibly infarcted or infected with gas-producing coliform or clostridial organisms. Localised areas of ischaemia can be inverted by oversewing, but evidence of more extensive infarction or deep infection indicates the need for hysterectomy. The prognosis in such cases is serious, and intensive fluid and antibiotic therapy is essential. The widely adopted and valid view that a single retained fetus, no matter how decomposed, is best removed with forceps per vaginam might seem to disregard the fact that the uterus is an ideal medium for the proliferation of anaerobic organisms. The high recovery rate after such deliveries probably suggests that fetal putrefactive changes in this species are due more often to coliform than clostridial infection.

Elective hysterectomy is often requested for bitches that require a caesarean operation. Whether the additional risk is warranted is entirely a matter for clinical judgement, although with proper supportive therapy the risk is not great. In cases where caesarean hysterectomy is planned, a preliminary hysterotomy incision should be avoided wherever possible and the uterus and ovaries should be removed en bloc. In some cases, however, it is necessary to remove an impacted fetus before the vagina can be ligated. The major problem with the en bloc procedure is the availability of a sufficient number of assistants to remove and revive all fetuses at the same time.

Most caesarean operations result in uterine adhesions. These are not always confined to the area of incision. Such adhesions may seriously interfere with exposure and exteriorisation of the uterus if a subsequent caesarean is necessary.

Postoperative management

After caesarean operation, most bitches accept their puppies and lick and suckle them normally, particularly if one or two were born naturally before surgery. Occasional bitches, where the litter was delivered entirely by caesarean operation, may be less receptive and behave aggressively towards the pups. Such bitches should initially be gently restrained to allow the pups to suck, and most settle quickly. If the aggression persists, it may be necessary to protect the pups in a cage in the whelping box and place them on the bitch every few hours or so for feeding until she shows signs of normal maternal acceptance. The puppies’ prime requirement immediately after birth is not food but warmth and the maintenance of an ambient temperature of 30–32°C. Delay in feeding for up to 6 hours or so after birth is of no consequence. Bitches that are allowed to eat their placentae usually have some degree of diarrhoea for a day or two afterwards.

Two particular problems may require veterinary attention during the initial postoperative period. It is normal after caesarean operation for a considerable volume of blood and other uterine fluids to be voided as a result of uterine involution. A continuing vulval discharge of blood may indicate serious haemorrhage from areas of placental attachment, especially if placentae have been forcibly detached. This is a life-threatening complication, especially in animals of a small size, and indicates the need for further oxytocin therapy immediately. The animal’s cardiovascular status should be carefully assessed by monitoring pulse and respiratory rates, and particular attention should be paid to pallor of mucous membranes and palpable uterine distension. Packed cell volumes have little meaning in rapid blood loss of this sort, and parenteral haemostatic agents are ineffective in arresting the haemorrhage. The only beneficial treatment for these cases is immediate blood transfusion or fluid replacement therapy if whole blood is not available. If the blood loss continues, such therapy may have only a temporary effect, and the need for hysterectomy may have to be considered once the animal’s circulatory status has been stabilised. This is an avoidable, but not uncommon, cause of death after caesarean operation in the bitch.

The second cause for concern may be the persistence of compulsive panting or hyperventilation, to the extent that it interferes with the bitch’s natural inclination to suckle the puppies or even to sleep. It is occasionally caused by the unnecessary provision of extra heat from an overhead lamp or other appliance, but most often it develops spontaneously in bitches, especially of the brachycephalic types, which have behaved in a similar way during the first and second stages of parturition. Blood calcium concentrations, should be measured since some of these cases are hypocalcaemic. When values are normal, little can be done to allay this exhausting condition except to sedate the bitch, but sedative drugs may be excreted in the milk and thus affect the young. It generally subsides over a period of 2 or 3 days.

Like all other species, the bitch is susceptible to infective peritonitis after caesarean operation, but good surgical technique and routine antibiotic therapy minimise the risk of this complication.

Intermittent uterine bleeding, generally attributed to subinvolution, may follow natural parturition or caesarean operation and persist for several weeks afterwards. It has little effect on the bitch's packed cell volume and is best left to resolve spontaneously because hormonal therapy is ineffective, the uterus by this stage being no longer sensitive to oxytocin. In some countries, occasional animals with a haemorrhagic vulval discharge during the suckling period will be found, on closer examination, to have lesions of transmissible venereal tumour contracted at coitus.

Maternal recovery rate, causes of death and postoperative fertility

Currently, rates of maternal mortality related to caesarean operation are lower than 5%. However, published data are only available for studies released more than 30 years ago (Mitchell, 1966). In that report, there was a 13.3% maternal mortality in 120 bitches subjected to hysterotomy or caesarean hysterectomy. Five of the 16 bitches that died failed to recover from the anaesthetic, and the remainder died during the next 5 days. Only three of the animals that died had living fetuses in utero. Deaths during or immediately after caesarean surgery are due principally to the combined effects of toxaemia and surgical shock, or to uterine haemorrhage. The choice of a safe anaesthetic technique, routine fluid infusion and proper management of the placenta will reduce maternal deaths to a minimum.

There are no data on postoperative fertility in the bitch, but it is certainly high, probably because the ovary and oviduct are completely protected by the bursa and are unlikely to be affected by adhesions.

The queen cat

Prolonged gestation does not normally occur in the queen unless there has been unnoticed uterine inertia or dystocia. Prediction of the time of expected parturition can normally be achieved by counting the number of days from mating, although in many non-pedigree queens this is often not observed by the owner. Nevertheless, measurement of plasma progesterone concentration using an ELISA method, as described above, can be clinically useful; queens that are still within their normal physiological pregnancy length will have high plasma progesterone concentrations, whilst queens which have had primary uterine inertia will have low plasma progesterone concentrations.

The indications for caesarean operation in the queen are not well documented, and it is likely that gravid ovariohysterectomy is performed more frequently than hysterotomy, except in pedigree animals. Joshua (1979) suggested that inertia and oversize are less common in this species than faulty disposition, or fetal deformities such as hydrocephalus and anasarca. Maternal causes of dystocia include pelvic distortion after fractures and uterine torsion affecting either the entire uterus or only one horn (Figure 21.2).

The operation is performed under general anaesthesia using similar considerations to those described above. The surgical approaches and technique described for the bitch are equally suitable for the queen. Except in animals intended for further breeding, gravid hysterectomy may be considered preferable to hysterotomy, and is generally well tolerated in this species. Antibiotic and supportive fluid therapy is advisable after protracted dystocia or if the uterus is grossly infected. The presence of fetuses in the peritoneal cavity as a result of uterine rupture is usually of little consequence, and affected animals may survive indefinitely without surgery, the fetal remnants

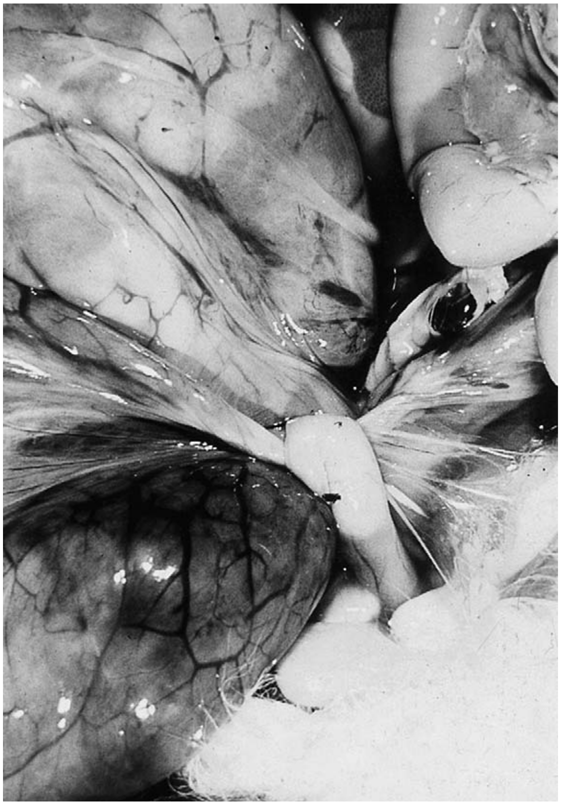

Fig. 21.2 Unicornual torsion in a queen.

becoming encapsulated by the omentum or mesentery.

OVARIOHYSTERECTOMY

Indications

This operation is most frequently performed electively as a means of preventing unwanted pregnancies and the nuisances associated with oestrus in pet animals. An important clinical justification for spaying is its protective effects against the subsequent development of mammary tumours, but for this purpose it must be performed before the first or second oestrus (Schneider et al., 1969). It has no effect on mammary tumours that have already developed, although it is frequently performed when mammary tumours are identified in the hope that removal of the ovaries will reduce the development of new lesions that may be stimulated by either progesterone or oestrogen. Ovariohysterectomy may have a sparing effect on the development of vaginal leiomyomata later in life (Kydd and Burnie, 1986). The most important clinical indication for ovariohysterectomy is the treatment of pyometra (which is discussed in detail below). Surgery is still the treatment of choice for this disorder, although there are reports of successful treatment with prostaglandins, prolactin inhibitors and combinations of these products. Ovarian neoplasms are not common in the bitch but granulosa cell tumours and ovarian cystadenoma (Figures 21.3 and 21.4) are successfully treated by ovariectomy, provided, with the latter tumour that metastases are not evident, either locally on the serosa or in the lymphatics on the dome of the diaphragm. Removal of the ovaries is also believed to be bene-

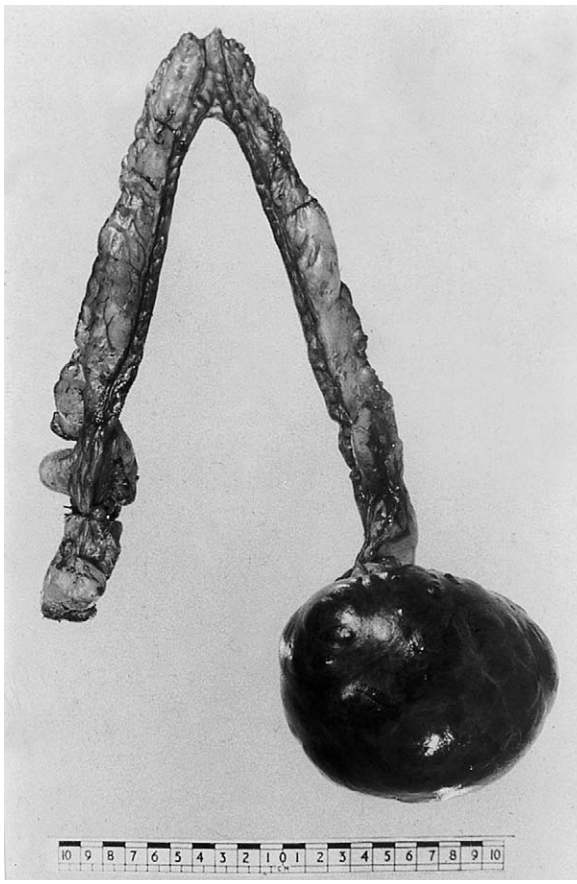

Fig. 21.3 Unilateral granulosa cell tumour in a bitch, responsible for a haemorrhagic vaginal discharge, vulval discharge and sexual attractiveness.

Fig. 21.4 Bilateral cystadenomata associated with haemoperitoneum in a bitch.

ficial for cases of diabetes mellitus that can be difficult to stabilise during the luteal phase. Gravid hysterectomy is usually taken to imply removal of the uterus during caesarean operation, either electively or as an emergency procedure, because of uterine infection or infarction, but it applies equally to spaying during pregnancy. It is a fact, paradoxically, that mid- to late pregnancy is the safest time for elective spaying because the ovarian attachments are then stretched and haemostasis is easily achieved.

An unusual indication for ovariohysterectomy in the cat is postparturient eversion of the uterus (Figure 21.5), which can be removed in situ by exposure and ligation of blood vessels through a vaginal incision. Unless the tissues are grossly oedematous or traumatised, the operation is better performed at laparotomy after the eversion has been reduced by gentle traction.

Elective ovariohysterectomy in the bitch should not be performed during oestrus because of the increased vascularity of the subcutaneous, uterine and ovarian vessels and the friability of the genital tract at this time. Ovariohysterectomy is also often considered by some to be premature before the first oestrus on the assumption that it:

- increases the risk of urinary incontinence
- allows the development of infantile vulva

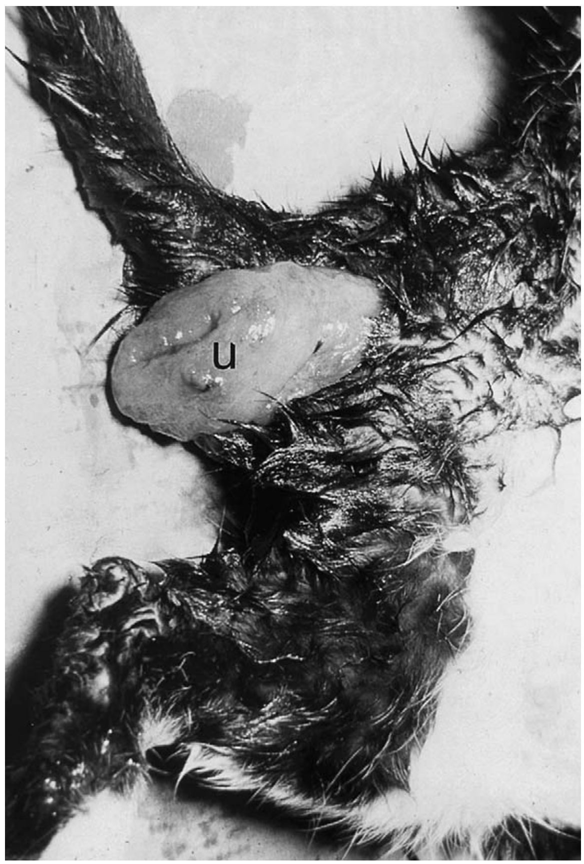

Fig. 21.5 Postparturient uterine eversion (u) in a queen.

- results in poor hair growth
- delays growth plate closure and increases the risk of physeal fractures especially in cats
- leads to obesity.

These contentions remain to be proven in suitably controlled studies.

Surgical technique

Ovariohysterectomy is a routine operation in small animal practice, and it is often regarded as a simple procedure which can be performed quickly, without assistance, through a small laparotomy incision. This is often not the case, and the routine ovariohysterectomy can be a technically demanding procedure especially in large and obese animals. It is essential to have good relaxation of the abdominal musculature and an incision of adequate length. In obese deep-chested bitches undergoing elective ovariohysterectomy, it may be impossible to expose the ovaries without significant traction. In such cases, section of the ovarian ligament, which is easily recognised if the mesovarium is tensed, facilitates ligation, but the tissues are then more likely to tear on traction.

The choice of suture material for ligation is important because the use of non-absorbable multifilament ligatures, especially in combination with poor surgical technique, may result in the formation of retroperitoneal abscesses and granuloma. In these cases, there is ultimately a single or numerous sinus tracts which discharge externally in the sublumbar region (Pearson, 1973). The chosen suture material must also be of adequate thickness to allow proper tightening.

The correct haemostatic technique for ligation of the ovarian pedicle consists of the application of three haemostatic forceps. The most proximal is subsequently removed to allow the ligature to be placed at the site of the crushed tissue, ensuring a snug fit with good compression of the tissue. However, in many cases there is insufficient exposure, much fat is present and the tissue is very friable. In these instances, it may only be possible to place two forceps, with the ligature being placed proximally to these. In these cases, the ligature may be placed through some of the perivascular tissues in the manner of a transfixing ligature. A common technical fault is to ligate immediately adjacent to forceps placed below the ovary; tissues fixed in a clamp cannot be adequately compressed by ligation until the clamp is released. It is therefore essential to ligate well below the clamp. Once the ligature has been placed, the pedicle is transected between the two distal forceps, allowing the ovary to be lifted from the abdomen. The pedicle should then be grasped with atraumatic forceps and the remaining haemostatic forceps should be removed to allow inspection of the pedicle for haemorrhage. The broad ligament is relatively avascular, but should be ligated in cases of pyometritis and in advanced pregnancy. The procedure is then repeated for the other ovary and with gentle traction the cervix and vagina should be visible in the incision. The lateral uterine vessels are normally ligated at the level of the proximal vagina using either a tight encircling ligature or a transfixing technique. Transfixing ligatures may become contaminated in the vaginal lumen and subsequently act as a focus of infection and predispose to secondary haemorrhage. In cases of pyometritis and gravid hysterectomy, it is a wise precaution to ligate each pair of uterine vessels separately close to the main vaginal ligature. The tissue is then transected at the cranial vagina, and the stump is lifted through the incision to inspect for haemorrhage before replacement in the abdomen. The cut end does not require closure or inversion. The ovarian pedicles and vaginal stump should be inspected prior to routine closure of the incision.

Complications

The complications of ovariohysterectomy are well documented (Pearson, 1973; Dorn and Swist, 1977). After surgery for pyometritis and dystocia, the animal may require intravenous fluid therapy (see below). Haemorrhage is the most common cause of death, and most frequently results from ligature failure at the ovarian pedicle, vaginal stump or broad ligament vessels. In most cases, careful inspection of these prior to closure of the incision will reveal blood leakage, although in some cases haemoperitoneum develops immediately post-surgery. This should always be considered in the animal that takes longer than expected to recover from anaesthesia. In addition, there may be a tachycardia, tachypnoea, pale mucous membranes, weak pulse and a prolonged capillary refill time. Blood may leak from the wound and there may be abdominal distension. In the post-operative period it may be difficult to decide whether to manage these cases conservatively or to intervene surgically. If the condition is progressive, the animal should be stabilised with intravenous fluid therapy prior to laparotomy. In this instance, the abdomen should be approached through the initial incision, but this should be extended to allow careful inspection of the pedicles and vaginal stump. Once the peritoneum is opened, it is best to exteriorise the small intestine on to saline-soaked swabs to allow inspection of the ovarian pedicles. Suction is extremely useful to allow careful examination of the site. The right ovarian pedicle can best be located by identifying the duodenum (lying against the right lateral abdominal wall) and retracting it across to the left side. This moves all abdominal contents away from the right side (since they are trapped in the mesoduodenum) and allows an unobstructed view of the right ovarian pedicle. A similar procedure can be performed on the left side using the descending colon. The vaginal stump is best approached by retroflexing the urinary bladder through the incision, allowing inspection of the vagina dorsally. The broad ligament can be identified in the dorsal abdomen. New ligatures should be placed on any site where haemorrhage has been identified. If there are multiple sites of haemorrhage there may be a clotting disorder.

In some cases, there may be a haemorrhagic vulval discharge some time after ovariohysterectomy. This may be due either to necrosis around vaginal stump ligatures or infection at this site. Rarely, the haemorrhage is severe and requires immediate resection of the stump. Many cases resolve spontaneously and are supported by antimicrobial and fluid therapy until that time.

In some cases, there may be inclusion of a distal ureter in the vaginal stump ligatures, or of the proximal ureter in the ovarian pedicle ligature. Usually this is unilateral and results in renal enlargement and hydronephrosis. If diagnosed shortly after surgery the ligature may be removed with some recovery of renal function. Otherwise animals become ill and renal function is lost, necessitating a nephro-ureterectomy.

Rarely a uterine stump pyometra may develop following ovariohysterectomy. This occurs only if the hysterectomy is performed to a level proximal to the cervix and either an ovary is left in position, or exogenous reproductive steroids are administered to the bitch. The clinical signs are similar to those of a conventional pyometra. In most cases leaving an ovary or a portion of ovary results in recurrent oestrus behaviour that is discussed below.

A serious potential long-term complication of spaying is urinary incontinence. The most common cause of incontinence in bitches after ovariohysterectomy is sphincter mechanism incompetence. The exact aetiology is poorly understood, but the condition appears to be multifactorial, and ovariohysterectomy appears to be a contributing factor. In a survey, Ruckstuhl (1978) recorded an overall incidence of 12% in 79 animals within 1 year of surgery and a frequency in larger breeds of almost 18%. The exact relationship is somewhat contentious but Thrusfield (1985), analysing a first opinion clinic population, found a positive association between all forms of acquired urinary incontinence and ovariohysterectomy in bitches aged 6 months or more. In a review of sphincter mechanism incontinence in the bitch, Holt (1985) found that 35 of 39 adult incontinent bitches in his series had been surgically neutered. Most cases develop clinical signs within 1 year of surgery. Arnold (1993) also found that 20% of bitches became incontinent after ovariohysterectomy, and showed that in 12 bitches there was a reduction in urethral pressure profile and urethral closure pressure after surgery, although none of those dogs developed incontinence. Once an accurate diagnosis of the condition has been made, it may be controlled by increasing urethral tone either by the administration of exogenous oestrogen or by alpha-adrenergic drugs such as phenylpropanolamine. Surgical treatment includes urethropexy techniques designed to increase the effective length of the urethra.

A further adverse effect of ovariohysterectomy is that bitches may lose the ability to regulate food intake. This can be controlled and obesity can be prevented by careful monitoring of feeding and exercise regimes by the owner.

Some workers have suggested that normal endocrine status can be maintained for at least a short period of time by the transplantation of ovarian tissue into an area of splanchnic venous drainage (Le Roux and Van der Walt, 1977). This technique has had limited study, but transplantation of segments of one ovary into the wall of the stomach caused their secretions to be metabolised in the liver in such a way that cyclical signs of oestrus waned after a curtailed pro-oestrus phase. More importantly, there was a high incidence of neoplasia at the site of transplantation (Arnold et al., 1988), such that the technique can no longer be recommended.

OVARIECTOMY

In the UK and USA, ovariectomy is an uncommon procedure for surgical neutering of bitches and queens, and it is frequently and erroneously thought that removal of the uterus is essential for the neutering procedure. In fact, ovariectomy alone is widely practised among veterinary surgeons in many European countries. Removal of the ovaries alone has several advantages over ovariohysterectomy including the following:

- The procedure is faster and less traumatic.
- The incision can be made more cranially allowing good exposure of the ovarian pedicle.
- There is some evidence, although anecdotal, which suggests a lower incidence of post-surgical urinary incontinence.

After removal of the ovaries, the uterus becomes small and atrophic and subsequent disease is unlikely unless exogenous reproductive steroids are administered to the bitch. In fact, the only common spontaneously occurring uterine disorder, pyometritis, is dependent on cyclical ovarian activity. The technique of ovariectomy also prevents operative haemorrhage due to inadequate ligation of uterine vessels, and the delayed but occasionally fatal bleeding associated with infection of the vaginal stump ligature. It also obviates the risk of accidental inclusion of ureters in the ligature and delayed uterine stump adhesions. The procedure has similar advantages to ovariohysterectomy in that it can protect against pyometra and other uterine disease, and if performed before the first or second oestrus it can prevent mammary neoplasia.

OVARIOHYSTERECTOMY FOR PYOMETRA

There have been advances in the medical treatment of canine pyometra including the use of prostaglandins, prolactin inhibitors combined with prostaglandin and progesterone receptor antagonists such as aglepristone. Non-surgical treatment by catheter drainage of the uterus per vaginam has also been described (Funkquist et al., 1983; Lagersted et al., 1987). Despite these, surgery remains the first-line treatment for most cases.

The surgical technique for ovariohysterectomy for pyometra is similar to that for surgical neutering. There are, however, a number of problems that are frequently encountered including:

- fluid electrolyte and acid–base imbalances
- renal dysfunction
- sepsis (the peritoneal cavity may be filled with pus)
- hypo- or hyperglycaemia
- hepatic damage
- cardiac dysrhythmias
- clotting abnormalities (White 1998).

In all cases, intravenous fluid therapy is mandatory and in cases which are collapsed initial infusion rates of up to 90 ml/kg/hour may be necessary. A balanced electrolyte is normally administered and since the most common acid–base abnormality is metabolic acidosis, Hartmann's solution is probably most useful. Ideally, the electrolyte and pH status should be monitored since both severe acidosis and hypokalaemia may develop. Similarly renal function should be assessed and fluid input should result in a urine output of more than 1 ml/kg/hour. In such an environment renal function will correct many of the acid–base imbalances. Hypoglycaemia is a common sequela to sepsis and any concurrent acidosis will impair gluconeogenesis such that it is important to establish blood glucose concentrations and to treat hypoglycaemia. In most cases of sepsis the commonly isolated organisms include *Escherichia coli, Staphylococcus* spp. and *Streptococcus* spp., such that cephalosporins are often the first-choice antimicrobial agent.

The surgical technique is similar to that for elective neutering, although the uterus is more friable and there is an increased risk of rupture during exteriorisation (Figure 21.6). Once exteriorised, the uterus should be packed off from the abdomen using saline-soaked swabs. The ovarian pedicles are ligated in the normal manner. Often vessels within the broad ligament are large and require ligation rather than simple tearing as in an uncomplicated ovariohysterectomy. There is some debate over the optimal excision at the uterine stump. Normally, the pedicle should be transected through the cranial vagina using absorbable suture material, and the stump should not be oversewn or inverted. If the stump is thought to be contaminated, omentum should be sutured over the stump prior to closure of the abdomen. If there is gross contamination of the abdomen with pus, this should be removed by suction and the abdomen should be lavaged using several litres of warmed physiological saline. Open peritoneal drainage may be necessary in severe cases. Postoperative complications following removal of a pyometra are similar to those following routine ovariohysterectomy.

Ovariohysterectomy for the treatment of pyometra is occasionally complicated by incarceration of a segment of one horn in an inguinal metrocele. Simultaneous herniorrhaphy and laparotomy may be necessary, but preoperative aspiration of pus should first be attempted to relieve the incarceration and allow the uterus to be excised in the normal way. Conversely, it may be possible to remove the entire uterus at herniorrhaphy, but this approach is not to be recommended.

OVARIAN REMNANT SYNDROME

In both the bitch and the queen, ovarian remnant syndrome is usually a result of incorrect surgical technique where a whole ovary (most commonly the right ovary) or a portion of it is left behind. In the author's experience seeding of the abdomen with ovarian cells is very rare, probably because of the presence of the ovarian bursa in these species.

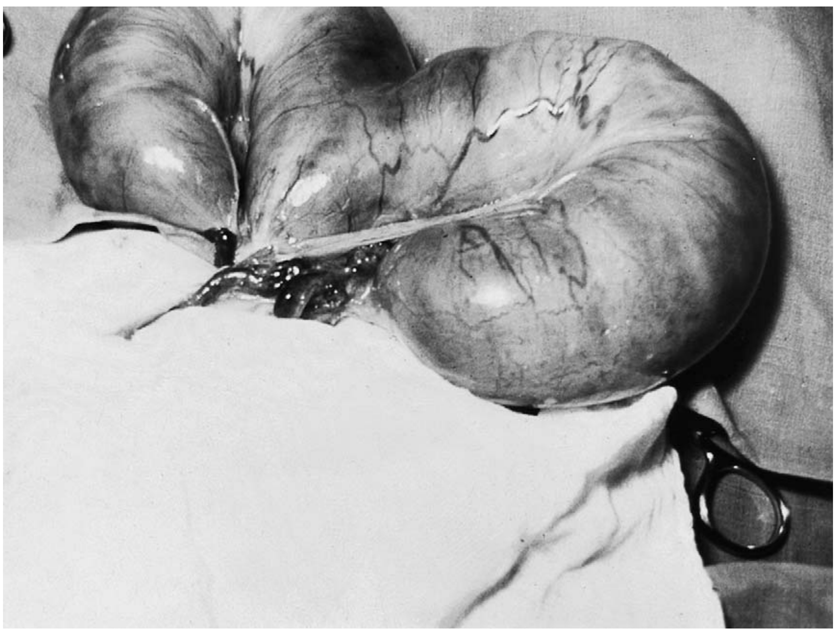

Fig. 21.6 Exteriorised uterus of bitch with pyometra. Note the grossly distended uterine horns causing increased fragility of the uterine wall.

Usually the female exhibits a regular return to oestrus, although in the bitch there may be no red-coloured discharge if a complete hysterectomy was performed. There may be pseudopregnancy after the oestrus, although care should be used in interpreting this sign since some cases of pseudopregnancy are the result of removal of the ovaries during the luteal phase. True signs of oestrus may be useful for the diagnosis of ovarian remnant syndrome although some bitches demonstrate sexual behaviour at various stages of the oestrous cycle as well as after surgical neutering, and others may be attractive to males because of the presence of a low-grade vaginitis. Accurate diagnosis requires the examination of a vaginal smear during oestrus. This demonstrates large anuclear epithelial cells (red blood cells may be absent). Alternatively in the bitch, plasma progesterone concentrations can be measured 2 weeks after the clinical signs of oestrus have disappeared. A high concentration of progesterone indicates the presence of luteal tissue from an ovarian remnant. In the queen, progesterone will only be produced after ovulation, which can be stimulated by the administration of human chorionic gonadotrophin (hCG) during the signs of oestrus (England, 1997).

Surgical exploration is best performed during oestrus or in the early luteal phase when the ovary is at its largest size. Usually the ovary can be palpated within the fat of the ovarian pedicle. If no tissue can be detected, it is prudent to remove both ovarian pedicles which usually contain the remnant material.

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22 Infertility in the cow: structural and functional abnormalities, management deficiencies and non-specific infections

GENERAL CONSIDERATIONS

Fertility is one of the key determinants of the lifetime performance of a cow. For beef cows and for pastoral dairy cows, it is necessary for a calf to be produced every 365 days. For intensively managed dairy cows, such as those of the UK and North America, the need to produce a calf each year is less of an imperative; yet even for these animals, regular calving is still essential for the establishment of lactations.

Regular breeding depends upon the normal function of the reproductive system. In order to breed regularly, the cow has to have functional ovaries, display oestrous behaviour, mate, conceive, sustain the embryo through gestation, calve, and resume oestrous cyclicity and restore uterine function after calving. Each of these aspects of reproductive function can be affected by management, disease and the genetic make-up of the animal. When the function of the reproductive system is impaired, cows fail to produce a calf regularly. When this occurs, the term 'sterility' is used to mean an absolute inability to reproduce; whereas the term 'infertility' either is considered to be synonymous with sterility, or may imply a delayed or irregular production of the annual live calf. The term 'subfertility' is probably a more appropriate term for the latter.

Prevalence and cost of infertility

Estimates of the prevalence of infertility in dairy cows have been made over many years, and it is interesting to compare historical trends. In 1955, Asdell estimated that at any time 10% of cows were experiencing some form of breeding trouble. At a similar time in the UK, Grunsell and Paver (1955) estimated that 4% of cows per year were treated for infertility and other pathological conditions of the genital organs. Leech et al. (1960) found that 3.7% of cows were culled for infertility. Gracey (1960) quoted a similar figure, of 5.2%, for Northern Ireland.

Examination of culling rates and the reasons for culling cows have been used as indicators of the prevalence of subfertility in herds. Analysis of cow disposal figures for European and American dairy herds suggested that about a third of all cows were culled because of reproductive disturbances, that 4–5% of heifers were sterile and about 5% of calves were stillborn or died at birth (Johannsson, 1962). In a survey carried out in the state of Kansas, 22% of cows were culled for breeding problems (Bozworth et al., 1972), which ranked second only to low production as a reason for disposal. In two studies on the reasons for the disposal of dairy cows in England and Wales for the years 1972–73 and 1976–77 the percentages that were culled for reproductive conditions declined from 43.2% to 33.0% (Beynon, 1978). Despite the evidence of some reduction in the numbers of cows with breeding disorders in these two surveys, no specific explanation could be found for the apparent improvement.

However, information on culling rate and reasons for culling should be interpreted with some care in estimating the prevalence of infertility. For example, it is common for cows that are slaughtered for apparent failure to conceive to be found to be pregnant (Singleton and Dobson, 1995). Moreover, it is of great importance to know the production system from which infertile cows have been culled. Pastoral dairy cows and beef cows, which need strictly annual calving patterns, are more likely to be culled for infertility than animals for which the calving date is of little importance. In year-round calving systems, persistence of lactation is also of importance in the decision whether or not to cull for infertility (Dijkhuizen et al., 1985).

Comparisons have also been made of the relative importance of reproductive and other disorders. For example, in a study of 32 dairy herds in South Ontario, Canada, comprising 2876 lactations, the records for 1979–81 showed that the lactational incidence rate for reproductive disorders totalled 43.2% compared with 16.8% and 5.0% for mastitis and locomotor disease (Dohoo et al., 1983). In the US state of Michigan, the mean incidence densities for breeding problems, mastitis and birth problems per 100 cow-years were 49.86, 33.06 and 13.81, respectively. In a study of 43 dairy herds in California, a mean of 24.8% of the cows were culled each year, with reproductive failure the most common cause (Gardner et al., 1990). In Ohio State, the mean numbers of cases per 100 cow-years were: reproductive disease, 73 (42%), mastitis, 37 (21%) and pneumonia, 19 (11%) (Miller and Dorn, 1990). A similar trend occurred in a survey of 34 herds in New York State, in which 26% of culls were removed for reproductive problems (Milian-Suazo et al., 1988). Reproductive disease or involuntary culling for failure to conceive were the major reasons for cows being culled in each of these surveys. In a recent large-scale survey of reproductive performance in New Zealand dairy herds, 13.6% of cows were culled during each season. Of these, 42.5% were culled for failure to conceive by the end of the breeding season and an additional 4.4% were culled for other reproductive problems. Mastitis was by far the most important disease; 10.4% of cows had mastitis during each year, whereas retained fetal membranes and uterine infections together occurred in only 2% of animals (Xu and Burton, 2000).

Infertility is a considerable problem in the beef herd, although relatively few data exist detailing its prevalence. Nevertheless, postpartum and lactational anoestrus are significant problems in beef herds, which, if animals are culled on the basis of calving pattern, can lead to significant losses of animals due to conception failure. New Zealand data indicate that between 7 and 11% of beef cows fail to conceive (Morris and Cullen 1998). Under the range conditions of Northern Australia, 75% pregnancy rates can be achieved, although this can drop to as low as 15% (O'Rourke et al., 1991). However, for feedlot and intensively managed beef cattle, pregnancy rates of up to 98% have been reported (e.g. Warren et al., 1988), with first service conception rates of between about 75 and 80% (Brown et al., 1991; Mann et al., 1998).

Economic consequences of infertility

Studies of the consequences of the losses due to infertility upon dairy herds have attempted to quantify its effects upon production and financial performance. All agree that infertility is expensive, although the level is highly dependent upon the production system. Infertility leads to a loss of milk production, a loss of income from calf sales and an increase in the replacement rate of cows with first-calving heifers. Its effects may, to some extent, be mitigated by the income that is derived from cull animals, although this is largely a matter of perception than actuality, for the costs of replacing mature cows with first-calving heifers are substantial.

Early studies of the economics of infertility were undertaken in the USA, where it was found that in Holstein cows the consequence of extending the calving interval from 12 to 14 months resulted in an average reduction in the annual financial return over feeding costs of 8.8% (Speicher and Meadows, 1967). The same extension of the calving interval resulted in an average loss of 144 kg milk per cow and 0.15 calves per cow (Lauderdale, 1964).

In the early 1990s, US data suggested that the total cost of breeding problems was $24.46 per cow per year, compared with $35.54 for mastitis (Kaneene and Hurd, 1990). In the UK, Esslemont (1992) calculated that for dairy herds, at 1992 prices of such items as milk, feed, calves, replacement heifers and culled cows, the cost to the farmer for each day's extension of the calving interval beyond 365 days was as much as £3.35. Data from France over the same period showed that an improvement of 1% in conception rates was worth FF10–20 per cow per year (Boichard, 1990). Estimates from the USA also suggested that a 1% increase in conception rate was worth up to $7.36 per cow per year (Pecsok et al., 1994a). Improvements in oestrus detection rates have also been associated with improved herd economic performance, Pecsok et al. (1994b) considering that an improvement from 60% to 70% detection efficiency was worth $6 per cow per year.

Bozworth et al. (1972) stated that 'infertility is one of the important economic losses in high producing herds and that modern feeding and management practices in large herds may accentuate the problem.' This comment is equally true today.

Overview of the causes of infertility

In many parts of the world, the last 40–50 years have seen a noticeable change in the relative importance of causes of infertility in cattle. In some places, the importance of the classical infectious diseases of reproduction has been dramatically diminished. The recognition of *Trichomonas fetus* infection (Stableforth et al., 1937) and *Campylobacter fetus* infection (Sjollema et al., 1949) as causes of widespread infertility constituted major advances. For example, control measures, in particular the widespread use of artificial insemination, have largely eliminated these diseases from the UK. Similarly, the eradication programmes for bovine tuberculosis and brucellosis have reduced the importance of both of these diseases as causes of reproductive loss. Although non-specific infections due to opportunist pathogens are still important, by far the greatest cause of infertility is due to management. In the dairy industry, this has been largely due to the increase in herd size, the increases in the mechanisation of farming and the concomitant reduction in the number of people attending the herds. At the same time, the demands put upon the dairy cow to produce more milk and the genetic selection for high yield have inevitably resulted in functional aberrations of the reproductive and endocrine systems. Changes in fertility, associated with such factors as those just described, are illustrated by a study that evaluated the fertility of dairy herds in New York State which were under the Dairy Herd Improvement Testing Scheme (Butler and Smith, 1989). In 1951, the mean overall pregnancy (conception) rate was 66% for both cows and heifers; in 1973 the figure had fallen to 50% for cows, during which time the average annual milk production per cow had risen by 1500 kg (33%). In a more recent survey, the same authors have shown that whereas milk production has increased by approximately 1500 kg from 1973 to 1985, mean overall conception rates in 1985 were 51%. Pregnancy rates for heifers are virtually the same as they were in 1951.

Infertility in the individual cow

Both congenital and acquired abnormalities of the genital system can influence fertility. The latter type are more frequently encountered, as demonstrated in a survey by Kessy (1978), who found that amongst 2000 genital tracts from abattoirs that were examined only six specimens (0.3%) had evidence of congenital abnormalities, compared with 194 (9.65%) with acquired lesions. Since most of the latter were identified in the tracts from parous specimens, the importance of conditions that might occur during pregnancy, and especially at parturition and during the puerperium, is demonstrated. Similar results were demonstrated by Al-Dahash and David (1977a). A summary of the results of Al-Dahash and David (1977a) is given in Table 22.1. Anatomical abnormalities usually affect individual cows or heifers, and are therefore unlikely to have a major influence on fertility in a herd.

LESIONS OF THE OVARIES

Congenital lesions of the ovaries

Congenital lesions of the ovaries are rare. A few reports exist of instances in which one or both ovaries are absent (ovarian agenesis), accompanied by an infantile genital tract and an absence of cyclical behaviour. Fincher (1946) saw an apparently hereditary condition of 'virtual absence of ovaries' in three maternal half-sister heifers.

Ovarian hypoplasia is a little more common. In this condition, one or both ovaries are small, functionless and composed of largely undifferentiated

Table 22.1 Incidence of reproductive abnormalities (from Al-Dahash and David 1977a)

Abnormality	*Number*	*Percentage*
Total specimens	8071	
Total pregnant	1885	23.36
Non-pregnant	6186	Percentage of non-pregnant specimens
Cystic ovaries	200	3.23
Cystic ovaries with mucometra	13	0.22
Ovarian cysts with normal corpus luteum	94	1.52
Ovarian tumours	14	0.23
Ovaro-bursal adhesions	148	2.39
Hydrosalpinx	65	1.05
Non-involuted uterus	136	2.20
Mummified fetus	22	0.36
Macerated/emphysematous fetus	8	0.13
Pyometra	68	1.10
Mucometra	14	0.23
Segmental aplasia	3	0.05
Uterine adhesions	19	0.31

parenchyma. Oocytes and follicles are virtually absent. Ovarian hypoplasia is generally a sporadic condition, except in the gonadal hypoplasia syndrome of the Swedish Highland breed. A high incidence of gonadal hypoplasia was recognised in males and females of this breed; Lagerlöf (1939) found an incidence of 13.1% of ovarian hypoplasia amongst 8145 cows. Where both ovaries were hypoplastic the genital tract was infantile and oestrous cycles did not occur. Eriksson (1943) concluded that the condition is inherited as an autosomal recessive with incomplete penetrance. There was a marked association of gonadal hypoplasia with white coat colour. By the adoption of a vigorous control programme, in which veterinary examination of breeding cattle led to the recognition and culling of cases of unilateral hypoplasia, the incidence of gonadal hypoplasia in Swedish Highland cattle was reduced from 17.5% in 1936 to 7.2% in 1952 (Lagerlöf and Boyd, 1953). Ovarian hypoplasia also occurs as an occasional finding in most breeds of cattle. No inherited basis for the condition has been demonstrated in these animals although, interestingly, Arthur (1959) reported a small number of cases in white Ayrshire heifers, which were acyclical and had hypoplastic ovaries.

Acquired lesions of the ovaries

The most common of the acquired lesions of the ovaries, cystic ovarian disease, is considered to be a functional disturbance of ovarian function and, hence, is considered later in this chapter.

Ovaritis (oophoritis; Figure 22.1) is a very rare lesion of the ovary. The authors have seen one or two cases as adventitious findings at post-mortem examination. McEntee (1990) describes cases of tuberculous oophoritis, brucella-induced oophoritis and ovarian abscessation in animals that have had generalised pyaemia. He further suggests enucleation of the corpus luteum as a cause of ovarian abscesses, possibly when it has been undertaken in cows suffering from perimetritis.

Granulosa cell tumours (Figure 22.2) and fibromas are generally the most common neoplasms of the bovine ovary. Lagerlöf and Boyd (1953) found 3 granulosa cell tumours and 1 fibroma in a survey of over 6000 bovine reproductive tracts, while Al-Dahash and David (1977a) found 7 fibromas and 2 granulosa cell tumours amongst 8000 bovine tracts. Lagerlöf and Boyd (1953) also found 3 carcinomas in their survey. In fact, most of the large cystic neoplasms of the bovine ovary are granulosa cell tumours. These tumours have been seen in pregnant as well as non-pregnant cattle. Granulosa cell tumours can produce any of the main ovarian steroids, although reports of oestrogen or androgen production are the most common in the literature (see McEntee, 1990). Tumours that secrete oestrogen cause animals to display nymphomaniacal behaviour, at least in the early stages of their development. Androgen-secreting tumours are more commonly associated with anoestrus, although in long-standing cases virilism may occur. The non-affected ovary is small and inactive, although Roberts (1986) reports that conception has been reported in a cow with a unilateral, non-steroidogenic tumour. Granulosa cell tumours are generally regarded as benign, although in one series, 9 out of 13 tumours had

Fig. 22.1 Infection and inflammation of the ovary (oophoritis) of an infertile cow; U = uterine horn. The ovary is arrowed.

Fig. 22.2 A granulosa cell tumour (t) involving the right ovary. The left ovary (o) is normal.

metastasised (Norris et al., 1969). McEntee (1990), on the other hand, considers metastasis to be very uncommon.

Other tumours of the bovine ovary have occasionally been reported. These include carcinomas, fibromas, thecomas and sarcomas. These tumours are generally benign and are often massive. Some of the largest tumours seen by the authors have been those of the ovary. For example, they have seen a Friesian cow with a granulosa cell tumour which weighed 24 kg; the cow showed successive phases of nymphomania, anoestrus and virilism. Another cow was presented with an ovarian carcinoma that had enlarged to occupy the caudal third of the abdomen and had metastasised widely throughout the mesenteries.

ABNORMALITIES OF THE UTERINE TUBES, UTERUS AND CERVIX

Segmental aplasia of the paramesonephric ducts

Developmental defects of the paramesonephric (Müllerian) ducts lead to a wide range of anomalies of the vagina, cervix and uterus. Depending upon the site of the aplasia, the cow may be subfertile or sterile. However, the ovaries develop normally and, consequently, affected animals show normal cyclic behaviour. Moreover, normal levels of steroid hormones are present, and there is a significant level of secretory activity within the tubular parts of the genital tract. Hence, when a developmental obstruction of the tubular tract occurs, cyclical secretions distend the lumen of the isolated portion of the tract.

Aplasia of each part of the tubular genitalia has been reported. In some cases, the whole of the vagina, cervix and uterine horns may lack patency. In these cases, as in the freemartin (see Chapter 4), the genital tract is difficult to locate per rectum but, unlike the latter, the ovaries are normal. More commonly, partial or segmental aplasia of the paramesonephric ducts occurs. In Kessy's survey (1978), the uterine tube was identified as a frequent site of congenital defects; unilateral aplasia was identified in 0.1% of the specimens and segmental aplasia in 0.05%.

In the case of uterus unicornis, only one uterine horn has a lumen, the other appearing as a narrow, flat band (Figure 22.3). It is more common for the right horn to be absent than the left. Provided the remainder of the genital apparatus is normal, individuals may conceive to ovulations from the sound side. A more serious type of aplasia occurs when isolated sections of uterine horn are present. Uterine secretion accumulates and causes sac-like dilatation of such isolated portions of the tract. These can become very large and can be confused with early pregnancy during examination per rectum (Figures 22.4 and 22.5). Animals with this deformity are sterile.

Abnormalities of the cervix also occur. Where there is duplication of the lumen of the cervix, each uterine horn connects with the vagina by a separate cervical canal. Affected animals conceive

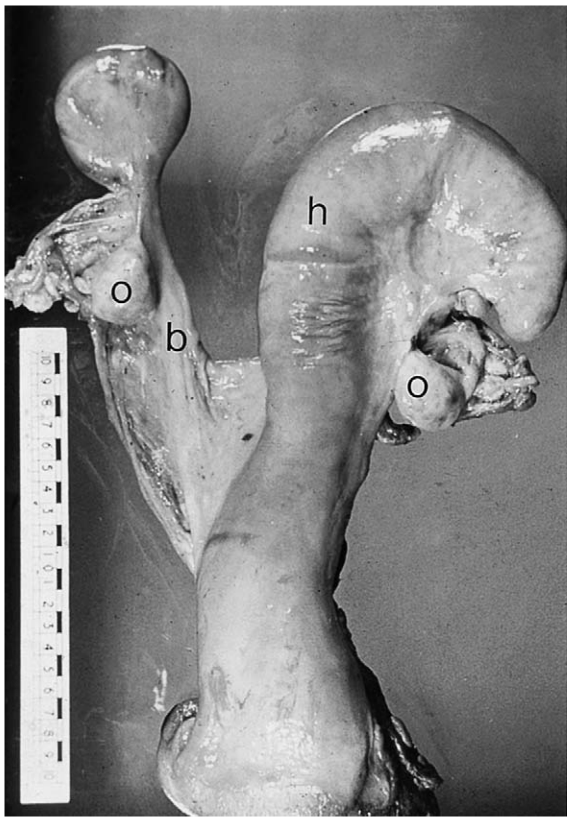


Fig. 22.3 Uterus unicornis. Note normal left and right ovaries (o) and complete right horn (h). The left horn comprises a flat band of tissue with no lumen (b) and a blind residual segment.

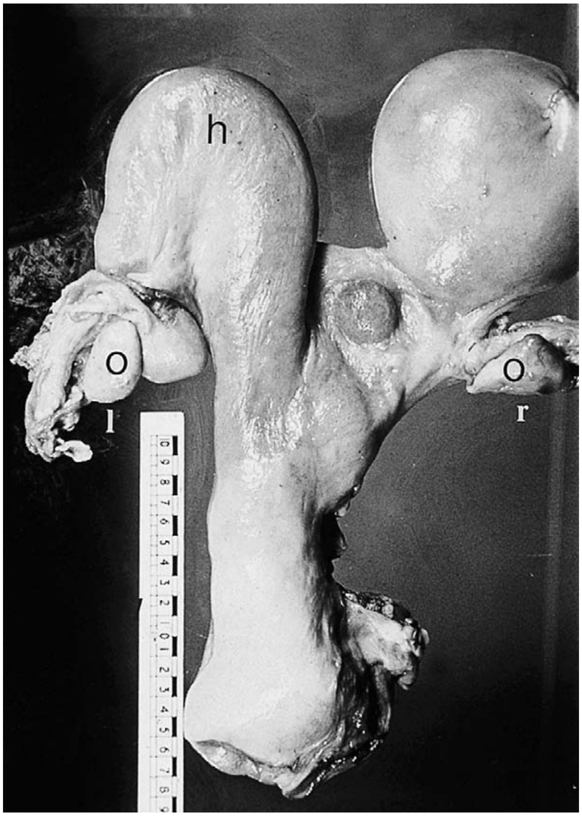


Fig. 22.4 Genital tract from a heifer with 'white heifer disease'. Note both ovaries (o) are normal with a corpus luteum present in the right and horns (h) distended with accumulated fluid.

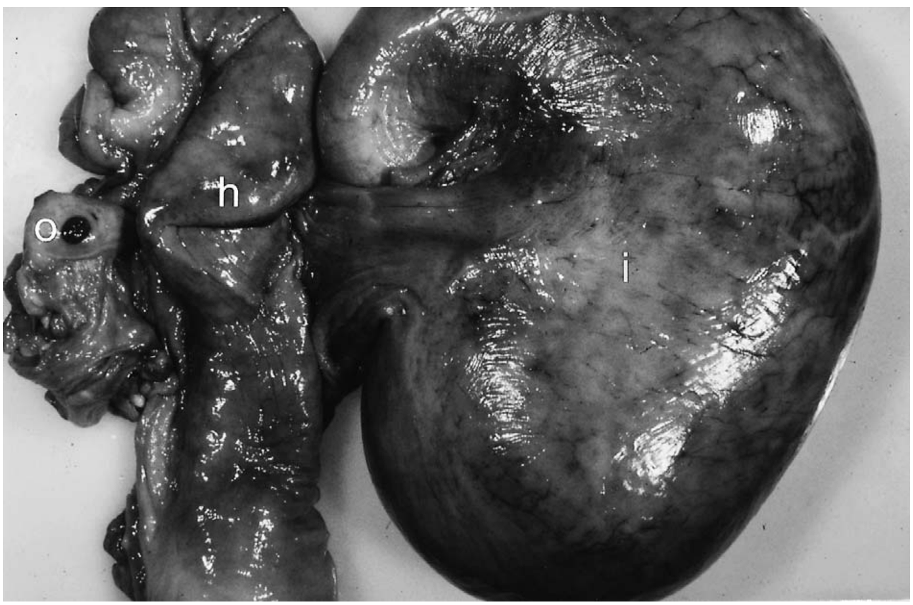


Fig. 22.5 Genital tract from a heifer with 'white heifer disease'. Note normal left ovary (o) and left horn (h), and the isolated portion of the right horn (i) greatly distended with accumulated fluid.

normally, but may show dystocia due to fetal limbs entering each cervical canal. A similar complication may arise in heifers with a single cervix opening into a double os uteri externum (Figure 22.6) and in cows with a dorsoventral postcervical band. The expulsion of the fetal membranes may also be impeded by these structural aberrations. Vertical vaginal bands can be easily divided with a fetotomy knife. In cases of uterus didelphys (Figure 22.7), a double cervix is present, the uterine body is divided and there may be division of at least the cranial part of the vagina. This condition represents a complete failure of fusion of the two paramesonephric ducts. Such animals may conceive, providing insemination takes place into the horn ipsilateral to the ovulation; and a number of reports exist of them carrying calves to term and giving birth normally.

The most common developmental aberration of the female tubular organs involves a variable degree of persistence of the hymen. This may appear as a vaginal constriction in front of the urethral opening, as a partition with a central aperture or as a complete partition between the vulva and vagina. The first type is likely to be discovered at parturition when it causes dystocia. The second and third types are likely to be found when investigating heifers which either strain forcibly after service, or cannot be inseminated artificially. Where hymenal obstruction is complete, there is an accumulation of secretions in front of the obstruction, which causes a fluctuating swelling of variable size that may be palpated per rectum. Following service, this retained secretion may become infected by pyogenic organisms. The less severe forms of hymenal obstruction may be rendered suitable for breeding by making cruciform incisions into the partition. Heifers with complete obstruction, which are ill because of retained pus, can be relieved by trocar and cannula and then fattened for slaughter. However, it should be noted that, in view of the probable hereditary

Fig. 22.6 Uterus didelphys with double external os uteri.

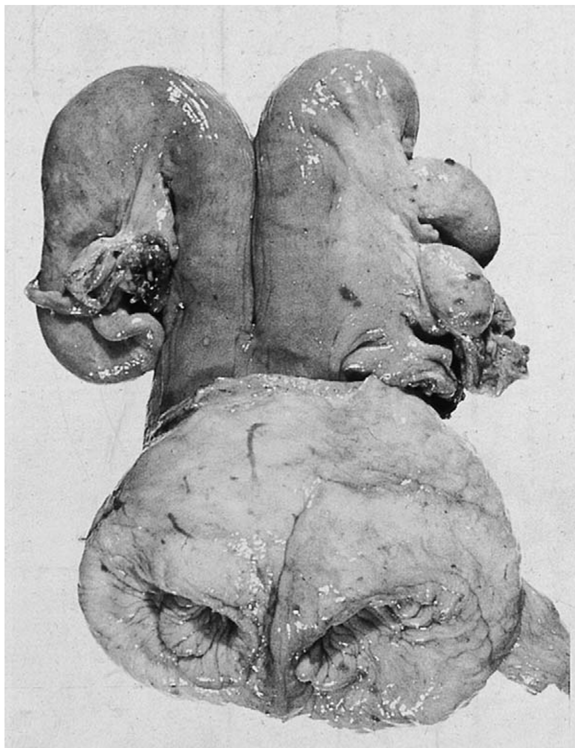


Fig. 22.7 Uterus didelphys showing two completely separate cervical canals.

origin of these developmental defects, surgical intervention in order to make breeding possible is not advisable. The genital organs of most heifers with hymenal constriction are otherwise normal, but occasionally segmental aplasias of other parts of the tubular organs are present.

The aforegoing developmental anomalies may arise in all breeds of cattle, although hymenal defects occur particularly commonly in white shorthorn heifers (which was the commonest breed in the UK up to the end of the 1950s). Thus, the syndrome of straining and illness after service has become known as 'white heifer disease'. This condition is considered to be due to a sex-linked recessive gene with linkage to the gene for white coat colour. The other developmental defects of the paramesonephric ducts are probably also due to sex-linked recessive genes; consequently they are likely to appear when in-breeding is practised. For example, Fincher and Williams (1926) reported that 56% of heifers were affected amongst the progeny that resulted from the mating of a Friesian sire with his daughters.

Freemartinism (see Chapter 4)

Freemartinism (Figure 22.8) is a distinct form of intersexuality which arises as a result of a vascular anastomosis of the adjacent chorioallantoic sacs of heterozygous fetuses in multiple pregnancies (Lillie, 1916). As a result, although the external genitalia of freemartin heifers appear normal, the internal genitalia are grossly abnormal. Typically, the gonads are either vestigial or have undergone masculinisation. The structures derived from the paramesonephric ducts are almost entirely absent or are grossly hypoplastic. In animals that have undergone a significant degree of masculinisation, the gonads resemble testes, to the extent that their parenchyma contains recognisable tubules and interstitial tissue. Development of the mesonephric (Wolffian) ducts is related to the degree of masculinisation of the gonad. In extreme cases, there are well-developed epididymides, vasa deferentia and vesicular glands (Short et al., 1969). Conversely, in the least affected cases, the female genital tract may be small, with a persistent hymen and hypoplastic ovaries (Wijeratne et al., 1977). These animals may have some occytes present in their gonads, and may even have small follicles and luteal-like tissue (Rajaoski and Hafez, 1963). More typically, the vestigial gonads of freemartins are devoid of oocytes and, hence, follicles, but have parenchyma that consists largely of degenerating sex-cords.

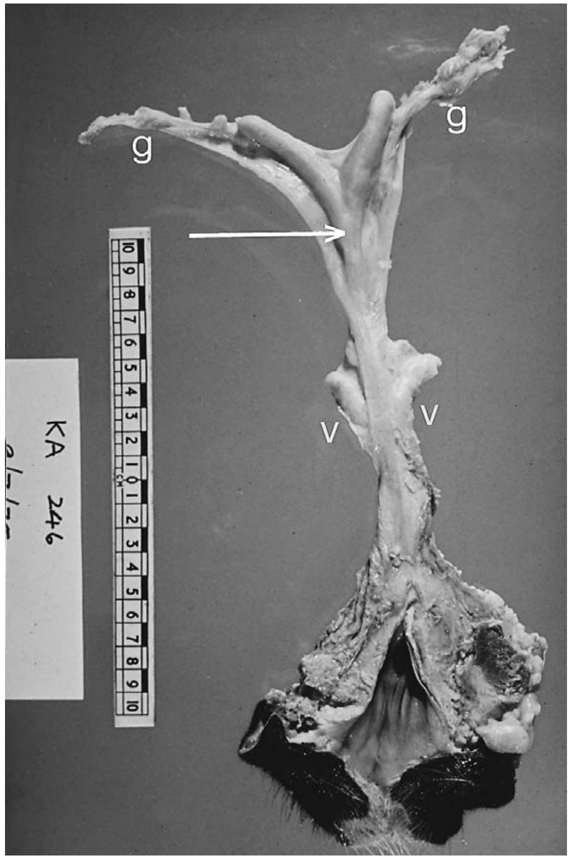


Fig. 22.8 Reproductive tract from a freemartin heifer. Note the vestigial gonads (g), underdevelopment of structures derived from the paramesonephric ducts (arrow) and rudimentary vesicular glands (v).

It is generally assumed that 92% of heifers which are born as co-twins to bulls are sterile freemartins (Biggers and McFeely, 1966). Vascular anastomosis occurs as early as 30 days of gestation; thus if there is death of the male twin of a heterozygous pair after this time with the other being carried to term, it is possible for a single-born freemartin to occur. This has been demonstrated as a cause of infertility in heifers with apparently normal external genitalia but with sex chromosome chimerism (Wijeratne et al., 1977).

The newborn freemartin can sometimes be recognised by its prominent clitoris with an obvious tuft of hair at the inferior commissure of the vulva, although these signs are not always reliable. Freemartins can be identified on the basis of the length of the vagina and the absence of the cervix. In the adult, the vagina is normally 30 cm in length, compared with 8–10 cm in the freemartin. Rectal palpation will fail to identify the cervix. In calves of 1–4 weeks of age, the vagina is normally 13–15 cm in length compared with 5–6 cm in a freemartin. Diagnosis at this age can be made using a blunt probe which should be inserted initially at an angle of 45° below the horizontal for 5 cm and then angled downwards to avoid impinging on the hymen (Long, 1990). It is easier when comparisons can be made between a number of animals.

The most accurate method of diagnosis, although not absolute, is the demonstration of sex chromosome chimerism in cultured lymphocytes. Heifer calves which are born co-twins to males and which show morphological changes in their reproductive tracts invariably show sex chromosome chimerism in blood and blood-forming tissues. Unfortunately, the distribution of male cell percentages in freemartins appears to be random; hence those with low male percentages in the blood will be as common as those with high male percentages (Wilkes et al., 1981). It is also possible to identify the presence of two populations of erythrocytes by haemolytic tests using a series of specific blood group reagents (Long, 1990).

The economic importance of early diagnosis of freemartinism has been shown by the survey of David et al. (1976), who found that a large number of heifers which were sold in markets for breeding were freemartins. Very high incidences of freemartinism can sometimes be found, therefore, in herds of heifers – most commonly those of heifer rearers – which are purchased from markets. This could also become important if induction of twinning by superovulation or embryo transfer becomes popular. At the time of writing, a move is being made in the UK to use Trade Description legislation to ensure that freemartin heifers (or those heifers that were co-twin to a bull) cannot be sold in markets as normal animals.

Very occasionally, other forms of intersexuality are found. A few cases of pseudohermaphroditism have been reported, as have rare cases of XY sex reversal and true hermaphroditism (see Chapter 4).

Parovarian cysts

Parovarian cysts are remnants of the mesonephric ducts that are commonly present in the mesosalpinx of cows. Tiny parovarian cysts, of a few millimetres in diameter, are very common incidental findings in slaughtered cattle. Larger cysts, of between 1 and 3 cm in diameter, may be felt during examination of the tract per rectum when they may be confused with ovaries. Parovarian cysts are of no consequence to the reproductive performance of the animal, except in the rare instances when they impinge on the uterine tube and reduce its lumen.

Ovarobursal adhesions and lesions of the uterine tubes

Acquired lesions of the uterine tubes and adnexa are common in cattle. In 1921, Carpenter et al. showed that 15.3% of cows which were examined during routine clinical work had such lesions of the uterine tubes and adnexa. Many subsequent studies have confirmed this high frequency of occurrence. The percentage incidence ranged from 0.95% in an abattoir study in Australia (Summers, 1974) to 100% in a similar study in Egypt (Afiefy et al., 1973). One of the most frequently observed lesions of the bovine reproductive tract is adhesions between the ovary and the ovarian bursa (Figure 22.9). The incidence of ovarobursal adhesions in the surveys described above ranged from 0.43% (Summers, 1974) to 46% (Afiefy et al., 1973). Al-Dahash and David (1977a) reported an incidence of 1.83%. The condition is uncommon in heifers, but its incidence increases with the age of the cow.

Much variation exists in the extent of the adhesions that are present, ranging from fine web-like strands in the depth of the bursa, which do not involve the uterine tube, through to complete envelopment of the ovary within a closely applied fibrous bursa. Infections of the ovarian bursa,

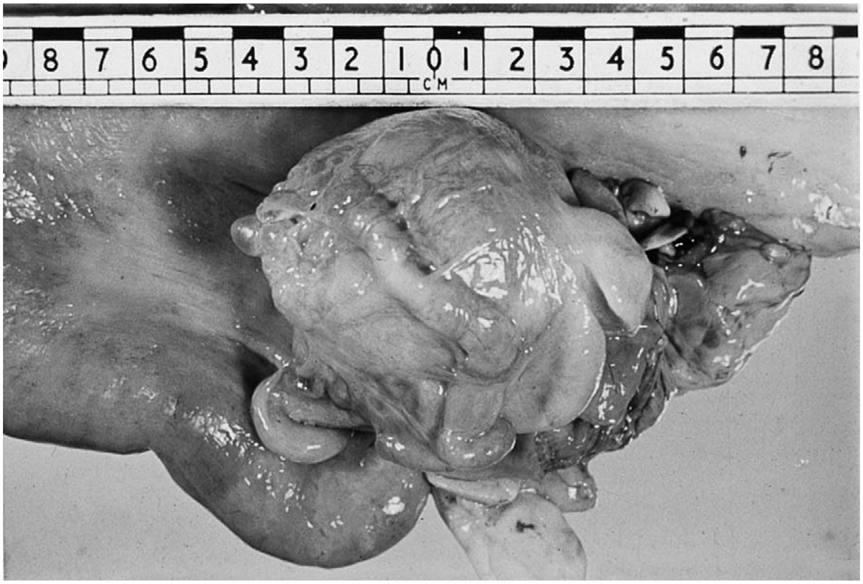


Fig. 22.9 Ovarobursal adhesions. Note that the ovarian bursa has completely enveloped the ovary.

which invariably result in large-scale adhesions between the ovary and bursa, also occur, often in association with metritis or salpingitis.

Edwards (1961) found the web-like adhesions in 62% of slaughterhouse cattle; it is unlikely that such lesions would interfere with fertility and they will not be discussed further. Intermediate cases of ovarobursal adhesions show fibrinous or fibrous strands of varying thicknesses connecting the fimbriae or bursae to the ovary. These strands are often attached to the ovary at the site of a scar of a regressed corpus luteum.

Of the remaining more severe types, between 25 and 50% are bilateral and likely to interfere with ovulation or to impede sperm or egg transport through the uterine tube. Of the unilateral cases, the right side is more frequently involved. Conception is unlikely to occur to ovulations from the affected side. Where the bursa is diffusely applied to the ovary, ovulation is prevented and luteinisation of the follicle occurs; the orange rim of the follicle being several millimetres thick. Ovarobursal adhesions are not infrequently associated with cystic ovarian disease, although it is not clear which lesion is causal. In such animals, regressed luteinised follicles from past cycles are often present in the same ovary.

Where the uterine tube is involved in the adhesions, its lumen may become occluded. A consequence of such occlusion is the accumulation of secretions, which causes distension and thinning of the wall, described as hydrosalpinx (Figure 22.10). Quite often a hydrosalpinx becomes secondarily infected by *Arcanobacterium (Actinomyces, Corynebacterium) pyogenes*, to produce a pyosalpinx or pyobursitis. Intra-ovarian and periovarian abscesses have also been seen (Arthur, 1962) in association with ovarobursal adhesions. Similarly, Kessy (1978) found four specimens out of 2000 where the uterine tube ipsilateral to ovarobursal adhesions was occluded. Two of these cases were associated with pyometra and two with the presence of a macerated fetus. Indeed, the most likely cause of ovarobursal adhesions in the pluriparous cow is puerperal infection which arises from ascending infection of the uterus or, in severe cases, perimetritis. It has also been suggested that they can be induced by the intrauterine infusion of irritant substances such as Lugol's iodine in large volumes, particularly under pressure as might be achieved using an enema pump.

The strand-like adhesions arising from scars of old corpora lutea (which more commonly affect the right ovary) may be regarded as physiological

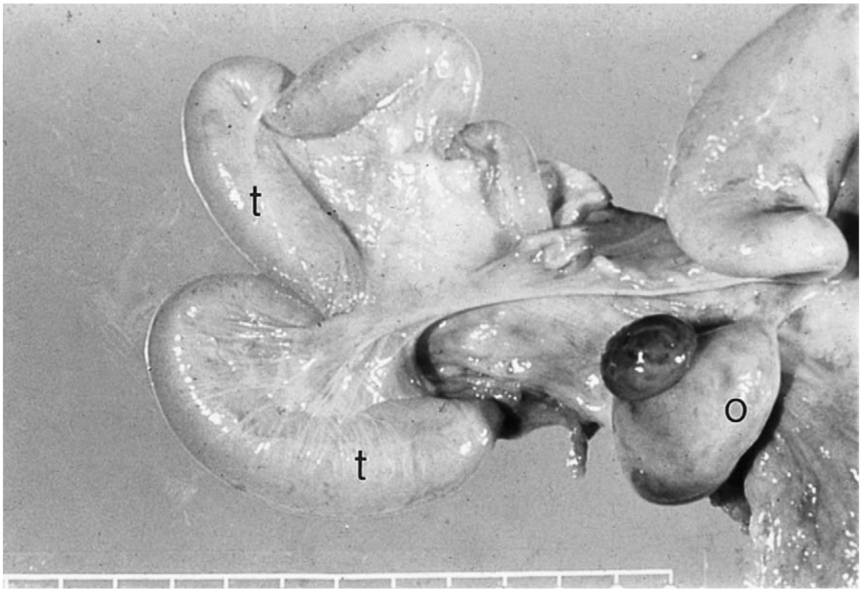


Fig. 22.10 Hydrosalpinx. Note distended ampulla of the uterine tube (t); the ovary (o) is unaffected and contains a corpus luteum.

hazards, which originate as slight haemorrhages from the site of ovulation. Interestingly, ovarobursal adhesions are also relatively common in sheep, a species which has a similar ovulating mechanism (see Chapter 25). It is possible that a proportion of ovarobursal adhesions can occur as a result of rough palpation of the ovaries, particularly where manual enucleation of the corpus luteum or rupture of an ovarian cyst is attempted. In the former, massive haemorrhage and death of the cow can occur, whilst in others, large haematomata attached to the surface of the ovary or filling the ovarian bursa have been identified. Since the availability of prostaglandins as luteolytic substances and with a more rational approach to the treatment of ovarian cysts, this cause should largely disappear.

Knowledge of other causes of ovarobursal adhesions is incomplete. The diffuse type of lesion, often with involvement of the uterine tube, was a relatively common accompaniment of tuberculous peritonitis. Adhesions may also occur as part of the more widespread peritonitis resulting from such conditions as traumatic reticular penetration or puerperal metritis. Mycoplasmas have also been suggested as a cause of ovarobursal adhesions. Hoare (1967) recovered mycoplasmas from a high proportion of ovarobursal and tubal lesions; although these organisms are commonly present in healthy cattle, the constancy of their occurrence in these particular lesions suggests an aetiological significance. It is believed that mycoplasmas become pathogenic when the resistance of the host is lowered: for example, as a result of a postparturient metritis or *Brucella* abortion. Hirst et al. (1966) have produced evidence of a causative relationship between mycoplasma-infected semen and infertility due to ovarobursal disease. In passing, it may be noted that ovarobursal adhesions are a feature of the viral epididymovaginitis of cattle in East Africa.

There is no doubt that ovarobursal disease is one of the major causes of individual cow infertility characterised by regular return to oestrus. There is no satisfactory treatment for the condition. Some cases may be prevented if rough manipulation of ovaries and irrigation of uteri with large quantities of irritant antiseptics are avoided. Prompt attention to cases of dystocia with a view to preventing puerperal metritis would also reduce the incidence of ovarobursal disease.

Several other acquired abnormalities of the uterine tubes can also cause infertility. A condition described as pachysalpinx has been identified in three genital tracts from parous animals (Kessy, 1978). The gross appearance of the tube resembles hydrosalpinx or pyosalpinx but no fluid is contained within the lumen; instead there is a mass of connective tissue. Enlargement and distension of the uterine tube can also occur as a result of the presence of multilocular mucosal cysts containing periodic acid–Schiff (PAS)-staining gelatinous material.

Diagnosis of ovarobursal disease and impatency of the uterine tubes

Diagnosis of ovarobursal adhesions in the live animal is difficult. In consequence, only about one-third to one-half of the lesions that cause infertility are diagnosed by rectal palpation. Neilson (1949) described a technique of rectal palpation which was designed to explore the patency of the ovarian bursa and to detect the uterine tube. Using the left hand, the method involves rotation of the right ovary so as to free it from the bursa; then while this is held lightly between thumb and forefinger, the other three fingers are extended forwards medially and downwards to engage the anterior free edge of the ovarian bursa on the dorsal surface of one or more of these fingers.

The fingers are then extended into the bursa and spread fan-wise to detect the presence of adhesions between the ovary and bursa. If the palm of the hand is turned upwards, the uterine tube may then be rolled between the fingers inside the bursa and the thumb outside the bursa. The left bursa may be examined by holding the left ovary between the last two fingers and thumb; by extending the forefinger and second finger forwards, downwards and medially it is possible to engage the edge of the bursa and then to explore the bursa and uterine tube as described for the right side.

In the more gross cases of ovarobursal adhesions, the periphery of the ovary loses its clear definition. The ovarian outline is more bulky and irregular and the ovarian mass lacks mobility. In occasional difficult cases, laparotomy with direct vision or endoscopic examination of the ovaries may be used. Palpation of distended uterine tubes per rectum is usually indicative of the presence of advanced lesions of hydrosalpinx or pyosalpinx, but most cases of impatency of the uterine tubes can only be determined by undertaking functional tests of the tube.

Two fairly simple tests are available to assess the patency of the uterine tube. The first is based on a technique that was first described for use in women (Speck, 1948). He demonstrated that if phenolsulphonphthalein (PSP) was placed in the uterine lumen it was not absorbed but, if the uterine tubes were patent, it passed along them into the peritoneal cavity. From this site it was readily absorbed into the circulation. The PSP was then excreted by the kidneys into the urine, where it produced a red or pink colour if alkaline. If the uterine tubes were occluded there was no passage of dye and, hence, no discoloration of the urine. The test has been used in the cow (Berchtold and Brummer, 1968; Kothari, 1978); the latter author was able to demonstrate, using laparoscopy, the escape of the dye from the ostium. The test involves the infusion of 20 ml of a 0.1% sterile solution of PSP into the uterine lumen using a Nielson's catheter. This has to be done carefully so as to avoid any trauma to the endometrium enabling absorption to occur. The bladder should then be catheterised and a small sample of urine kept for a control. A urine sample is then collected 30–60 minutes later. The urine is made alkaline by the addition of 0.2 ml volume of 10% trisodium orthophosphate buffer to 10 ml of urine. In the presence of PSP, the liquid becomes red or pink; in its absence the urine remains the same colour as the control. The test should be performed during the luteal phase of the cycle, preferably about day 10, since false negatives can be obtained during the follicular phase (Kessy and Noakes, 1979a, b). False positives can arise if there is endometrial erosion due to infection and inflammation; it is not very effective in differentiating between bilateral and unilateral patency (Kessy and Noakes, 1979a, b).

A more accurate method of evaluating the patency of each uterine tube separately has been described by Coulthard (1980). A Foley-type embryo flushing catheter (see Chapter 35) is introduced into one horn, the cuff inflated and a small volume of dye infused into the tip of the horn. If the tube is patent the dye will pass via that uterine tube to the peritoneal cavity and hence to the urine (the cuff prevents reflux of the dye to the other side). The procedure is repeated on the other side several days later.

The second test involves the use of starch particles to simulate the transport of the oocyte or zygote. This method was first described for the cow by McDonald (1954) and subsequently used by Kessy and Noakes (1979a). Briefly, starch grains are spread over the surface of the ovary and are picked up by the infundibulum. They are transported thence to the vagina, from where they can be recovered after staining with iodine.

The prognosis for the condition is, at best, guarded. In cases of bilateral occlusion of the uterine tube, the animal is normally irreversibly sterile. Methods have been described for 'unblocking' uterine tubes by insufflation of the uterus with carbon dioxide, but, due to the risk of inducing uterine rupture, these are now rarely used. Manual rupture of ovarobursal adhesions has, likewise, been described, and a proportion of animals may manage to conceive after this procedure.

Uterine tumours

Tumours of the uterus are rare in cattle. Leiomyomata and fibromyomata are sometimes seen; pregnancy may occur in the neoplastic uterus. The larger uterine tumour may be confused on rectal palpation with a mummified fetus (Figures 22.11 and 22.12).

Benign tumours of mesenchymal tissues are the most common of the occasional uterine tumours of cattle. In a 2-year abattoir survey in Denver (Anderson and Davis, 1958), 24% of the cattle tumours (excluding 'cancer eye') were in the genitalia. Amongst these were adenocarcinoma of the uterus, 26 cases; lymphosarcoma of the uterus, 6; leiomyoma of the uterus, 4; granulosa cell tumours

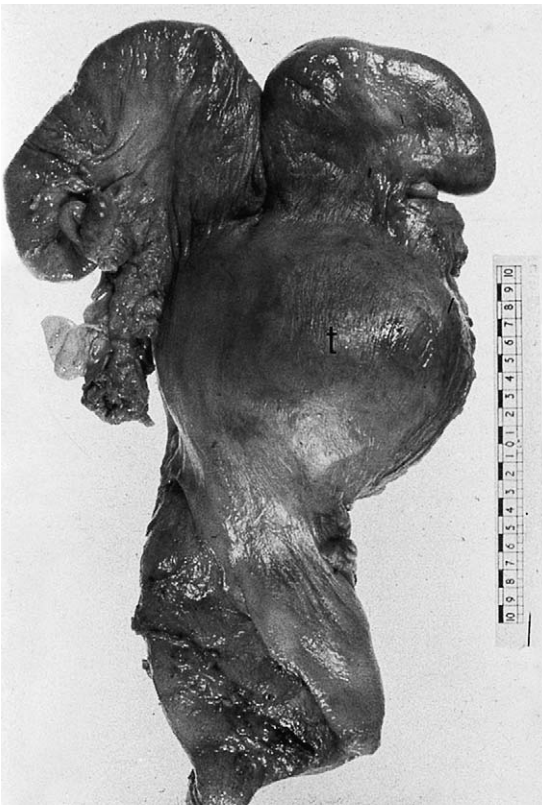


Fig. 22.11 Fibroma (t) involving the base of the uterine horns and body.

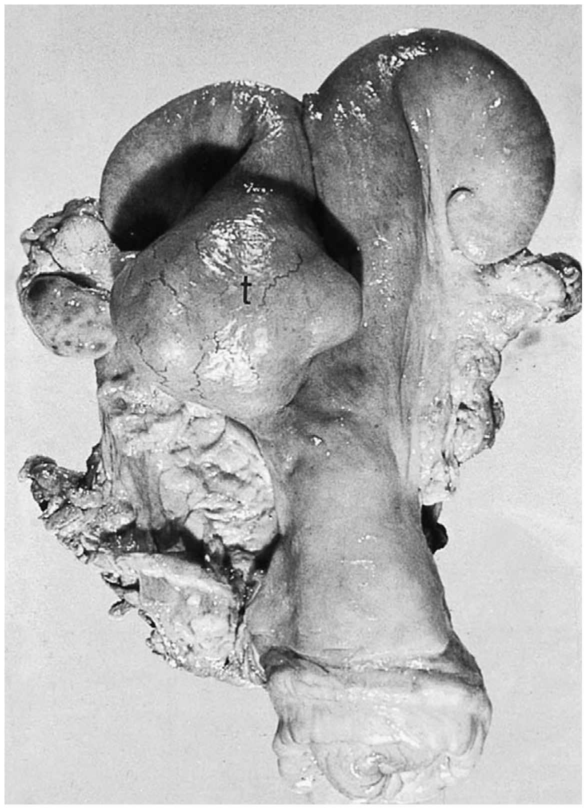


Fig. 22.12 Fibroma (t) involving the left uterine horn.

of the ovary, 6; cystadenoma of the ovary, 1; and squamous epithelioma of the vulva, 1. In a meta-analysis of abattoir surveys of reproductive abnormalities, Roberts (1986) found leiomyomas, fibromyomas and fibromas accounted for 77% of tumours. These benign tumours are often incidental findings at the time of slaughter, although they affect fertility if they occlude or occupy a significant proportion of the uterine lumen. Occasionally, the tumours are massive.

However, other series of cases have reported the adenocarcinoma and lymphosarcomas to be the most important tumours of the bovine uterus (Brandley and Migaki, 1963; Smith, 1965). Adenocarcinomas present as moderately enlarged, firm, constricted lesions of the uterine wall (McEntee, 1990) and have a high rate of metastases to the lung and abdominal structures. Affected animals often present clinically as having chronic wasting disease. These tumours are very rare in Europe; most cases have been reported from North America.

Uterine adhesions

A troublesome sequel to the caesarean operation is adhesion of the uterus to the omentum, intestines or abdominal wall. Similar lesions may follow uterine rupture. Such lesions may accompany ovarobursal disease and may follow tardy involution of the uterus and metritis. They are frequently associated with sterility.

Lesions of the cervix

The most common congenital lesions of the cervix are those which occur as part of the 'white heifer' syndrome (see above).

Since the cervix is the main physical barrier between the uterine lumen and the external environment, cervical lesions make it vulnerable to ascending infections. Inflammation of the cervix is likely to follow obstetrical trauma incurred during the relief of difficult dystocia. Cervicitis almost invariably accompanies puerperal metritis (see below) and is common in cases of delayed involution of the uterus and/or retention of the fetal membranes. The organisms present in such infections are those normally found in the posterior vagina, including *Escherichia coli*, streptococci, staphylococci and *A. pyogenes*. The latter organism is most prominent in established infections.

Rarely, parturient laceration of the cervix is followed by fibrosis and obstruction of the cervical canal, with infertility. Occasionally, cirrhosis of the cervix may prevent proper dilatation of the organ at parturition, but most cases of failure of cervical dilatation are of functional origin.

Prolapse of one or more of the cervical folds is commonly seen in the plurigravid cow. It is a physiological hazard of parturition and is not a cause of infertility.

Tumours of the cervix (Figure 22.13) occur very occasionally. Leiomyomas and, to a lesser extent, fibromas are the most common of these lesions, whereas adenocarcinomas (the most common human cervical tumour) are very rare indeed. The benign tumours of the cervix are only of clinical significance as space-occupying lesions or when they cause mechanical interference.

CONDITIONS OF THE VAGINA, VESTIBULE AND VULVA

Atresia of the vulva

An abnormally small vulva has been described as a cause of dystocia in Friesian and Jersey heifers. In such cases episiotomy or caesarean operation may be required to allow delivery. The defect has been seen to affect many of the progeny of a particular Jersey bull (Hull et al., 1940), thus indicating that it is likely to be of hereditary origin.

Cysts of Gaertner's canals

Cysts in linear series, which may be 6–8 cm in diameter, often occur on the floor of the vagina. They can be easily punctured and are not a cause of infertility.

Obstetrical damage to the vagina

Parturient trauma of the tubular genital tract is a frequent consequence of dystocia. Fetomaternal disproportion is the common cause of dystocia in cattle, particularly in the Friesian–Holstein breed.

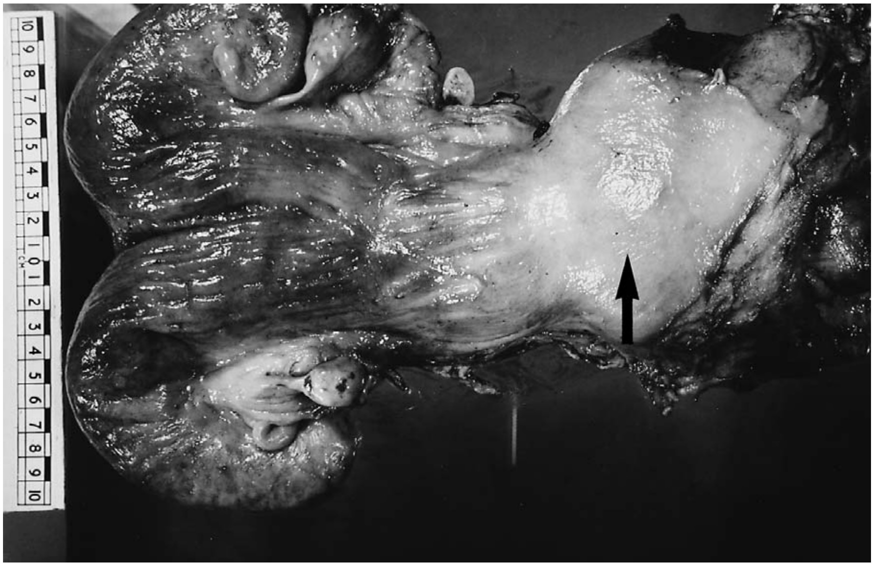


Fig. 22.13 Fibroma of the cervix (the cervix is arrowed).

Delivery of large calves by forced traction frequently damages the birth canal to such an extent that the animal is rendered sterile. Obstetric contusion of the vagina, especially in fat heifers of the beef breeds, is particularly likely to be followed by necrotic vaginitis associated with *Fusobacterium necrophorum* infection. Likewise, in other instances involving the removal of dead, emphysematous calves in unhygienic circumstances, parturient trauma may be followed by severe toxaemia due to invasion by anaerobic bacteria. Treatment of such animals requires the use of parenteral broad-spectrum antibiotics and, in severe cases, supportive fluid therapy. Some practitioners have found that local emollient creams are also helpful. Since severe vaginitis causes persistent straining, caudal epidural anaesthesia will provide temporary relief.

Other sequelae of dystocia include laceration or bruising of the vulva, which may be followed by cicatrisation and distortion, with imperfect closure of the vulval sphincter and aspiration of air. These sequelae are similar to, but less severe than, those of rupture of the perineum. Some of these cows are infertile to natural service but conceive to intrauterine insemination. At subsequent parturition, dystocia owing to fibrosis of the vulva may arise. Gross fibrosis of the vagina may also follow pyogenic infection of lacerations, also causing a narrowing of the birth canal and dystocia. Caesarean section may then be required at subsequent births.

Obstetrical damage to the perineum

Lacerations of the perineum can also result in impaired fertility of affected cows. Second-degree perineal lacerations may give rise to a pneumovagina if the conformation of the vulva is compromised. Surgical correction of such malconformation of the vulva is possible by performing Caslick's operation (see Chapter 18).

A third-degree perineal rupture may occur at calving, usually as a result of dystocia and severe calving trauma. In this situation, the whole thickness of the vagina and rectal wall ruptures, so that the rectum and vagina are confluent (i.e. the cow has a cloaca; see Plate 3). Third-degree perineal tears do not heal; thus air and faeces are aspirated into the vagina, inevitably resulting in vaginitis, cervicitis and metritis. Affected cows have a chronic mucopurulent vulval discharge, although their general health is not impaired. Normal cyclic behaviour resumes but conception does not occur because of the metritis (see below). The condition can be cured only by surgical reconstruction of the perineum using the techniques described by Götze's or Aanes (see Chapter 18).

Rupture of the perineum may be prevented by sound obstetric technique, including episiotomy.

Urovagina

An increasing number of cows are being diagnosed as having vaginal urine pooling or urovagina. In such animals, urine accumulates in the anterior vagina, where it impinges upon the cervix, and causes inflammation of both cervix and vagina. The inflammation then extends into the uterus, causing endometritis. There seems to be a greater prevalence in certain breeds, particularly the Charolais and Holstein. The cause of the condition is not known, although stretching of the suspensory apparatus of the genital tract as a result of several pregnancies may be a factor. In many Holstein cows, the disorder is diagnosed at the time of a post-calving examination to assess the degree of uterine involution; in most cases it resolves spontaneously. Surgical treatment has been described (Hudson, 1972, 1986), although the procedure is far from straightforward.

Tumours of the vagina and vulva

Fibropapillomata of the vagina and vulva of cattle are not uncommon. They do not cause infertility but may interfere with birth. They are usually pedunculated and may be removed surgically. There is a possibility that vaginal fibropapilloma are of viral origin and are transmitted venereally. They occurs in young cattle and undergoes spontaneous resolution.

All other tumours of the vagina and vulva are rare. A squamous cell carcinoma of the vulva occurs in unpigmented areas of the skin of cattle that are exposed to high levels of solar radiation (McEntee, 1990). Lymphosarcomas have also been occasionally found in the vagina.

METRITIS, ENDOMETRITIS, PYOMETRA AND RETAINED FETAL MEMBRANES

One of the most significant causes of infertility in cattle is the complex of diseases that includes retained fetal membranes (RFM), puerperal metritis, endometritis, pyometra and other nonspecific infections of the uterus. These diseases share common aetiological factors, predispose to one another and, to a large extent, share common treatments.

A degree of bacterial contamination of the uterus almost always occurs during, or immediately after, parturition. Bacterial contamination of the uterus may also occur during coitus or insemination. Whether or not a persistent infection of the uterus becomes established depends upon the level of contamination, the animal's uterine defence mechanisms and the presence of substrates (such as devitalised tissue) for the growth of bacteria.

Under normal circumstances, there are several mechanisms which prevent opportunist pathogens from colonising the genital tract. Firstly, the uterus is protected by the physical barriers of the vulval sphincter and cervix. It should be noted that, although the vulva may appear of little consequence as a barrier, it is, in fact, remarkably efficient at preventing faecal contamination of the tubular genitalia. Secondly, the uterus is protected by local and systemic defence mechanisms; both are is influenced by the reproductive steroid hormones, oestrogen and progesterone. In general, it is considered that the genital tract is more resistant to infection when it is under oestrogen dominance, whilst under progesterone dominance it is more susceptible. The reproductive endocrine system therefore has a significant influence on the resistance of the genital tract to infection. It is not surprising that on the two occasions when the physical barriers are breached (i.e. at coitus, or insemination; and at the time of parturition, especially immediately postpartum) the genital tract is in its most resistant state, since it is under the dominance of oestrogens and progesterone concentrations are low.

The high oestrogen concentrations that occur at oestrus and parturition cause changes in the numbers and proportions of circulating white blood cells, with a relative neutrophilia and a 'shift to the left'. Moreover, at oestrus, the blood supply to the uterus is increased under the influence of oestrogens, whilst at parturition there is a massive blood supply to the gravid uterus. This increased blood supply, coupled with the migration of white cells from the circulation to the uterine lumen, enables vigorous and active phagocytosis of bacteria to occur. Oestrogens also cause an increase in the quantity and nature of vaginal mucus, which also plays an important role in defence of the uterus against bacteria by providing a protective physical barrier and by flushing and diluting the bacterial contaminants.

Hence, despite the massive contamination with opportunist pathogens that occurs at oestrus and parturition, the bacteria are normally eliminated quickly (see Chapter 7) and there is rarely impairment of health.

Since the genital tract is generally able to overcome the potential challenge of massive non-specific bacterial contamination it is important to consider the reasons for failure. Firstly, damage to the mechanical barriers that protect the uterus make it more vulnerable to the establishment of infection. Thus, obstetrical damage to the vulva impairs its ability to act as an effective sphincter, causing aspiration of air, ballooning of the vagina, dehydration of the mucosa and the development of vaginitis. Likewise, damage to the cervix may allow heavy contamination of the uterine lumen, especially if there is concurrent damage to the vulva. Since the main cause of both these conditions is poor obstetric practice, they should largely be preventable (see Chapter 6). It is possible to restore the barrier function of the vulva after injury or even after perineal laceration/rupture (as described in Chapter 18), enabling the cow to eliminate the infection. Surgical repair of the cervix is virtually impossible.

Secondly, failure of the natural defence mechanisms around the time of calving may be caused by a number of factors; these include dystocia, RFM, metabolic diseases and fatty liver disease. Injured and devitalised tissue is less resistant and is readily infected; as a result, a severe and sometimes fatal puerperal metritis can occur. Other factors which delay uterine involution have been described in Chapter 7.

Finally, since progesterone domination of the genital system increases its susceptibility to infection, any condition which results in prolongation of the luteal phase can enable non-specific contaminants to become pathogenic. A persistent corpus luteum, either of dioestrus or of a degenerate pregnancy, or luteal cysts, are sometimes associated with infection of the uterus. Moreover, since infection of the uterus inevitably causes damage to the endometrial epithelium, the uterus becomes unable to secrete luteolytic patterns of $PGF_{2\alpha}$. Hence, the corpus luteum is retained and a self-perpetuating infection results.

Puerperal metritis

Puerperal metritis occurs within a few days of parturition. It usually follows an abnormal first or second stage of labour, especially when there has been a severe dystocia. The disease is also associated with uterine inertia, twin births, RFM, prolonged traction and damage to the vulva and/or birth canal.

Bacteria colonise the non-involuted uterus, producing toxins which are absorbed and cause severe symptoms. Many species of bacteria can be recovered from cases of puerperal metritis. The most important infecting organisms are *A. pyogenes*, group C streptococci, haemolytic staphylococci, coliforms, and Gram-negative anaerobes, particularly *Bacteroides* spp. In rare cases, clostridia are present which rapidly produce disease that is serious and often fatal.

Affected animals show both local and general symptoms. It is very common for toxaemia, septicaemia and pyaemia to occur. The temperature of affected cows may be elevated to 40–41°C, but is more often subnormal. There is a rapid pulse rate (in the region of 100/minute) and the respirations may be sufficiently frequent to suggest a respiratory disease. Animals are anorexic and dehydrated; they often have a toxaemia-induced diarrhoea and exhibit signs of shock. It is common for the infection to extend through the uterine wall into the peritoneum, causing a localised or generalised peritonitis. The uterus contains a large volume of toxic, fetid, reddish, serous exudate, containing pieces of degenerating fetal membranes; the exudate is discharged from the vagina by frequent expulsive straining efforts. Vaginal and uterine exploration of an affected case causes acute discomfort and is accompanied and followed by the most severe and persistent expulsive efforts. The cotyledons are swollen and the fetal membranes often remain firmly attached. The vulva and vagina are swollen and deeply congested. Puerperal metritis must be differentiated from (primary) pneumonia, traumatic reticulitis and pericarditis, and from milk fever and acute mastitis. Many animals with puerperal metritis also develop mastitis, particularly if they are recumbent, and many also have concurrent hypocalcaemia.

The treatment of puerperal metritis requires both good nursing care and vigorous medication. The cow should first be kept warm and made as comfortable as possible by, for example, transferring it to a well-bedded and warm loose-box. An attempt should be made to remove the fetal membranes by very gentle external traction, but no attempt should be made to enter the vagina and uterus with the hand. It should be appreciated that the uterus is particularly friable and that it contains a voluminous mass of septic material. Rough attempts at removal of the fetal membranes or even careful exploration of the vagina and uterus can cause severe damage and predispose to the absorption of toxins and entry of bacteria. If the cow is continually straining, caudal epidural anaesthesia can be used; local anaesthetic alone gives transient relief for 1–2 hours and sometimes it will 'break the cycle' and stop the straining, which is often self-perpetuating and debilitating. However, by using xylazine, either alone or in combination, the duration of effect can be prolonged (see Chapter 12). If the case is seen within 2–3 days of parturition, 50 i.u. of oxytocin by intravenous injection may cause contraction of the uterus and expulsion of fluid and debris.

The disease is best treated by systemic administration of broad-spectrum antibiotics and supportive therapy. The choice of antibiotic and the route of its administration have been the subject of much debate. Intrauterine antibiotics are unlikely to eliminate the infection and some, such as nitrofurazone, neomycin and some sulphonamides, may be detrimental to the endometrium. Likewise, intrauterine infusions of dilute iodine are considered to be more harmful than helpful. Intrauterine infusions of tetracyclines may be effective against mild cases of endometritis, but they do not penetrate far enough into the uterine wall to be effective against full-thickness metritis. The materials used for compounding some boluses are irritant and delay involution (Olson et al., 1984). Hence, the authors are of the opinion that intrauterine antibiotics are of little value at this stage of the disease. Systemic broad-spectrum antimicrobials, fluid therapy and non-steroidal anti-inflammatory drugs are widely recommended. The use of oestrogens is contra-indicated in cases of acute puerperal metritis since, although they potentially increase the resistance of the genital system, oestrogens also increase the blood flow to the uterus and, thereby, increase the absorption of bacterial toxins.

Once the temperature approaches normal and the cow shows some signs of improvement, some benefit can be obtained by uterine lavage and drainage. This can be done with a wide-diameter, soft rubber tube, at one end of which a large number of holes are made (a horse's stomach tube is ideal), to which is attached a large funnel. The perforated end is carefully inserted through the cervix into the uterine lumen and several litres of warm (49°C) sterile saline are poured down the tube through the funnel. The funnel end is quickly lowered before the tube empties, thus establishing a siphon. The interior end inevitably becomes blocked but the obstructing material is flushed out with more saline and the siphonage repeated over and over again, until the uterus is as empty as possible. The warm saline solution is believed to exert both a soothing and a stimulating effect on the uterus, and this, together with the evacuation of exudate, promotes involution. Parenteral antibiotics should be continued and, at this stage, intrauterine antibiotics may be beneficial. Ideally, the patient should be given daily treatment as outlined above. A favourable turn is shown by resumption of appetite, cessation of diarrhoea and the presence of a less fetid but thicker vaginal discharge.

Recovered cases inevitably show a mucopurulent discharge or leucorrhoea, due to chronic endometritis (see below). The prognosis for subsequent fertility should always be guarded, since cows that have suffered a severe puerperal metritis very often develop lesions such as ovarobursal adhesions, uterine adhesions and occluded uterine tubes, as described above. Other complications of metritis include pneumonia, polyarthritis and endocarditis. In pyaemic cases, abscesses may develop in the lungs, liver, kidney or brain.

Endometritis

Endometritis, which implies inflammation of the endometrium, is a common condition of the cow. Unlike metritis, it does not affect the general health of the cow, although it does have a profound effect upon the fertility of the animal. Most of the specific pathogens which cause infertility (such as *Campylobacter fetus* and *Trichomonas fetus*) do so because of the endometritis that they produce (see Chapter 23). However, the most important cause of endometritis is non-specific, opportunist pathogens that contaminate the uterus during the peri-calving period.

Causes of endometritis

The causal organisms usually reach the uterus from the vagina at coitus, insemination, parturition or postpartum, although it is possible in some circumstances for infection to arrive by the circulation. The great majority of cows suffer from bacterial contamination of the uterus after calving, but, under normal circumstances, this flora is rapidly eliminated (see Chapter 7). In cows that develop endometritis, the bacterial flora is not eliminated from the uterus, causing the endometrium to become inflamed. Hence, whilst the degree of bacterial contamination of the uterus is undoubtedly important in determining whether or not endometritis occurs, the pathogenesis of disease is largely concerned with the factors that impair the cow's ability to eliminate the infection, rather than with the bacteria themselves. There are therefore many factors that are associated with the development of endometritis (Andriamanga et al., 1984; Markusfeld, 1984, 1985), as described below.

Retained fetal membranes. In virtually every survey of the factors causing endometritis, retained fetal membranes are identified as being of major importance. In one survey, the incidence of endometritis was 25 times higher in cows with retained fetal membranes than in normal cows (Sandals et al., 1979), while more recently, Kaneene and Miller (1995) demonstrated significant statistical association between retention of membranes and endometritis.

Hence, conditions that lead to RFM are also associated with the development of endometritis. These include:

- multiple births (Muller and Owens, 1974)
- abortion (Faye et al., 1986)
- induced calving. The high incidence of RFM and, hence, endometritis is one of the most significant risks associated with induced calving.

Dystocia. Difficult calvings predispose to endometritis for several reasons. Firstly, there is a higher than normal incidence of retained fetal membranes in animals that suffered dystocia. Secondly, there is often damage to maternal tissues causing devitalisation. The vulval seal may be damaged. Thirdly, the obstetrical interventions to correct the dystocia increase the load of pathogens within the uterus. All of these factors have previously been mentioned as predisposing to acute puerperal metritis, a condition which almost always leads to endometritis during its resolution.

Management factors. Many management factors affect the incidence of endometritis. Thus, high milk yield is associated with an increased incidence of endometritis (Grohn et al., 1990). Markusfeld (1984) found that postpartum metritis was more prevalent in first calvers that yielded less in the last 5 months before calving than those that yielded average or above. However, this association is probably dependent upon state of nutrition, rather than milk yield per se. Overfeeding is associated with endometritis (Markusfeld, 1985; Kaneene and Miller, 1995), particularly where animals develop ketosis and fatty liver syndrome (Reid et al., 1979). Conversely, underfeeding has also been associated with endometritis. Debate exists as to whether hypocalcaemia is associated with endometritis (Curtis et al., 1983; Faye et al., 1986). Curiously, in the study of Kaneene and Miller (1995), the incidence of endometritis was associated with the intensity of veterinary attention that the herd received; however, this was considered to represent an increased rate of diagnosis rather than more affected animals. Season of the year may also affect the incidence of endometritis; cows calving during the winter or spring are more prone to endometritis than those calving at other times.

Return of cyclical ovarian activity. It has been known for some time that the uterus of the cow is more resistant to infection at oestrus than during the luteal phase of the cycle (Rowson et al., 1953). Since cellular defence mechanisms are potentiated during oestrus (Frank et al., 1983), it has been generally assumed that a delay in return to cyclical activity would predispose cows to endometritis. This has been shown by Andriamanga et al. (1984), who found that 34% of the cows that were cyclical by 37 days postpartum had endometritis, compared with 49% that were acyclical by the same stage. However, Olson et al. (1984) found that in the cows that developed pyometra, the average interval from calving to first ovulation was 15.5 days compared with 21.8 days for the normal, non-infected animals. In these cows that ovulated early, the bacterial contamination was such that it was probably not eliminated at the oestrus, so that when a luteal phase followed the bacteria were able to proliferate and colonise the uterus.

Bacterial loading. The environment in which the parturient and postparturient cow is kept affects the incidence of endometritis. In particular, a dirty, unhygienic calving environment predisposes to the disease. This is probably the explanation for the effect of season of year, since cows calving in the winter or indoors in the spring are likely to be in a more heavily contaminated environment. Notwithstanding this association, in a bacteriological study of uterine flora in postpartum cows from farms with hygienic or unhygienic calving accommodation, there was no qualitative or quantitative difference in the bacterial flora, despite vastly different incidences of endometritis (2% versus 15%; Noakes et al., 1991).

Even so, the nature of the flora is important. In the studies of Hartigan et al. (1974a) it was found that endometritis is almost invariably a sequel to invasion with *A. pyogenes*; histopathological lesions of endometritis were observed in 97.4% of the uteri infected with this organism. More recently, the role of obligate anaerobes in the pathogenesis of endometritis has been demonstrated (Ruder et al., 1981; Olson et al., 1984). There is good evidence that there is synergism between *A. pyogenes* and *Fusobacterium necrophorum*, the latter organism producing a leucocidal endotoxin which interferes with the host's ability to eliminate *A. pyogenes*. Similarly, *Bacteroides* spp. also produce substances that interfere with the phagocytosis and killing of bacteria. These synergistic activities that allow the establishment of *A. pyogenes* infections are of some importance to the outcome of endometrial disease, since duration of *A. pyogenes* infection determines the degree of damage that the endometrium suffers.

Prevalence

World-wide figures for the prevalence of endometritis are varied, ranging from 43 to 35% in France (Andriamanaga et al., 1984; Martinez and Thibier, 1984) and 37% in Israel (Markusfeld, 1984) to 10% in Belgium (Bouters and Vandeplassche, 1977) and 6.25 and 10.3% for Jersey and Holstein cows, respectively, in the USA (Fonseca et al., 1983). In the UK, an incidence rate of 10.1% was recorded (Borsberry and Dobson, 1989) whilst in a study involving 20 000 cows in 63 herds during the calving season 1989–90, a mean incidence rate of 15% was reported for cows with a vulval discharge. The lowest and highest quartile values were 3.7 and 26.9%, respectively (Esslemont and Spincer, 1992).

Although the differences in incidence rates may be genuine and related to predisposition factors (see below), they may be due to differences in clinical opinion about what constitutes endometritis. In most cases a diagnosis is based upon the presence of an abnormal vulval discharge, usually with the presence of varying amounts of pus. When small quantities of pus are present, particularly in the absence of an unpleasant odour, then it is usually indicative of the spontaneous recovery phase. In addition, some cows produce a more copious than normal volume of postpartum lochial discharge (see Chapter 7).

Clinical signs

Clinical signs of endometritis are the presence of a white or whitish-yellow mucopurulent vaginal discharge (known as leucorrhoea or 'whites') in the postpartum cow. The volume of the discharge is variable, but frequently increases at the time of oestrus when the cervix dilates and there is copious vaginal mucus. The cow rarely shows any signs of systemic illness, although in a few cases milk yield and appetite may be slightly depressed. Rectal palpation frequently shows a poorly involuted uterus which has a 'doughy' feel. Studer and Morrow (1978) found a close correlation between size and texture of uterus and cervix, the nature of the purulent exudate and the degree of endometritis determined by biopsy and the nature of the bacterial isolation.

Uterine biopsy has been used to study both the incidence of clinical and subclinical endometritis. Biopsies can be collected (Ayliffe, 1979), using an instrument (Figure 22.14) modified from that described by Hartigan et al. (1974b), although interpretation of biopsy material requires considerable experience of the normal cyclical changes that occur in the endometrium. In most endometrial biopsy studies of the uterus, subclinical cases of endometritis have been diagnosed, which have not exhibited clinical signs. For example, Sagartz and Hardenbrook (1971) reported that 77% of infertile cows had endometritis; bacterial infection was found in 64% of these cows and biopsies revealed that 80% showed evidence of lesions. Morrow et al. (1966) found that 63% of infertile cows that had no clinical abnormalities exhibited histological evidence of endometritis. Schmidt-Adamopolou (1978) reported that a group of 49 infertile cows, which had been clinically diagnosed to be infertile from causes other than endometritis, had, upon biopsy examination, lesions of endometritis in 92% of cases. In a study by Hartigan et al. (1972), 50% of the genital tracts obtained from an abattoir showed histological evidence of endometritis, yet only 12.5% showed gross lesions. Hence, it is likely that subclinical endometritis is a major contributor to the 'Repeat Breeder' syndrome of bovine subfertility (see below).

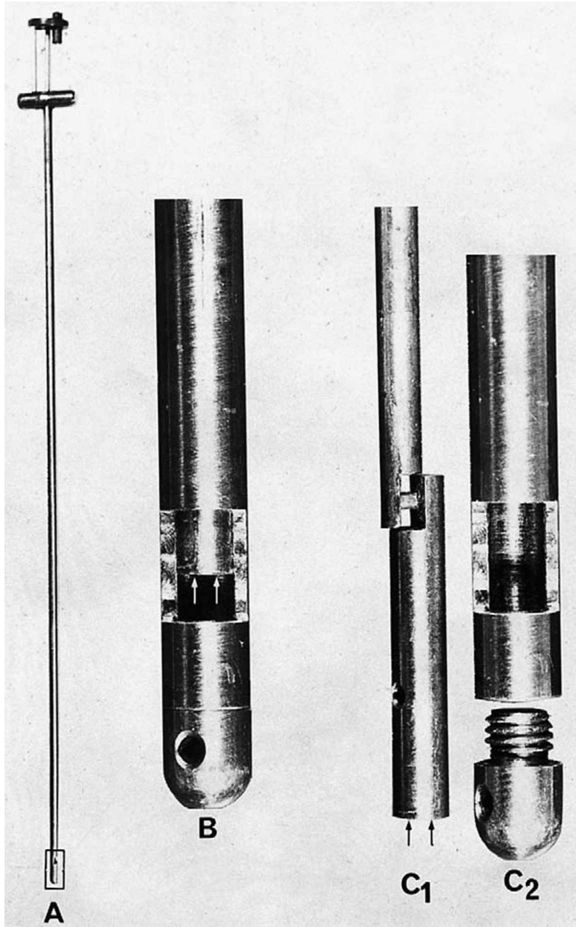


Fig. 22.14 Uterine biopsy instrument. A, whole instrument showing window with cutting edge; B, 'close-up' with edge partially withdrawn (arrowed); C_1 and C_2, 'close-up' views showing the cutting edge (arrowed) and interchangeable tip C_2.

Treatment

Few aspects of theriogenology have attracted more debate than the treatment of endometritis. Nevertheless, there is a consensus that there is little value in performing routine swabbing and bacterial sensitivity tests before treatment. This is because of the variable nature of the flora, the problems associated with the collection of uncontaminated uterine swabs and the difficulty of handling material for anaerobic culture.

A wide range of antiseptics, antimicrobial agents and hormones have been used as treatments for endometritis. Objective studies of the effectiveness of these agents have been difficult because of the multifactorial nature of the disease and the large numbers of animals needed to generate statistically valid data. Moreover, many cases of endometritis are self-limiting and resolve after the resumption of oestrous cyclicity. The self-cure rate has been estimated at 33% (Stephan et al., 1984). Other work has shown that, by 35 days after calving, 46% of animals have no evidence of endometritis a figure that decreases by 10% per week thereafter (Griffin et al., 1974). Yet, although endometritis is frequently self-limiting, with spontaneous recovery after a spontaneous oestrus, there is a danger that non-treatment will lead to the development of pyometra.

In the treatment of chronic endometritis with antimicrobial substances, it is preferable to administer the substance by the intrauterine route. Provided an adequate dose rate is used, this will result in effective minimum inhibitory concentrations (MICs) reaching the endometrium and being established in the intraluminal secretions. The latter point is important for the effective treatment of the disease, since subtherapeutic dose rates are frequently used. Hence, some clear principles underlie the choice of antimicrobial and/or antiseptic agents:

- Its efficacy against the wide range of aerobic and anaerobic, Gram-positive and Gram-negative bacteria that will be present.
- Its efficacy within the generally anaerobic environment of the uterus.
- Whether an effective bactericidal (or bacteriostatic) concentration can be achieved at the site of infection by the route of administration. When the intrauterine route is used, the substance must be evenly and rapidly distributed throughout the uterine lumen with good penetration into the deeper layers of the endometrium.
- It must not inhibit natural uterine defence mechanisms, particularly the cellular component.
- It must not traumatise the endometrium. Several of the vehicles which have been used at various times in the formulation of pharmaceutical preparations can damage the endometrium. Examples include: propylene glycol, which can cause a necrotising endometritis; oil, which can cause granulomata; and chalky bases, which can cause irritation and blockage of glands.
- Treatment must not reduce fertility by producing irreversible changes in the reproductive system.
- Treatment must be cost-effective by enhancing fertility.
- Details of its absorption from the uterus and excretion in the milk must be known so that appropriate withdrawal times can be followed.

In consequence, several antibiotics are inappropriate. Nitrofurazone is irritant and has an adverse effect on fertility. Aminoglycosides are not effective in the predominantly anaerobic environment of the infected uterus. Field trials have also provided evidence for a lack of effectiveness of these drugs in the treatment of endometritis. Sulphonamides are ineffective because of the presence of para-aminobenzoic acid metabolities in the lumen of the infected uterus. Penicillins are susceptible to degradation by the large numbers of penicillinase-producing bacteria that are present.

A broad-spectrum antibiotic, such as oxytetracycline, used at a dose rate of up to 22 mg/kg, will provide effective MICs in the lumen and uterine tissues. Considerable concentrations of antibiotic reach the endometrium following intravenous or intramuscular injection (Ayliffe and Noakes, 1978; Masera et al., 1980). Intrauterine infusions of penicillin are also effective in long-standing cases in which *A. pyogenes* has become dominant, since the organism is sensitive to penicillin, and high concentrations of antibiotic are maintained within the uterine wall for up to 24 hours.

When there is a palpable mature corpus luteum on the ovary it is arguable that the best method of treating clinical endometritis is with $PGF_{2\alpha}$ or its synthetic analogues. When a corpus luteum is present (i.e. during the luteal phase of the oestrous cycle or when there is a pathologically retained corpus luteum) $PGF_{2\alpha}$ causes luteolysis, thereby stimulating the return of oestrus and reducing the high progesterone concentrations. Frequently, clinical signs of endometritis, as characterised by leucorrhoea, are seen by the herd manager at oestrus; in such a case no responsive corpus luteum will be present and the cow will require re-examination in 6–8 days when prostaglandin therapy can be used. The cow will return to oestrus 3–5 days after treatment and, unless the purulent discharge is severe, it is advisable to serve or inseminate at the induced oestrus. Good results, as determined by conception at the induced oestrus, have been reported by

Gustafsson et al., (1976), Coulson (1978) and Jackson (1981). Intrauterine $PGF_{2\alpha}$ has been advocated as a means of combining luteolytic and ecbolic actions; no advantage over systemic administration has, however, been observed. However, administration of $PGF_{2\alpha}$ to cases of endometritis with no corpus luteum has also been reported to increase the cure rate (Steffan et al., 1984).

Several intrauterine therapeutic preparations also contain oestrogens, whilst the administration of oestrogens by intramuscular injection at the same time as intrauterine infusion of antibiotics has also been recommended. Such hormones increase uterine blood flow and simulate the changes that occur during the follicular phase of the oestrous cycle. However, high dose rates of oestrogens can influence folliculogenesis, resulting in transient or irreversible changes, including ovarian cysts, and can result in long periods of infertility. Nevertheless, in cows where no corpus luteum can be palpated, intramuscular injection of 3–5 mg of oestradiol benzoate has been used with some success, although these dose rates produce much higher concentrations in the peripheral circulation than occurs at oestrus.

Sheldon and Noakes (1998) compared the effectiveness of intrauterine infusion of 1500 mg oxytetracycline hydrochloride with intramuscular injection of 500 μg cloprostenol (a $PGF_{2\alpha}$ analogue) or 3 mg oestradiol benzoate, as treatments for endometritis. They concluded that, provided a corpus luteum was present, $PGF_{2\alpha}$ was the most successful treatment, both in terms of cure rate and calving to conception interval. Oxytetracycline was more effective than oestradiol, but marginally less so than $PGF_{2\alpha}$. Pepper (1984) produced very similar results when he compared a commercial antibiotic preparation with $PGF_{2\alpha}$ and oestradiol. Not unsurprisingly for all of the aforegoing treatments, the severity of the disease adversely affected the cure rate, with the best results obtained in mild cases in which self-cure would have occurred.

Consequences

Endometritis reduces fertility by extending the calving to conception interval and increasing the number of services per pregnancy (see Chapter 24). Studer and Morrow (1978) found a significant correlation between the state of the uterus, as determined by rectal palpation, and the calving–conception interval, especially in relation to the amount of pus in the discharge. Extension of the calving–conception interval has been variously reported at 12 days (Tennant and Peddicord, 1968), 20 days (Erb et al., 1981), 10 days (Bretzlaff et al., 1982) and 31 days (Borsberry and Dobson, 1989). First service conception rates were reduced from 49% in normal cows to 32–44% in cows with vaginal discharges (Morton, 2000), whilst the services per conception have been increased from 1.67 and 2.16 to 2.0 and 2.42, respectively (Tennant and Peddicord, 1968; Bretzlaff et al., 1982).

Secondly, endometritis can cause long-term, irreversible changes to the genital tract. The consequence of this long-term effect is clearly shown by the increased culling rate, which, in the survey of Bretzlaff et al. (1982), changed from an average of 5% for the herd in general to 20.6% for those which suffered from metritis. Comparable figures of 6.2 and 13.6%, respectively, were obtained by Tennant and Peddicord (1968). Other workers have demonstrated pathological evidence of endometritis in cows culled for infertility, particularly the Repeat Breeder cow (Brus, 1954; Fujimoto, 1956; Dawson, 1963). Morton (2000), in a large-scale survey of Australian dairy cows, reported that 21-week in-calf rates were reduced from 89% in normal animals to 55–58% in cows with vaginal discharges. Likewise, in New Zealand, uterine infection was associated with a reduction of final pregnancy rates from 92.3% to 75.4% (Xu and Burton, 2000).

Endometritis reduces the profitability of a dairy enterprise; the cost can be calculated by relating it to the increase in the calving–conception interval. In the study by Esslemont and Kossaibati (1997) a total cost of £166 per cow was calculated. Losses were mainly due to an extended calving–conception interval, increased culling rates, reduced milk yield and the cost of treatment. This equated to £833 per hundred cows in a herd.

Pyometra

Pyometra is characterised by a progressive accumulation of pus in the uterus and by the persistence of functional luteal tissue in the ovary.

In most cases, pyometra occurs as a sequel to chronic endometritis when, as noted above, as a result of inflammation, the uterus ceases to produce or release the endogenous luteolysin (see Chapter 1). The corpus luteum of dioestrus persists and, since the genital tract remains under the continuous influence of progesterone, the infection is not eliminated. Because the cervix remains fairly tightly closed the purulent exudate accumulates within the uterine lumen, although occasionally there is a slight purulent discharge. Occasional cases of pyometra occur in the presence of a luteal cyst.

The second main cause of pyometra is the death of the fetus, invasion of the uterus by *A. pyogenes* and retention of the corpus luteum of pregnancy. This is a relatively infrequent cause of the condition. Thus pyometra usually results from contamination of the uterus during dioestrus, such as occurs after insemination during the luteal phase. Venereal infection with organisms such as *Trichomonas fetus*, which cause embryonic death, also causes pyometra.

Cows which suffer from pyometra show few or no signs of ill health; the main reason for them being examined is the absence of cyclical activity, or, perhaps, the presence of an intermittent vaginal discharge. The uterine horns are enlarged and distended (Figure 22.15), quite often to an unequal degree, owing to incomplete involution of the previously gravid horn or to recent conceptual death. Differentiation of pyometra from a normal pregnancy can sometimes be difficult, but there are a number of distinguishing points:

- The uterine wall is thicker than at pregnancy.
- The uterus has a more 'doughy' and less vibrant feel.
- It is not possible to 'slip' the allantochorion.
- In most cases of pyometra, no uterine caruncles can be palpated. However, when the infection occurred in a non-involuted uterus, involution of the caruncles is delayed and they may remain palpable for quite a long time.
- Transrectal ultrasonography will demonstrate the absence of a fetus and the presence of a 'speckled' echotexture of the uterine contents compared with the black anechoic appearance of normal fetal fluids.

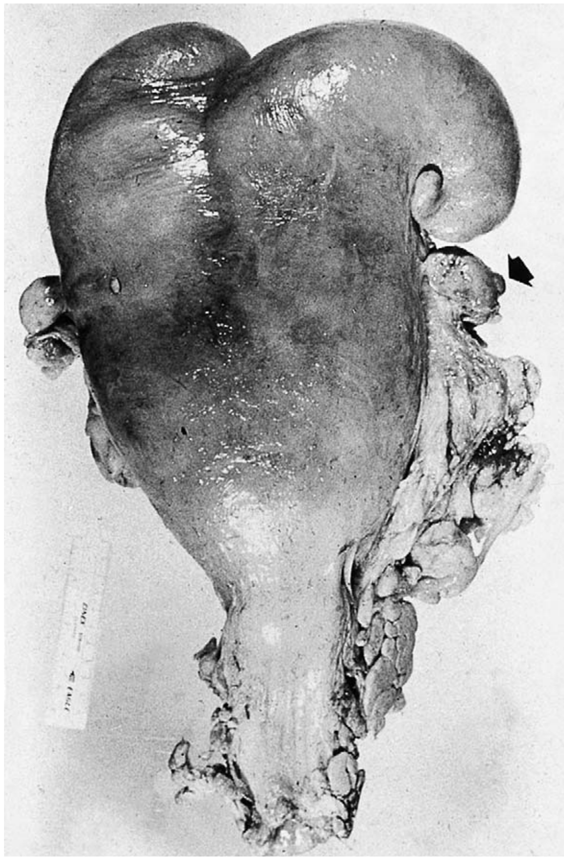


Fig. 22.15 Cow's uterus with pyometra. Note the distended horns and a corpus luteum present in the right ovary, indicated by the arrow, and fibrin tags over the dorsal surface of the uterine horns and body.

Pyometra associated with *T. foetus* infection presents features which are different from those previously described. Uterine pus is, as a rule, much more copious and may attain a volume of many litres. It is generally more fluid and is greyish-white or white. The uterus undergoes much greater distension. The mucus occupying the cervix is moist and slippery, rather than sticky and tenacious, and motile trichomonads can generally be found in it.

The best treatment is the use of $PGF_{2\alpha}$ or its analogues. They result in regression of the corpus luteum, dilatation of the cervix and expulsion of the purulent fluid, with oestrus occurring 3–5 days later. If there is any doubt about the diagnosis of pyometra the cow should be left untreated and re-examined 2 weeks later for evidence of change.

Provided that the condition is not too long-standing and therapy is instituted quickly, there is a reasonable possibility that the cow will eventually conceive again. However, long-standing cases are associated with more severe degeneration of the endometrium, reducing chances of reconception. Roberts (1986) associated larger volumes of pus with a poorer prognosis, while the presence of perimetritis precluded reconception. Neilson (1949) and Roberts (1971) quoted reconception rates of 51% and 46%, respectively.

Retained fetal membranes (RFM)

Retention of fetal membranes is a recurrent theme in considerations of the metritis–endometritis–pyometra complex of diseases. It is a common complication of bovine parturition and, although of little consequence per se, its role in predisposition to infections of the uterus means that retention of the fetal membranes is an important contributor to bovine infertility.

Aetiology

Retention of the fetal membranes (Figure 22.16) occurs when the normal processes of dehiscence and expulsion (see Chapter 6) fail to take place. There appear to be three main factors involved in the separation and expulsion of the fetal membranes, namely:

- maturation of the placenta
- exsanguination of the fetal side of the placenta when the umbilicus ruptures, which causes collapse and shrinkage of the trophectodermal villi and their physical separation from the maternal crypts
- uterine contractions, which aid the exsanguination of the fetal side of the placenta and cause physical separation of the placenta by distorting the shape of the placentomes (thereby causing 'unbuttoning' of the cotyledon from the caruncle), expulsion of the dependent and detached parts of the fetal membranes can then occur.

Hence, the factors that cause retention of fetal membranes are those which interfere with the separation of the fetal microvilli from the maternal cotyledons and those which interfere with the patterns of uterine contractility, particularly of third-stage labour.

Fig. 22.16 Cow with retained fetal membranes. Photograph by courtesy of N. B. Williamson.

Maturation of the placentomes. The main changes that occur during maturation of the placentome are:

- flattening of the maternal crypt epithelium (Bjorkman and Sollen, 1960)
- changes in the molecular structure of the collagen in the placentome from Type 1 to Type 2
- migration and increased activity of leucocytes (Gunnink, 1984)
- reduction in the number of binucleate cells in the trophectoderm from 20% to 5% in the last week of gestation, even though the rate of their migration remains the same (Gross et al., 1985)
- hyalinisation of the blood vessel walls in the placentome (Grunert, 1984)

- changes in the composition of the 'glue line' adhesive proteins between the cotyledonary and caruncular epithelium (Bjorkman and Sollen 1960).

Preparatory changes for loosening of the placentomes are not confined to the peripartum period, but begin during the last months of gestation (see Grunert, 1986). These changes are largely dependent upon the rising oestrogen concentrations that occur during the latter stages of pregnancy, and are complete at between 2 to 5 days before parturition.

Experimental investigations of the endocrine basis for RFM have been made in cows in which premature birth has been induced, or which have been ovariectomised and given steroid replacement therapy. These studies (Agthe and Kolm, 1975; Chew et al., 1977, 1979; O'Brien and Stott, 1977; Stott and Rheinhard, 1978) suggested that the endocrine control of placental detachment is dependent upon a critical sequence of changes in oestradiol-17β and progesterone secretion, in which not only the ratio between the two hormones, but also their absolute concentrations and the temporal patterns of their changes of concentration are important in determining whether or not retention occurs. However, field data of oestradiol-17β concentrations in cows with spontaneous or corticosteroid-induced retention are not clear-cut (Laven and Peters, 1996), since, in many studies, no differences have been found between normal and affected animals (e.g. Matton et al., 1979).

Since prostaglandins play an important role in placental separation, it might be expected that differences in prostaglandin dynamics might exist between normal cows and those with RFM. In vitro studies of placentomes from cows that had RFM showed that they produced less $PGF_{2\alpha}$ and more PGE_2 than those from normal cows (Gross et al., 1987). However, it is unclear whether this is a causal relationship, since these differences could simply reflect interconversion of the two prostaglandins within the binucleate cells of the placentome (Laven and Peters, 1996).

Nevertheless, it is clear that anything which interferes with the process of maturation of the placentomes, or which causes birth to occur before maturation is complete, results in RFM. Premature birth is very commonly associated with RFM. Cattle twins are usually slightly premature; hence, their birth is often followed by retention. Morrison and Erb (1957) stated that 43% of cattle twin births were followed by retention, while Erb et al. (1958) reported that 37.4% of 760 cases of retention studied by them were accounted for by twin births and abortions. Likewise, when twinning is induced by embryo transfer, an increased incidence of retention occurs (Anderson et al., 1978). If a calf is removed prematurely by elective caesarean operation there is delay in expulsion of the afterbirth, while the high incidence of RFM that follows induction of premature calving is well known.

In a similar manner, heat stress can reduce gestation length and increase the incidence of RFM in dairy cattle. Thus, Dubois and Williams (1980) found that cows which calved during the warm season in Georgia, USA, where the mean daily temperature was 26°C, had a reduction of 2.82 days in gestation length and an incidence of 24.05% retention, compared with 12.24% for the remainder of the year. The gestation lengths for retaining cows were, on average, 5.25 days shorter than those of non-retaining cows.

Placentitis. Both placentitis and RFM occur in cases of abortion due to *Brucella abortus, Campylobacter fetus* and moulds such as *Aspergillus* or *Mucor* spp. Roberts (1986) considered the relationship between placentitis and RFM to be causal. Roberts (1971) also studied retention of fetal membranes in American herds (which were free from infections such as brucellosis, campylobacteriosis, leptospirosis, infectious bovine rhinotracheitis (IBR) and moulds), and concluded that genital infection around the time of parturition was associated with 'failure to cleanse'. The mechanism by which such infections cause retention was not clear. Inflammatory swelling could affect the physical union between the maternal caruncle and fetal cotyledon; the involvement of the endometrium could interfere with the endocrine changes of the third stage of labour; or bacterial toxins could affect the myometrium. Retention was most likely to occur when many cows calved in the same accommodation in quick succession, perhaps due to a build-up of virulent organisms (i.e. group C *Streptococcus, E. coli, Staphylococcus,* *Pseudomonas* and *A. pyogenes*) in the environment. Such outbreaks of retention were associated with metritis and calf scour. Laven and Peters (1996), however, questioned Roberts's interpretation of these data, and argued that the metritis caused by such organisms were more likely to occur as the result of RFM rather than as a cause thereof. However, Roberts (1986) did note that, in situations where placentitis could be considered to be causal of membrane retention, oedematous, necrotic and haemorrhagic changes could be seen in the leathery placenta.

Enlargement of the placentomes, in the absence of placentitis, also leads to retention. Such enlargement may occur in the presence of oedema of the chorionic villi, hyperaemia of the placentomes, advanced involution of the placentomes in postmature fetuses and prepartum necrosis of the villous tips of the fetal placentome (Grunert, 1984; Paisley et al., 1986; Laven and Peters, 1996). These abnormalities are considered to mechanically prevent the separation of fetal and maternal villi.

Uterine inertia. Uterine inertia is frequently suggested as a predisposing factor for RFM, and a number of early studies (Benesch, 1930; Jordan, 1952; Venable and MacDonald, 1958) concluded a positive effect of uterine contractions on expulsion of the afterbirth. Conversely, Zerobin and Sporri (1972) considered that uterine atony was not a cause of retention, while no relationship was established between early postpartum uterine motility and retention in cows induced to calve prematurely (Martin et al., 1981). Grunert (1984) considered that less than 1% of cases of retention were caused by uterine inertia and that, even when inertia had occurred, detachment of the placenta was easily accomplished. On the other hand, Grohn et al. (1990) found parturient paresis to be a risk factor for RFM (and, indeed, for metritis), while Arthur and Bee (1996) also quote hypocalcaemia as being associated with retention. Curiously, there is also clinical evidence of a reverse association between retention and parturient hypocalcaemia; Roine and Saloniemi (1978) reported that retention in one year was likely to be followed by milk fever the following year. Interestingly, McKay (1994), who administered oral calcium to cows in the immediate postpartum period, reported no effect upon the incidence of RFM. However, Arthur and Bee (1996) considered that RFM occurs by a process analogous to uterine exhaustion in polytocous animals, and the incidence of retention has been reduced by giving oxytocin or $PGF_{2\alpha}$ (Laven and Peters, 1996) in at least some studies. Sucking has been associated with a reduced incidence of retention (Vinattieri et al., 1945), which has also been cited as evidence of an oxytocin-mediated stimulation of uterine contractility. Hence, the relationship between lack of uterine contractility and failure to expel the fetal membranes is tenuous, and a causative relationship is by no means universally accepted (Grunert, 1984; Paisley et al., 1986; Laven and Peters, 1996).

Hence, there are factors other than hormone imbalance which can cause uterine inertia: hypocalcaemia, particularly in dairy cattle; over-stretching of the myometrium (as with hydrallantois or grossly oversized fetus); and degeneration of the myometrial fibres as a result of bacterial toxins. Secondary uterine inertia, which results from exhaustion of the myometrium in obstructive dystocia, may also result in RFM.

The immune system. Joosten and Hensen (1992) have shown a link between RFM and MHC (major histocompatibility complex) class 1 compatibility of the calf and dam, perhaps pointing to a failure of alloreactivity to the fetal membranes by the dam. In addition, retention may also be related to failure of the release of inflammatory mediators (Slama et al., 1993). The importance of the immune system in the development of RFM has only recently been appreciated, but the link is now sufficiently well established that, from reviewing the work of Gunnink (1984) and Heuweiser and Grunert (1987), Laven and Peters (1996) stated that the primary problem in the condition is, in fact, a reduced immune response of the uterus.

Other factors. There is some evidence of a hereditary predisposition to RFM. Cows of the beef breeds are much less often affected than those of dairy breeds, and in the latter the incidence is higher in Ayrshires than Friesians. Old cows are more affected than young ones. Springtime calving exerts a predisposing influence; it might be connected with a vitamin A deficiency which has been shown to produce retention under experimental conditions. There is evidence of a high incidence of RFM in areas deficient in selenium (Trinder et al., 1973; Julien et al., 1976a, b) and of a reduction of incidence after selenium supplementation of the diet. Gwazdauskas et al. (1979), however, found no reduction in RFM after 28 days of prepartum supplementation with selenium. Hence, it is concluded that selenium deficiency may be responsible where there is a high incidence of RFM in certain deficient areas, but that sporadic cases of retention are not associated with selenium deficiency.

In Utah, USA, Lamb et al. (1979) found that Holstein heifers which were kept in confinement but which had been driven 1.5 km daily for 4–6 weeks before parturition had easier calvings and showed earlier expulsion of the fetal membranes and quicker involution of the uterus. Older cows showed no benefit from the exercise.

Incidence

The results of surveys of incidence of retained fetal membranes are shown in Table 22.2. It is noteworthy that the lowest incidence was in New Zealand, where the cows were at pasture the whole year. Apart from this and the British figure of 3.8%, the average incidence for all calvings would seem to be about 11%; for normal calvings it is about 8% and for dystocias 25–55%. Additional data relating to incidence indicate that retention tends to increase with parity, that there is an individual tendency to recurrent retention and that the incidence is very high with twins and late abortions (but not with early abortions in which the whole conceptus is easily expelled). Also, genetically high-yielding dairy cows and cows on high nutritive planes at parturition are more prone to retention (Whitmore et al., 1974).

Table 22.2 Published incidences of retention of fetal membranes

Authors	*Country*	*Incidence (%)*
Vandeplassche and Martens (1961)[a]	Belgium	55.0
Vandeplassche and Martens (1961)[b]	Belgium	8.0
Ben-David (1962)	Israel	8.4
Banerjee (1963)	Holland	11.2
Moller et al. (1967)	New Zealand	1.96
Geyer (1964)[a]	Germany	25.0
Muller and Owens (1974)	USA	7.7
Pandit et al. (1981)	India	8.86
Arthur and Abdul-Rahim (1984)	Saudi Arabia	6.3
Bendixen et al. (1987)	Sweden	7.7
Putro (1989)	Indonesia	30
Samad et al. (1989)	Bangladesh	39
Majeed et al. (1991)	Iraq	12.8
Mee (1991)	Ireland	4.1
Zaiem et al. (1994)	Tunisia	15
Esslemont and Peeler (1993)	UK	3.8
Esslemont and Kossaibati (1997)	UK	3.6

[a] Dystocia cases
[b] Herds free from brucellosis, vibriosis and trichomoniasis

Clinical features

It should be noted that cows which fail to expel the fetal membranes within 36 hours or so are likely to retain it for 7–10 days. Myometrial contractions largely cease from 36 hours after the birth of the calf, so, if the membranes have not been expelled by this time, freeing of the fetal villi from the maternal crypts eventually occurs as a result of autolysis and bacterial putrefaction. This process starts within 24 hours of birth but takes several days to complete. Natural sloughing of the maternal caruncles also contributes to the subsequent dehiscence of the membranes, such that eventual expulsion of the membranes depends upon uterine involution. The duration of retention seems to depend on several factors, such as the extent of the areas of attachment of the fetal membranes, the rate of uterine involution, the amount of uterine exudate and the proportion of the afterbirth which had already passed through the cervix when retention began.

The toxic products of putrefaction accumulate within the uterus causing a fetid odour which pervades the atmosphere and, more importantly, taints the milk. The milk from affected cows must not be sold for human consumption, and it is for this economic reason and for aesthetic considerations as much as for cows' ill-health that farmers are concerned about retention.

Delayed involution of the uterus and a variable degree of metritis commonly accompany retention. Because of this association, it is difficult to assess either the morbidity and mortality, or the pathogenic importance of retention per se. Nevertheless, it is generally considered that there is little departure from health in cows with retained membranes which have calved spontaneously after a normal length of gestation. On the other hand, when retention follows extensive obstetric interference for dystocia, a severe metritis and toxaemia can supervene within 2 or 3 days which, if untreated, can be fatal. Whether these cases can be directly attributed to the retention is, however, unclear, since in similar cases cows may be equally ill if the placenta was removed at the time of delivery.

Mortality is commonly put at 1–4% and morbidity, as denoted by some temporary impairment of appetite and reduction of milk yield, at 55–65% of cases. Early series of cases of RFM quoted mortality rates of 2.8–4.2% (Fincher, 1946); and see Arthur, 1975; Roberts, 1986). In an important early investigation of the morbidity of RFM, Palmer (1932) observed the pathogenicity of retention in 44 cattle; no treatment was given except to four cows which became quite ill and in which proflavine and saline were infused into the uterus. During the fortnight after calving, appetite was good in 31.8%, fair in 54.5% and poor in 13.6%; body weight was unaffected in 88.6%. When the 44 cases of retention were mated and compared with 44 cows in the herd which had cleansed normally, there was no significant difference in the subsequent breeding records of the two groups.

Indeed, there has developed a consensus of veterinary opinion which supports Palmer's findings that uncomplicated retention does not significantly affect the fertility of cows which are mated beyond 60 days after the last calving. The significance of retention is, therefore, dependent upon the degree of metritis that occurs. Sandals et al. (1979) clarified this aspect of the condition by means of a retrospective analysis of 652 parturitions of 293 dairy cows in Canada. Their study revealed that RFM alone did not impair subsequent reproductive performance. The animals which developed the metritis complex, with or without RFM, did suffer significant increases in 'days open', services per conception, calving to first oestrus interval and days from calving to first service. Borsberry and Dobson (1989) also reported that RFM extended the calving to conception interval by 25 days, but when it was associated with endometritis the interval was extended by 51 days. Esslemont and Peeler (1993) and Esslemont and Kossaibati (1997) reported that RFM increased the calving to conception interval, the number of services per conception and the culling rate, whilst reducing milk yield (probably because of reduced appetite); the latter authors calculated that each case cost £238. The conclusion from these various studies has been that the influence of RFM upon fertility depends on the proportion of cows with retention that develop metritis. Yet, for the majority of cases of retention, natural resolution occurs and the breeding potential is normal by 2–3 months after calving. The data of Morton (2000) only partly support this notion, however. In this survey of Australian dairy herds, it was found that 21-week in-calf rates of cows with RFM were not significantly different from normal cows (83% versus 89%). However, the proportions that were pregnant after 6 weeks were much different (45% versus 58%), as were the first service pregnancy rates (39% versus 49%). McDougall and Murray (2000) likewise showed that the interval from calving to conception and the first service pregnancy rate were poorer in cows with RFM than normal cows; moreover, the affected cows were more likely to be culled for infertility at the end of the breeding season.

When retention is accompanied by metritis, the symptoms depend upon the severity of the uterine disease. As described earlier, severe disease is accompanied by increased pulse and respiratory rates, raised temperature, anorexia, diarrhoea, depression, reduced milk secretion, straining, fetid vaginal discharge and, occasionally, laminitis. Jordan (1948) found that the bacterial flora of the uterus in retention cases was the same as in cases of metritis, streptococci (particularly *Streptococcus dysgalactiae*) appearing first and being followed next by staphylococci (often coagulase-negative) and finally by diphtheroids (*A. pyogenes* predominating). Coliform and anaerobic bacteria were also present. In uncomplicated cases, a blood leucocyte picture characteristic of pyogenic infection was present after the third day, with neutrophilia and a 'shift to the left'. In toxaemic cases, there was severe leucopenia, neutropenia and eosinopenia. More recently, Noakes et al. (1991) found *A. pyogenes* and Gram-negative anaerobes such as *Bacteroides* spp., *Fusobacterium necrophorum* and *F. nucleatum* to be the commonest isolates.

Treatment

The treatment of animals with retained fetal membranes has long been a contentious subject. A number of approaches have been taken to animals with this condition, especially:

- manual removal
- administration of ecbolic agents
- no treatment
- treatment for metritis/endometritis, but no specific treatment of retention itself.

Laven (1995) surveyed the methods used by British veterinarians for treating cases of retention, finding that manual removal was used in at least some cases by 92.5% of respondents. Ecbolic agents (oxytocin, $PGF_{2\alpha}$) were sometimes used by 84.2% of respondents, with 15.7% using oestradiol to try to potentiate the effects of oxytocin. A few gave calcium borogluconate. Of the treatments used to control metritis, 67.5% of respondents used pessaries and 17.5% intrauterine infusions of oxytetracycline. Most veterinarians reserved parenteral antibiotics for animals that were systemically ill, but 18% used them in animals with no illness. The 'no treatment' option was only used routinely by 1.6% of respondents.

Manual removal. The techniques used for manual removal of RFM range from externally applied gentle traction, through to forced extraction and separation of each cotyledon and caruncle. Manual removal is a superficially attractive method, in that it immediately removes the stinking mass of decomposing tissue, thereby improving milking hygiene. However, there is increasingly incontrovertible evidence that manual removal is detrimental to the cow (Laven, 1995).

The traditional method of manual delivery has been described by Roberts (1986) and by Arthur and Bee (1996). In this method, the post-cervical portions of the placenta were twisted together into a 'rope', then a hand was inserted into the uterus and each cotyledon was squeezed out of the base of the maternal caruncle. Continuous steady traction and rotational force were applied with the other hand to withdraw the detached membranes.

Even when this procedure is undertaken with careful cleansing of the perineum and as high a standard of asepsis as possible, it causes considerable damage to the uterus. The deeper parts of the membranes are often left behind (Grunert and Grunert, 1990) and the endometrium is damaged (Vandeplassche and Bouters, 1982). Roberts (1986) also found that, if fetal cotyledons were torn off and remained attached to the caruncles during forced extraction of membranes, they would detach into the uterus some time later and, since the cervix would by then have closed, would remain within its lumen as foreign bodies.

Hence, although Boyd (1992) considered fertility was improved and the risks of illness were mitigated by removal, the converse has generally proved to be the case. Most evidence shows that manual removal of fetal membranes has a detrimental effect upon fertility. At best, there is no difference between removal and conservative treatment (Laven and Peters, 1996) and, in the studies of Banerjee (1963), Hammerman (1963), Ben-David (1968) and Bolinder et al. (1988), manual removal was associated with subsequent poorer fertility than conservative treatment. Similarly, the prevalence and severity of uterine infection are worse after manual removal than conservative treatment (Penavin et al., 1975; Bolinder et al., 1988; Bretzlaff, 1988; Laven, 1995), a conclusion also reached in Roberts's (1986) consideration of the condition. Roberts also concluded that the presence of pyrexia was an absolute contraindication to the forced removal of fetal membranes.

The current recommendations for the manual removal of fetal membranes, therefore, are that cows should not be examined until 96 hours after calving (Laven, 1995; Arthur and Bee, 1996) and that removal should be gentle (DeBois, 1982;

Watson, 1988); ideally it should be limited to the withdrawal of the membranes from the genital tract after they have become spontaneously detached from the caruncles (Roberts, 1986). While in many animals, spontaneous detachment may have occurred within 96 hours, Roberts (1986) considered that it is quite acceptable to leave membranes for 10 or even 15 days before removal if this length of time was needed for their detachment. In this context, farmers should also be discouraged from attempting to undertake forced removal from their own cows, since they are very likely to use too much force and to attempt removal too soon after calving.

Ecbolic agents. The most rational measure for both the prevention and treatment of RFM would be to stimulate adequate myometrial contractions so that a 'natural' dehiscence and expulsion could occur. Shaw (1938) found that, in herds which experienced an unusually high incidence of retention of the afterbirth, the administration of 10 ml (100 i.u.) of oxytocin to all cows immediately after calving reduced the rate of retention from 10 to 1%. However, using the same dose of oxytocin but at 3–6 hours after calving, and injecting every other cow in a 200-cow herd, Miller and Lodge (1984) found no significant difference in rates of retention between treated and control cows. However, in cattle practice generally the veterinarian is not consulted until after 24 hours of retention because until then the farmer has hoped for a spontaneous expulsion. By this time, the response to oxytocin has become unpredictable and generally poor (Arthur and Bee, 1996).

In order to attempt to achieve a more reliable response to oxytocin, oestrogenic substances have also been given, in the hope of both increasing the sensitivity of the myometrium to oxytocin and enhancing the natural uterine defence mechanisms. For these reasons, the synthetic oestrogens, stilboestrol dipropionate and oestradiol monobenzoate, have been widely applied to cows with RFM in the form of parenteral injection, or uterine infusion and pessary, and their use has sometimes been followed by injections of oxytocin (Roberts, 1986; Arthur and Bee, 1996). Most of these clinical trials were uncontrolled, and accordingly the results are impossible to appraise. However, Moller et al. (1967), in a well-documented record of the use of stilboestrol by parenteral injection on cows with retention in New Zealand, found that this treatment was of no value. It should also be noted that the use of stilbenes in food-producing animals is now prohibited in most countries, while the contraindication for the use of oestrogens in animals with severe metritis also applies to cases in which there is also retention of the membranes.

Prostaglandin $F_{2\alpha}$ and its derivatives have been used as ecbolic agents and, in the study of Laven (1995), their use was more common than that of oxytocin. Prostaglandins may assist in detachment of the membranes through direct actions upon the placentomes (Gross et al., 1986), rather than just by an ecbolic action. In cows that have been treated with $PGF_{2\alpha}$ at 1 hour or 12 hours after calving, Herschler and Lawrence (1984), Studer and Holtan (1986) and Zaiem et al. (1994) found beneficial effects, while Hopkins (1983), Bretzlaff (1988), Gross (1988) and Garcia et al. (1992) found no effect (Laven and Peters, 1996).

No treatment. Arthur and Bee (1996) were convinced, by the poor response to manual removal and the dubious effects of ecbolic agents, that uncomplicated cases of RFM require no treatment. They noted, however, that a certain strength of conviction was required to prescribe no treatment, and that it would be imprudent to adopt a rigid attitude of non-interference. When called to treat a cow with retention, the veterinarian must enquire about the animal's general health and, if there is any doubt from the stockperson's answers, a visit should be made and a clinical examination carried out. If the cow is ill with metritis, antibiotic treatment and/or uterine drainage are probably indicated. On the other hand, when the stockperson's replies imply that the animal is normal, no visit need be made unless the patient's health deteriorates.

Treatment for metritis/endometritis. Some degree of endometritis is invariably associated with RFM. Hence, many therapeutic regimens have been used either to attempt to prevent endometritis, or to treat it once it has occurred. Antibiotics can be given in the form of pessaries or as infusions that have been formulated for intrauterine use. Antibiotics that have been formulated for intrauterine infusion include oxytetracycline and cephapirin, both of which are active in the uterine environment and have a broad spectrum of action. However, some of the antibiotics that are present in intrauterine pessaries are inactivated in the presence of the debris that is contained within the uterus (Paisley et al., 1986; Laven, 1995), a problem that is often exacerbated by veterinarians failing to use the recommended dose (Laven, 1995).

It has been common practice, after forced extraction of RFM, or after unsuccessful attempts at extraction, to place antibiotics into the uterus in an attempt to prevent endometritis. Intrauterine antibiotics reduce odour (Roberts, 1986), but they also reduce the rate of putrefaction of the membranes and reduce the level of intrauterine phagocytosis (Paisley et al., 1986), thereby prolonging retention (Roberts, 1986). Whether antibiotic therapy improves subsequent fertility is questionable. Bannerjee (1965) studied intrauterine oxytetracycline treatment of animals with RFM: as a result of which he advocated the institution of oxytetracycline treatment within 72 hours of non-delivery of the placenta. Some other trials have also found the use of oxytetracycline to be beneficial (El-Naggar, 1977; Squire, 1980). Nevertheless, most evidence suggests that such usage of antibiotics is of little or no benefit to the subsequent fertility of the cow; Moller et al. (1967) found that cows which had received intrauterine tetracycline medication showed worse conception rates than others which had not been treated. Duncansson (1980) and Garcia et al. (1992) also found no benefit from such use.

The use of systemic antibiotics in cows that are ill with metritis is, however, far less controversial. Most studies agree that, where retention is associated with septic metritis or systemic signs of illness, appropriate, vigorous treatment regimens should be instituted.

ANOESTRUS AND OTHER FUNCTIONAL CAUSES OF INFERTILITY

Abnormalities of the reproductive endocrine control systems constitute functional forms of infertility. When these forms of infertility occur in substantial numbers of individuals within a herd, a significant impairment of the herd's reproductive performance can result. Some abnormalities occur as a result of inherited factors, but most result from failures of some aspect of management. Paramount amongst these are the stress of production and nutritional deficiencies or excesses. However, cows are also subject to social stresses which arise from modern husbandry methods, such as the interference with the establishment of a stable social hierarchy that results from groupings of large numbers of cows.

Most forms of functional infertility result in anoestrus, i.e. a failure of the cow to display oestrus. The anoestrus syndrome is both a common and an economically important problem of world-wide dairy farming. It is also a significant problem of the beef industry, given the critical economic dependence of much of that industry upon regular annual calvings.

Causes of anoestrus include:

- pregnancy
- ovarian inactivity, resulting in anovulatory anoestrus
- failure to observe oestrus
- ovulation that is not accompanied by signs of oestrus ('silent heat')
- cystic ovarian disease, which can result in anoestrus, or other abnormal patterns of reproductive behaviour
- miscellaneous conditions, such as spontaneous prolongation of the life span of the corpus luteum; that associated with infection has been described above.

Pregnancy

It is remarkable how many pregnant cows are presented for examination for diagnosis of anoestrus. Herd managers forget that animals have been artificially inseminated, or that bulls were running with the herd, or that bulls may break their way through fences and mate with cows, or that male animals that were thought to have been castrated may still have functional testicular tissue. Hence, whenever anoestrous cows are presented for examination, the possibility of pregnancy should not be overlooked, however remote the possibility might be. The authors have regularly had cows presented for anoestrus examination that have been 6 or more months pregnant.

Anovulatory anoestrus

Oestrous cycles, which cease during pregnancy, do not resume straight away after calving. The high concentrations of progesterone that have prevailed throughout pregnancy cause negative feedback suppression of the hypothalamo-pituitary axis, with the result that follicular activity in the ovaries of full-term pregnant cows is minimal. Hence, a period of restoration of both gonadotrophin secretion and of ovarian follicular activity has to occur after calving before oestrous cycles can be resumed.

Thus, postpartum anovulatory anoestrus could almost be regarded as a normal facet of bovine reproduction. The significance of anoestrus is, therefore, where its duration is such that animals remain acyclic at the time when herd managers want to re-breed them. At that time, anoestrus then becomes regarded as a pathological problem that needs treatment. Hence, it is the duration of anoestrus in each individual cow, and its prevalence within the herd, that determine the significance of the 'condition' for the maintenance of regular calving patterns.

However, clinical anoestrus does not only occur as an over-extension of normal postpartum acyclicity. Other cows, which have started to cycle at the normal time after calving, may relapse back into anoestrus, often in response to nutritional (including micronutrient) deficiencies. These animals are associated with significant economic losses, especially if their return to anoestrus occurs after the start of the breeding period, in which case their failure to return to oestrus may well be regarded as a sign that they have successfully conceived.

Clinical findings

The clinical history of such animals is generally straightforward; they have not been seen in oestrus since the time of calving. There are, however, other animals which relapse into anoestrus after having started cycling. These may be identified as animals that have ceased cycling, or may only be discovered when they are presented for pregnancy diagnosis. On examination per rectum, the ovaries of affected cows are small, quiescent and usually flat and smooth, especially in heifers. Depth of anoestrus can be gauged, to some extent, by the size of the ovaries and the degree of development of the structures within them (Nation et al., 1998). Thus, cows with very small, inactive ovaries, which are devoid of any significant structures (i.e. no palpable follicles or luteal structures), are considered to be in a greater depth of anoestrus than are those with larger ovaries containing palpable follicles. Differentiation must be made from other causes of anoestrus, but the presence of a large corpus luteum within an ovary would easily permit detection of animals that were pregnant or had endometritis or pyometra, while the presence of cystic ovarian disease is also characterised by enlargement of the ovary.

In some anoestrous cows, follicles up to prematuration size of 1.5 cm may be present. Old cows frequently have roughened, irregular ovaries, because of the presence of old regressed corpora lutea and corpora albicantia. It is, however, often difficult to identify the presence of a small, developing or regressing corpus luteum, so that it is easy to confuse ovaries that contain such structures with anoestrous ovaries. The presence of uterine tone may help to identify animals that have recently ovulated. Ultrasonographic examination of the ovaries per rectum permits identification of ovarian structures for a greater proportion of the oestrous cycle than does simple manual palpation but, even so, there are stages of the cycle when differentiation between the cyclic and anoestrous ovary is not achievable. If there is sufficient time before the start of the breeding period, differentiation can eventually be achieved by waiting 10 days; at this time the ovary of a cyclic cow will have a mid-cycle corpus luteum, whereas the anoestrous animal will remain with inactive ovaries.

Milk or blood progesterone determinations are helpful in confirming a diagnosis; two samples can be taken at 10-day intervals or a single sample 10 days before a rectal palpation is made (Boyd and Munro, 1979). The more frequent use of milk progesterone assays from 25 days postpartum until the first service has been shown to be cost-effective (McLeod, 1990).

Incidence and predisposing factors

Anovulatory anoestrus is a multifactorial problem which occurs in response to a great variety of management or nutritional deficiencies.

Breed. Incidences vary between beef and dairy breeds, although this is highly dependent upon management system. A genetic effect has been implicated for the longer period for the return of ovarian function postpartum in beef breeds (36–70 days), compared with dairy breeds (10–45 days). On the other hand, it may be that the predisposition of beef suckler cows to anoestrus may be due to the inhibitory effect of prolactin, which is released during suckling, upon the pituitary's gonadotrophic activity. Suckling undoubtedly has a profound effect upon the duration of postpartum acyclicity (Lamming 1980). In an experiment with cross-bred beef cows, non-suckling cows exhibited their first oestrus 10–33 days postpartum, whilst identically bred and fed cows that suckled their calves did not return to oestrus for at least 98 days postpartum (Radford et al., 1978).

Season. The effect of the season and environment is shown by increased frequency of anovulatory anoestrus in autumn-calving herds housed indoors and fed on preserved fodder (Marion and Gier 1968; Oxenreider and Wagner, 1971). Much anecdotal evidence exists to suggest that this may be due to an inhibitory effect of short winter photoperiods upon reproductive activity. For example, Peters and Riley (1982) demonstrated a relationship between day length at the time of calving and the interval to the resumption of oestrous cyclicity of beef cows, suggesting that the effect was a direct manifestation of photoperiodism. Parkinson (1985) showed that dairy bulls exhibit a nadir of sperm production during the midwinter and similarly claimed a photoperiodic basis for the phenomenon. Even so, it remains unclear whether modern breeds of domestic cattle do experience photoperiod-induced limitation of reproductive performance during the winter. The effects which have been observed could equally be mediated by nutritional constraints (including the extra energy demands of maintenance during inclement winter weather), or the greater difficulties associated with both the expression and detection of oestrous behaviour amongst housed animals.

In late autumn- or winter-calving cows that have been in anoestrus, there is frequently a return to normal ovarian cyclic activity when they are turned out to grass in the spring. It has long been held that this effect is due to the presence of a specific 'substance' in grass, but it is more probable that the effect relates to an improved plane of nutrition, or to the effect of exercise and a new environment.

Plane of nutrition and metabolic workload. The reader is referred elsewhere for detailed information upon ration management for cows and for discussions of the interrelationships between energy, protein, intake and yield. In terms of the effect of inadequate nutrition, the most severe effect of inadequate nutrition is the cessation of cyclical activity, although other less severe manifestations are silent oestrus, ovulatory defects, conception failure and fetal and embryonic death. In particular, inadequate nutrition will increase the time interval to the first ovulation, which should normally occur 17–42 days postpartum (see Chapters 7 and 24). The time interval to the first insemination is thereby delayed (Table 22.3) if the period of postpartum acyclicity extends beyond the earliest service date (Butler and Smith, 1989).

However, in general terms, it should be noted that neither plane of nutrition nor micronutrients can be considered in isolation, but they must be considered in respect of the metabolic demands that are placed upon the cow. High-yielding dairy

Table 22.3 The relationship between loss of body condition during the first 5 weeks postpartum and reproductive performance (after Butler and Smith, 1989)

	*Body condition score**		
	A	*B*	*C*
Number of cows	17	64	12
Mean days to first ovulation	27 ± 2	31 ± 2	42 ± 5
Mean days to first observed oestrus	48 ± 6	41 ± 3	62 ± 7
Mean days to first service	68 ± 4	67 ± 2	79 ± 5
Mean first service pregnancy(conception) rate	65	53	17
Mean services per conception	1.8 ± 0.4	2.3 ± 0.2	2.3 ± 0.4

* Body condition scores:
A = < 0.5 body condition score lost,
B = 0.5–1.0 body condition score lost,
C = > 1.0 body condition score lost.

cows, because of their high energy demands for lactation, are probably at an intrinsically higher level of risk of anoestrus than are lower-yielding animals; yet, where their metabolic needs are met, there is little evidence that yield per se significantly predisposes to anoestrus. Beef cows, whose metabolic demands for lactation are more readily met, are less disposed to anoestrus than those whose demands are not met, yet neither of these animals are fed to remotely the same level as the high-yielding dairy cow. Hence, the effect of high milk yield on ovarian rebound is a matter of debate. Some have been convinced of a relationship (Oxenreider and Wagner, 1971), whilst others suggest that there is not a direct effect but a result of a concomitant loss of body weight and nutritional deficiency. The complexity of this interrelationship was demonstrated by Butler and Smith (1989), who showed that there is a direct correlation between milk yield and negative energy balance, and a direct correlation between negative energy balance and the time interval after calving to the first ovulation; the latter becomes significant within the first 2 weeks of lactation. Nevertheless, many studies of both beef and dairy cattle have shown that plane of nutrition (i.e. provision of energy and protein) is the key determinant of the incidence of anoestrus. In many dairy cows during early lactation the nutritional demands associated with the rate of increase in milk production exceeds their dry matter feed intake. As a consequence, there is a negative energy balance which results in the cow mobilising her energy reserves with a resultant loss of weight. The negative energy balance is at its maximum 1–2 weeks after calving and it can persist beyond the 5–6-week period when peak yields occur. If it is not corrected, the period of negative energy balance can extend well beyond the time when it would be appropriate to start serving cows, the earliest service date. In high-yielding cows, appetite, even when using energy density diets, may not be able to satisfy energy requirements until yields have started to decline. Signs of energy deficiency are usually first shown in first calf heifers, followed by second calf heifers, with mature cows least affected.

Cows which are in negative energy balance during early lactation are more at risk of becoming anoestrous than those which are in energy equilibrium, while many cows which are in negative energy balance remain anoestrous until equilibrium is restored. The effects of energy balance during the post-calving period are further modulated by the effects of condition score at calving, and the rate at which tissue stores are utilised to meet the energy demands of the cow. To some extent, reasonable body stores of fat at the time of calving can provide a store of energy that can mitigate the effects of undernutrition, but (as described below), excessive mobilisation of fat from overweight animals that are significantly underfed can itself worsen the effects of undernutrition. Conversely, in situations where feed availability is such that the energy demands of the freshly calved cow can be virtually fully met, calving in only a moderate condition score is preferable to calving with appreciable stores of fat. An example of this 'carry-over' effect of pre-calving feeding upon post-calving reproductive performance is from Ducker et al. (1985), who gave heifers different levels of energy intake during the last 10 weeks of pregnancy and during early lactation. They used 'high' levels of feeding (83.6 MJ/day) and 'low' levels (64.6 MJ/day) during pregnancy, and 'high' (146.8 MJ/day) and 'low' (119.8 MJ/day) in weeks 6–18 of lactation. High levels of feeding during pregnancy significantly reduced the interval to first ovulation.

Hence, within each farming system, practices have been developed empirically which best maximise feed intake and, consequentially, minimise the duration/incidence of anoestrus. In traditional British dairying systems, the preferred option has been to calve in a moderately fat condition, since the grass silages and cereals available to British dairy farmers were of inadequate quality to feed freshly calved cows fully. More recent British practice has followed that of North America, in which the dairy cows hold very small reserves of body fat, but whose appetite is large and, when given high-quality forages and energy-dense cereals, can virtually have their energy demands met. A pastoral dairying system cannot fully feed cows in the early post-calving period, so it tries to find an equilibrium point of calving condition score that allows enough fat reserves to meet the energy deficit, whilst not being so fat that appetite is limited. Farm advisors, nutritionists and, sometimes, veterinarians who operate within these systems become adept at optimally balancing these factors in order to both maximise lactation yields and minimise post-calving anoestrus.

The effect of protein upon anoestrus is less well understood. Protein undoubtedly significantly affects yield, but its effects upon the reproductive system are complex. On one hand, gross deficiencies of protein predispose to anoestrus but, more commonly, small changes in the availability and degradability of the protein component of the ration can markedly affect utilisation of energy and, hence, energy balance. It is probable that, in most nutritional schemes, the interrelationship between protein and energy are of greater consequence in affecting the incidence of anoestrus than is protein deficiency per se.

Some experiments have directly addressed the relationship between protein content of the diet and anoestrus. For example, in a study of high-yielding Friesian cows (Treacher et al., 1976), animals which were given 75% of the recommended crude protein intake had a mean calving to first oestrus interval of 46 days, compared with 35 days for the control group on a normal intake. Likewise, Jordan and Swanson (1979) found a definite effect of protein intake. Between the 4th and 95th days postpartum, groups of cows were fed isocaloric diets containing three different levels (12.7%, 16.3% and 19.3%) of crude protein. Cows on the highest level of protein had the shortest interval to first oestrus. Interestingly, in both studies, the final conception rate was not dependent upon the amount of protein fed; thus the interval to first oestrus was affected but ability to conceive was unaffected by protein level.

Micronutrients. The effects of micronutrient deficiencies upon the prevalence of anoestrus, together with their other effects upon reproductive function, are considered later in this chapter.

Stress. Many aspects of cattle productivity are believed to be affected by stress, of which there appear to be many sources (Wagner, 1974; Platen et al., 1995; Albright and Arave, 1997). Metabolic stresses, imposed by high yield, and social stresses, produced by group and space management of animals (Muller et al., 1986; Rind and Phillips, 1998), are widely considered to be of importance. Physical stressors, such as transport (Nanda and Dobson, 1990), temperature and handling (Thun, 1996), have also been implicated as limitors to production in cattle, as have the stresses of pain, intercurrent disease and lameness.

The social stresses to which cattle are subject have been well known to herd managers for a very long time. Situations in which dominance hierarchies cannot be stabilised are believed to be particularly stressful. Situations in which this occurs include excessively large groups of cows, groups that are continually mixed and the introduction of new animals to the group (see Albright and Arave, 1997). Newcomers are at a social disadvantage and, hence, are stressed; freshly-calved heifers, however, which both are newcomers and have the disadvantage of a significantly lower body weight than the established members of a herd, are believed to be significantly affected (Fielden and Macmillan, 1973). Animals are also stressed when housed in uncomfortable conditions, are over crowded or have inadequate feeding or watering facilities. Recently, these well-established observations have been given substance by quantifying behavioural or endocrine parameters of stress in such animals, and demonstrating that these are correlated with measurable outcomes of reproductive performance.

Other stressors whose direct effect upon the reproductive system have been investigated include transport, handling and temperature. Transport and handling stress have been used as experimental tools for investigating the effects of stress upon the reproductive system, since they provide sources of stress which are more readily quantifiable than is the stress produced by social interactions. Thermal stress (i.e. chiefly high temperatures) influences reproductive performance mainly by affecting embryonic survival, although extreme thermal stress also inhibits oestrous cyclicity (see Thatcher and Collier, 1986; Lee, 1993).

Lameness is considered to predispose to anoestrus, both due to its effects upon nutrition and directly via the stress produced by chronic or unrelieved pain. Lame cows usually have subnormal feed intakes and, therefore, often lose weight rapidly. Hence, where lameness occurs during early lactation, affected cows are in negative energy balance for a long period of time. An effect of bovine lameness upon corticosteroid release has not been clearly demonstrated, although such an effect occurs in sheep (Ley et al., 1994). Hence, it is conceivable that corticosteroid-mediated impairment of reproductive performance also occurs in lame cattle. Although some studies have failed to show any influence of foot disorders on fertility (Cobo-Abreu et al., 1979; Dohoo and Martin, 1984), a survey involving 770 cows over 1491 lactations has shown reduced fertility, as measured by calving to first service interval, calving to conception interval and overall conception rates (Lucey et al., 1986). The greatest effect occurred in cows that had solar or white line lesions during the 36–70 days after calving, the time when cows would be served first; the calving to first service interval was extended by 17 days and the calving–conception interval by 30 days. Overall conception rates during the 63 days before the lameness was diagnosed were 31% compared with 40% at other times. Heel lesions had a particularly serious effect on conception rates. Treatment of lameness was followed by improved fertility (Lucey et al., 1986). More recently, Morton (2000) has shown that submission rates and pregnancy rates are depressed in lame cows, especially when the lameness occurs within the first 6 weeks of lactation.

Incidence. True anoestrus is most frequently diagnosed in high-yielding dairy cows, first-calf heifers which are still growing and beef suckler cows. Observed incidences of anovulatory anoestrus are completely dependent upon the presence or absence of the factors which predispose to the development of the condition. Thus, a generalised figure for incidence can be relatively meaningless, for it depends upon both the type of cattle and the management system. Even within a single system, incidences of anoestrus vary from region to region, season to season and, especially, from year to year. Interactions between nutrient availability, climatic conditions and the establishment of lactation are responsible for enormous fluctuations in the incidence and depth of anoestrus.

Some cows resume cyclical ovarian activity within a few weeks of calving and then become anoestrous. In a study involving 535 dairy cows in four commercial herds, Bulman and Lamming (1978) found that 5.1% of the cows showed this pattern of activity, with the period of anoestrus exceeding 14 days. This compared with 4.9% which had not returned to oestrus 50 days or longer after calving. In a survey of 11 papers in a review by Stevenson and Call (1988), the mean incidence of relapse into anoestrus was 5.5% (range 2.3–22.5%).

Pathogenesis

The endocrine mechanisms which are involved in restoration of normal cyclic activity of the reproductive system after calving have been described in Chapter 7. The process is initiated by the hypothalamus regaining the ability to produce gonadotrophin-releasing hormone (GnRH) and the pituitary regaining the ability to respond to stimulation by GnRH by secreting gonadotrophins. Perhaps the critical step in the entire process is the return of pulsatile secretion of luteinising hormone (LH), since this facet of gonadotrophin secretion has been shown to play an important role in the return of ovarian activity. The anterior pituitary is virtually refractory to stimulation with GnRH in the immediate postpartum period, probably due to the duration of progesterone-induced negative feedback during pregnancy (Lamming et al., 1979).

Many of the factors which predispose to anovulatory anoestrus have also been shown to reduce the pulse frequency of LH in the postpartum cow. Although energy deficiency is clearly amongst the most important of these, little is known about the mechanisms by which it interacts with the GnRH-producing system. McClure (1994) reviewed the hypotheses on the subject that were extant at that time, concluding that, since the GnRH neurons of the ruminant are glucose-dependent (rather than VFA-dependent), the effects of energy deficiency may simply reflect a hypoglycaemic dysfunctionality of those neurons. Secondly, there is some evidence that hypoinsulinaemia occurs concurrently with hypoglycaemia during an energy deficit. Changes in insulin concentrations may directly or indirectly influence gonadotrophin release. In the rat, for example, it has been shown that an insulin receptor located in the hypothalamus may modulate GnRH output (McClure, 1994). Furthermore, the role of neural opioid peptides must also be considered, as an energy deficit causes an increased secretion of these substances (Dyer, 1985). Since opioids have been shown to reduce pulsatile GnRH, and hence LH secretion, they could also mediate the effects of energy deficiency. McClure (1994) also concluded that hypoglycaemia-dependent activation of opioidergic pathways is of significance in the pathogenesis of anovulation.

In beef cattle, the act of suckling stimulates bursts of prolactin secretion (Karg and Schams, 1974), which is considered to be responsible for the extension of the period of anoestrus in such animals. Although no causal relationship has been established in the cow, there appears to be a reciprocal relationship between the hypothalamic control of LH and prolactin release; opioid antagonist treatments (see below) increase LH and decrease prolactin secretion whilst agonists have the opposite effect (Peters and Lamming, 1990). Radford et al. (1978) demonstrated that in suckled cows at 40 days postpartum the LH release in response to stimulation with an injection of oestradiol benzoate was reduced in comparison with that in non-suckled cows. In sheep, the situation is more clear, with autocoid-like actions of prolactin within the pituitary being a critical moderator of both lactational and seasonal anoestrus (Brooks et al., 1999). Hence, given the markedly elevated concentrations that occur in the suckled cow, a significant role for prolactin in the generation of bovine lactational anoestrus seems highly likely.

Finally, the effects of stress upon the hypothalamo-pituitary axis have already been mentioned, but should be re-emphasised at this point, as a further potential contributor to the suppression of the reproductive endocrine axis of the cow which is experiencing anovulatory anoestrus.

In addition, insulin has been shown to exert a significant effect upon the ovary (Poretsky and Kalin, 1987). It has been postulated that low insulin concentrations may limit the responsiveness of the ovary to endogenous gonadotrophin secretion, thus affecting ovulation and corpus luteum formation (Butler and Smith, 1989). Studies of both laboratory mammals and ruminants over recent years have demonstrated a role for insulin-like growth factors (IGFs) as regulators of follicular development. It should also be noted that the high breeding-value Holstein dairy cattle, which seem to be particularly at risk from postpartum anovulatory anoestrus, have intrinsically higher levels of secretion of growth hormone and, consequentially, IGFs than do other cows. Hence, the relationships between the metabolic and reproductive endocrine systems which, operating primarily at the level of the ovary, cause follicular dysfunction, may well be the mechanism of generation of at least some aspects of the postpartum anoestrus syndrome.

Deficiencies of phosphorus, copper, cobalt and manganese and the ingestion of phyto-oestrogens can cause anoestrus, whilst diseases which cause severe weight loss and debility or metabolic disturbances, such as ketosis, can have a similar effect. McClure (1994) postulated that many of the effects of micronutrient deficiencies can be explained by their common role in regulation of glucose production or, in other words, their deficiencies are also mediated through hypoglycaemia.

Treatment

Anovulatory anoestrus can be treated in one of two main ways. Firstly, the predisposing factors can be identified and eliminated. For example, feeding could be improved, micronutrient deficiencies corrected, stress reduced. Alternatively, the animal can be treated with reproductively active hormones, in an attempt to 'restart' the reproductive endocrine system. In fact, although the latter treatments are to a greater or lesser extent successful, they can rarely be relied upon to resolve a situation of anovulatory anoestrus fully (especially at the herd level), unless attention is also paid to alleviating the predisposing factors.

Elimination of predisposing factors. Where micronutrient deficiencies have caused anoestrus, resolution can be quite rapid once appropriate dietary supplementation has been instituted. As an example, the authors recall a herd with hypophosphataemia in which the cows were anoestrous; when an animal did display oestrus, its pregnancy rate was about 30%. Supplementation with phosphorus produced an immediate improvement and, more spectacularly, 70% of the herd was in oestrus during the week after phosphorous feeding started.

Where energy deficiency has caused anoestrus, it is unlikely that a rapid response can be achieved by suddenly increasing energy intake. Stimulation of ovarian activity usually requires 3–4 weeks of improved feeding before a response occurs. Interestingly, an anecdotal observation that has frequently been reported by embryo transfer practitioners is that poor superovulation responses occur for at least 28 days after a period of feed deficit. Where poor responses are obtained, it is normally possible to identify a time when feeding was suboptimal. Hence, improvement of overall energy levels in the diet usually has to be part of a long-term strategy for the farm. Management of the dry period has to be improved, so that the animal calves in the optimum condition score. Management of the transition cow has to be improved, so that it does not suffer from hypocalcaemia, and its rumen is prepared to respond to post-calving changes in nutrition. Management of feeding has to be improved, ensuring that supplies and requirements are as closely matched as possible, that feeding regimens are used that will ensure maximum dry matter intakes and that all age groups of animals have equal (or, in the case of first-calvers, preferential) access to food. Most of these principles apply equally to dairy and beef cows, although the opportunities for modifying feed management practices of dairy cows are usually much greater than for beef suckler cows. In beef suckler cows, temporary weaning and restricted suckling together with the use of progestogens (see below) during the time of calf removal have resulted in reducing the time to the first ovulation postpartum.

Alleviation of other predisposing factors, such as stress, can be difficult. Avoiding mixing groups of cows at critical times seems obvious, yet management of 'high' and 'low'-yielding groups of cows can make this difficult to achieve in practice. Provision of adequate feeding space is more easily achievable, but reducing group sizes to numbers where cows can establish normal social hierarchies can be virtually impossible. The effects of climatic stress can be reduced by the provision of shade to cows that are heat-stressed. Provision of shelter to cows that have to stand around in cold, draughty collecting yards can also be remarkably effective. The authors recall that, in one herd, placement of wind-breaks around a badly sited collecting yard halved the incidence of both mastitis and anoestrus within the herd. The incidence of lameness can be almost entirely attributed to management practices, so anoestrus due to this cause should be regarded as being due to poor management rather than an inherent pathological problem.

Hormonal treatment. Many different hormonal treatments have been given to anoestrous cattle in attempts to cause a resumption of cyclic activity. They fall into two broad categories: administration of hormones with gonadotrophic activity, and administration of progestogens.

The most potent gonadotrophic drug that is available for use in cattle is equine chorionic gonadotrophin (eCG; also known as PMSG, pregnant mares' serum gonadotrophin). It can be used to stimulate ovarian activity and it can induce follicular growth and oestrus. The drawback to its use is that at a dose rate of 3000–4500 i.u. is as likely to cause superovulation rather than to initiate normal ovarian activity. When eCG has been used, it is therefore not advisable to serve or inseminate at the induced oestrus. But if the cow is not inseminated, there is a possibility that she will relapse into anoestrus. Hence, eCG is usually used in combination with other hormones unless superovulation is required.

The administration of GnRH to cows causes the release of LH (Kittock et al., 1973). A single dose of 5 mg GnRH has been used successfully to treat anoestrous diary cows (Bulman and Lamming, 1978). In their trial, it was considered that initiation of normal cyclical activity had occurred if the injection of GnRH was followed by a rise in plasma progesterone. However, in dairy cows in deep anoestrus, or in beef suckler cows, the administration of a single injection of GnRH was ineffective in stimulating ovulation, so the conclusion of Lamming et al. (1981) was that GnRH was only effective in causing ovulation when there was a large follicle already present within the ovary. In suckled beef cows, a second injection of GnRH 10 days later was necessary, after a transient rise in progesterone, to initiate normal cyclical activity (Webb et al., 1977). It has been suggested that the initial progesterone rise has a modulating effect upon endogenous gonadotrophin secretion (Lamming, 1978), and that the repeated dose may mimic the surges of LH that occur in normal cyclic activity (Kittock et al., 1973). Using 2-hourly injections of GnRH or constant release implants has stimulated the onset of cyclicity in lactationally anoestrous beef suckler cows. However, such a regimen is impractical for farm use, while the presence of constant levels of GnRH down-regulated pituitary GnRH receptors, resulting in treatment failure (Lamming and McLeod, 1988).

The newer synthetic GnRH analogues such as buserelin, at dose rates of 0.02 mg, will stimulate oestrus in 1–3 weeks after treatment. This effect is probably not mediated through LH, but may involve a longer-term stimulation of FSH secretion, which initiates new waves of follicular growth.

Progestogen treatment, often associated with other drugs such as oestrogens GnRH or $PGF_{2\alpha}$, has long been used to induce ovarian activity postpartum (Foote and Hunter, 1964; Britt et al., 1974; Wisehart and Young, 1974). These steroid regimens are effective because they either simulate the short luteal phase that usually precedes the first normal oestrous cycle (Lamming, 1980), or else cause an accumulation of gonadotrophin by exerting a negative feedback effect on the anterior pituitary. Whichever mechanism is responsible, rapid withdrawal of the progestogen is essential. Progesterone injections are not really feasible since they have to be given daily for several days, and concentrations do not decline sufficiently abruptly at the end of treatment. Hence, two other routes of administration have been devised. The first is the intravaginal route. The progesterone-releasing intravaginal device (PRID) or controlled internal drug release (CIDR) device (see Chapter 1) are easily inserted and readily removed, producing an abrupt decrease in concentrations. When these devices are placed in anoestrous cows for 7–14 days, most show oestrus within a few days of their removal. Alternatively, the synthetic progestogen, norgestomet, can be given as a subcutaneous ear implant, again for 7–14 days. In this case, the removal of the ear implant at a predetermined time after its placement produces the required abrupt decrease in concentrations. The latter should not be used in lactating dairy cows if the milk is entering the human food chain; it can be used in suckler cows.

A number of trials have reported low conception rates after treatment with progesterone-based regimens (e.g. Bulman et al., 1978), probably due to the effects of long, high progesterone concentrations (Robinson, 1979). Yet even in these animals, there is a reduction in the calving–conception interval compared with those of untreated controls (Lamming, 1980). In attempts to overcome such disappointing results, other hormones have been combined with the basic progestogen regimens. Several authors have found that the injection of low doses (viz. 750 i.u.) of eCG at the time of PRID or CIDR withdrawal improves the response (Mulvehill and Sreenan, 1977; Macmillan and Pickering, 1988). However, larger dose rates will probably have an undesirable superovulatory effect. When norgestomet-based regimens are used in beef suckler cows, a small dose of eCG is used to improve the ovulation response, especially where the cows are in poor body condition score at the time of treatment. Used in this way, the response to norgestomet plus eCG is highly acceptable.

The work of Hansel and co-workers in the 1970s and 1980s showed that good responses to progesterone treatment can be achieved with quite short periods of administration. Hence, they used PRIDs for 7 days, combined with $PGF_{2\alpha}$ towards the time of progesterone removal (in case animals had active luteal tissue), and found both good oestrus responses in anoestrous animals, and good conception rates in cows that were in oestrus. Subsequently, Macmillan and co-workers have progressively refined the use of the CIDR for treatment of anoestrus. They found that short-term progesterone treatment, combined with a small dose (1 mg) of oestradiol benzoate at the time of progesterone removal produced a better response, in terms of numbers of animals displaying oestrus and conception rates to that oestrus, than did progesterone alone.

Oestrogens, both natural and synthetic, have also been used to treat anoestrus. These hormones readily induce behavioural oestrus, but they do so without inducing ovarian follicular activity or ovulation. Moreover, oestrogen administration during the early postpartum period has a potentially detrimental effect upon the pituitary – hypothalamic axis. One of the important stages of the restoration of normal endocrine cyclicity during the postpartum period is the return of the ability of the hypothalamus to generate a positive feedback response to oestradiol. Administration of excessive doses of oestrogen during this phase of the postpartum period can down-regulate hypothalamic oestradiol receptors, resulting in an inordinately long delay before the resumption of normal patterns of ovulation. Low doses of oestradiol, such as are used in current CIDR regimens, avoid this problem. However, in years when the preponderance of cows are in deep rather than shallow anoestrus, a proportion of animals exhibit an oestrus, which is only behavioural rather than physiological, after receiving only 1 mg of oestradiol.

Many studies have shown that the response to any of the aforegoing steroid regimens is better in animals that are well rather than poorly fed. Both the anecdotal evidence of those veterinarians who use CIDRs to treat anoestrus and the documented response of controlled trials show that the response is better in animals whose plane of nutrition approaches equilibrium, rather than being in energy deficit.

Failure to observe oestrus

The use of artificial insemination (AI) as the main method of breeding dairy cows means that the responsibility for oestrus detection falls upon the staff who manage the herd, since if the breeding of the herd is to be controlled, no fertile bulls can be present during the AI period. Oestrus detection therefore becomes a vitally important aspect of dairy herd management, for, whilst good oestrus detection does not necessarily guarantee good reproductive performance, poor oestrus detection makes poor performance hard to avoid. On the other hand, in beef herds, which are usually naturally mated, the importance of oestrus detection is far less critical, largely being confined to ensuring that adequate non-return rates occur after service.

Efficiency of oestrus detection

Cows display oestrus behaviour in the absence of a bull, by mounting and standing to be mounted by other sexually active cows (see Chapter 1). Whether or not these behavioural signs are observed depends upon many factors (Table 22.4). Yet despite the importance of good oestrus detection to the economic performance of the dairy herd, it is often regarded as a 'chore' that has to be fitted between the many other tasks which occupy the herd manager's day, or is delegated to junior members of farm staff. Perhaps it is not surprising, therefore, that in many situations oestrus detection efficiency is poor. On the other hand, where oestrus detection is regarded as a priority task, and is undertaken by highly motivated farm staff under ideal conditions for observation, oestrus detection rates can be remarkably high.

Table 22.4 Factors associated with efficiency of oestrus detection

Time allowed for oestrus detection
How much?
How often?
When, in relation to the activity patterns of the cows?
Human social factors
What other activities are happening on the farm at the same time?
What other pressures exist for the herd manager's time?
Is the detector able to recognise the signs of oestrus?
Is the detector interested in detection of oestrus, or is it 'just another job'?
Calving pattern
How many cows?
How many cows in the sexually active group?
For how much of the year does oestrus detection have to be undertaken?
Housing
Are cows housed or at pasture?
Is there room for cows to display oestrus behaviour?
Can cows that are not in oestrus avoid being ridden?
Identification and records
Can cows be identified accurately?
When should individuals be observed for return to oestrus after artificial insemination?
Aids
Tail paint, heat-mount detectors, electronic aids, etc.?
Relative reliance that is placed on aids and primary observation?

It is therefore of some importance for the veterinarian to understand both the factors that promote efficient oestrus detection, and the limitations that are placed upon oestrus detection within each dairying system, so that appropriate advice can be given to farmers for maximising the proportion of oestrous cows that are inseminated at the correct time.

Most normal cows exhibit oestrous behaviour at the appropriate stage of the oestrous cycle.

King et al. (1976), using continuous observation of dairy herds, found that at the time of the third postpartum ovulation (and for subsequent ovulations) 100% of cows showed signs of oestrus. At the first postpartum ovulation only 50% showed signs, but by the second, 94% of animals displayed oestrous behaviour. Similar figures have been reported by Morrow et al. (1966). It is therefore unusual for normally fed, healthy cows to fail to show signs of behavioural oestrus once normal cyclical activity has been re-established postpartum. Hence, detection of 100% of animals that are in oestrus is not an unrealistic goal.

Regular observation of cows for an adequate period of time. In the previously mentioned study of King et al. (1976), when the same herds were subjected to casual observation for oestrus, only 64% of cows were seen in oestrus preceding the third ovulation, 44% for the second and 20% for the first ovulation. Hence, a significant discrepancy existed between the number of cows displaying signs of oestrus and those which were observed doing so. The problems of oestrus detection in the predominantly autumn/winter-calving British dairy herd have been extensively studied by Esslemont (1973). He examined the effect of the use of a rigid regimen, involving three or four periods of observation for 15 or 30 minutes, upon oestrus detection efficiency (Esslemont, 1973). In the study, three 15-minute periods of observation at 8.00, 14.00 and 21.00 achieved a detection rate of 69.6%. Increasing the duration to 30 minutes improved the rate to 81.2%, whilst four 30-minute periods of observations at 8.00, 14.00, 21.00 and 24.00 hours produced the best result of 84.1%. The absolute times of observation are not critical and can be varied to suit the timetable of the farm.

The relatively short duration of oestrus is one of the main reasons that observation has to be undertaken both regularly and frequently. Although early reports indicated that oestrus can last for 18–20 hours (Tanabe and Almquist, 1960), most studies have found it to last no more than 15 hours (see Albright and Arave, 1997). Esslemont (1974) found that although the mean duration of oestrus is 15 hours, 20% of cows are in oestrus for less than 6 hours.

There are, however, times when cows can be relied upon to exhibit few or no signs of oestrous behaviour. Williamson et al. (1972) showed that observations at pasture are more effective than during milking. Pennington et al. (1985) noted that sexual activity of Holstein heifers was minimal during feeding. Likewise, Pennington et al. (1986) reported heifers to display minimal sexual activity during milking and feeding. Esslemont and Bryant (1976) observed that housed cows were least likely to display oestrous behaviour in holding yards (i.e. whilst awaiting milking), but were most likely to do so in cubicle, or in feeding or 'loafing' areas. It has long been considered that cows are more likely to display oestrous behaviour during the night than during the day. Hence, late evening observations have always been considered to be of great importance in maximising oestrus detection efficiency, especially since the duration of oestrus can be short. In a recent review of oestrous behaviour in cattle, Albright and Arave (1997) concluded that the results of studies of the timing of oestrous behaviour were inconsistent with simple diurnal patterns and probably should be explained in terms of the periods of time for which cows were left undisturbed by other farm activities. Thus, it may be that the prevalence of oestrous behaviour that occurs in housed cows during the night may simply be an indication of the time when there is least other activity on the farm (see also Williamson et al., 1972; Hurnik et al., 1975; Esslemont and Bryant, 1976).

Effects of season and calving pattern. Patterns of oestrous behaviour vary with temperature, with the frequency of mounts and the repertoire of activities differing between hot, moderate and cold conditions (Pennington et al., 1985). In cold conditions, mounting behaviour was much less between 18.00 and 06.00 than at other times of day, whereas in hot conditions it was least during the hottest part of the day. Pennington et al. (1985) also noted that the duration of oestrus was reduced to 8–10 hours under extreme climatic conditions. Certainly, where autumn calving and winter mating is practised (as in the UK), a shortening of the duration of oestrus and a reduction in the intensity of oestrous behaviour are widely recognised as problems of mating management of such herds.

Our ability to detect oestrus also depends upon the seasonal pattern of calvings. This is largely dependent upon the numbers of sexually active cows that are present in the herd at any time. In a genuine year-round calving herd, only 2% of the cows will calve within any given week. Cows comprising the sexually active group (SAG) will normally include those in oestrus, plus those in pro-oestrus and those which have ovulated within the past 24 hours. Thus, cows might be in the SAG for a maximum of 3 days during any oestrous cycle. Hence, for a 100-cow, year-round calving herd, it is entirely conceivable that a typical SAG might consist of 2 cows. This presents a number of problems to the person detecting oestrus. Firstly, the duration and intensity of oestrus increase as the number of animals in the SAG increases (Hurnick et al., 1975; Esslemont et al., 1980; Kilgour and Dalton, 1984), making oestrus detection easier in the larger group. Secondly, there will be times when the sexually active 'group' consists of a single cow, making oestrus detection very difficult indeed. Thirdly, the person detecting oestrus has to be continually observant for signs of oestrous behaviour. Few herd managers can maintain high levels of oestrus detection efficiency throughout long periods of time. Detection can be highly efficient for short periods, but a high level of accuracy can rarely be sustained indefinitely.

Thus, oestrus detection efficiencies are generally much lower in year-round calving herds than in seasonal calving herds. In the former herds, it is generally accepted that oestrus detection rates are rarely better than 60%; in many cases they are less than 50%. Williamson et al. (1972) found that in one herd in Australia the herd manager correctly selected only 56% of cows that were known to be in oestrus as determined by continuous veterinary observation. Such a figure would also typify the results that are currently achieved in many herds throughout the northern hemisphere. On the other hand, some British, semi-seasonal, autumn-calving herds, in which breeding starts on a definite date, can achieve close to 90% oestrus detection efficiency over short periods (up to 9 weeks), a figure which is given as the target efficiency for strictly seasonally calving herds. Interestingly, when the size of SAGs declines in such herds towards the end of the breeding season, it is noticeable that oestrus detection efficiency is not much better than in year-round calving herds.

Herd size. Most observers agree that closely associated with increased herd size there is a reduction in the accuracy and efficiency of oestrus detection (Fallon, 1962; Esslemont, 1974; Wood, 1976). In part, this occurs because each herd manager has to look after more cows, so cows tend to lose their individual identity. Thus, they are not so accurately identified and the slight changes in behaviour, which in a small herd might warn the herd manager of approaching oestrus, are not noticed.

Housing, collection areas, races and paddocks. Crowded collection areas, confined spaces and muddy floors sometimes prevent cows that are not in oestrus escaping from the attentions of other mounting cows, and may not permit the ready grouping of sexually active individuals. A suitable 'loafing' area should be provided to enable cows to show oestrous behaviour.

Several studies have reported that the floor surface is of importance in the display of oestrous behaviour. Concrete surfaces are more slippery than dirt surfaces, and hence less conducive either to mounting or to being mounted (Britt et al., 1986; Vailes and Britt, 1990), whilst individuals which have previously slipped on concrete may be unwilling to attempt to mount in the future (Albright, 1994). Pennington et al. (1985) also noted that the majority of mountings took place in conditions which had the best footing and were less crowded (see Albright and Arave, 1997).

Paddocks that are dry underfoot are an ideal place to observe oestrus, once post-milking grazing behaviour has subsided. Detection of oestrus in races has most of the disadvantages that are associated with collecting yards.

Proficiency of detectors. The importance of trained staff to identify oestrus correctly was shown by Esslemont (1974), who found that in four units where such personnel were used the detection rate was 82–97%, yet with untrained staff it was 67%.

Where there is a defined breeding season, it is possible to use early (pre-season) heats as a means of retraining staff in the skills of oestrus detection. Many such herds record pre-mating heats, not only to identify anoestrous cows, but also to allow prediction of the time when the first heat can be expected during the mating period.

Aids to the detection of oestrus

There are several methods available which can be used to improve the oestrus detection rate.

Identification of cows. It must be possible to identify the individual readily from any position, so that the herd manager can then record the animal number immediately and permanently. Good freeze-branding on the rumps, together with numbered collars or large ear tags, should preferably be used for identification. Even in small herds, whose stockpersons think that they know the cows as individuals, many mistakes are made through misidentification or misrecording.

Provision of adequate lighting. This enables cows to be seen showing behavioural signs and allows their accurate identification. Lighting is obviously most important at night, but can also be significant during the day, if cows are housed in dark yards.

Heat mount detectors and other aids to the detection of mounting. A 'heat mount' detector such as the KaMaR or 'Beacon' can be used. These consist of a soft, translucent plastic dome attached to a rectangle of canvas in which there is placed a soft plastic vial of red dye which is fixed with adhesive just cranial to the base of the tail (Figure 22.17). When a cow is mounted and the

(a)

(b)

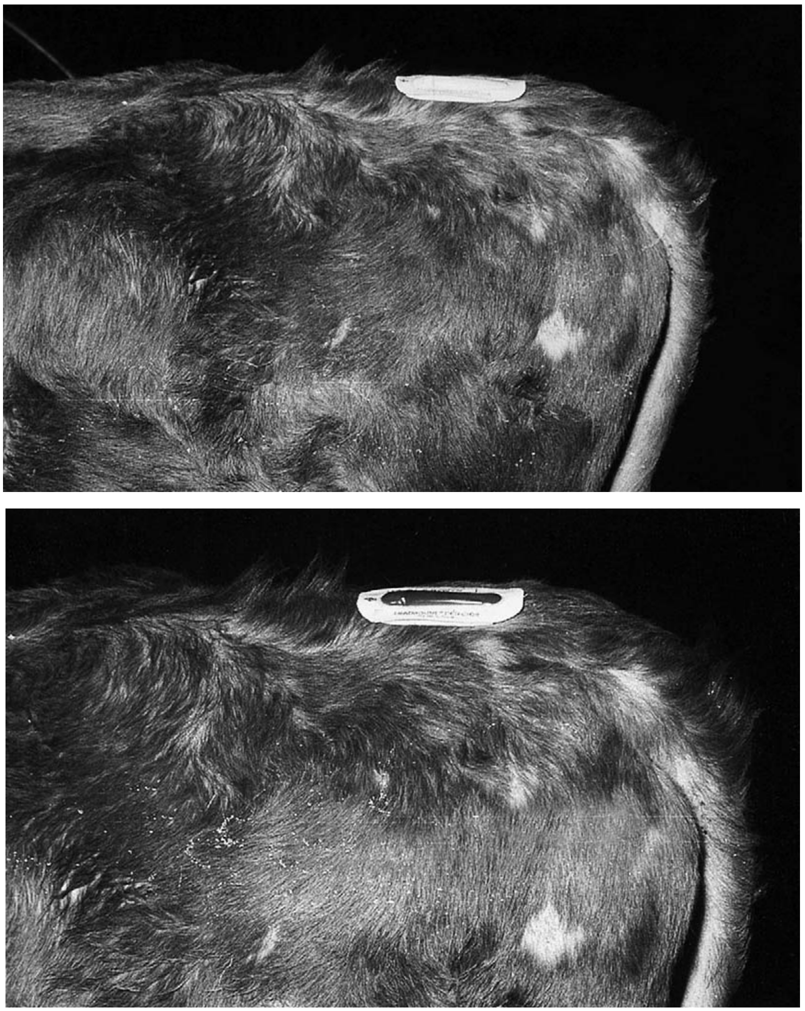


Fig. 22.17 KaMaR heat mount detector attached to the sacrum of a cow. (a) Before activation. (b) After activation.

vial subjected to sufficient pressure, i.e. at standing oestrus, it is compressed, the dye escapes and the dome becomes red.

False positives can occur when the detector is activated by a cow rubbing the underside of a rail or in crowded collecting yards when a cow that is not in oestrus cannot escape the attentions of mounting cows. Detectors can also become detached when placed on wet coats or when the winter coat is being shed.

In 1977, Macmillan and Curnow in New Zealand reported on the use of the technique of tail-painting using brittle, high-gloss enamel paint to improve the detection of oestrus in cows after $PGF_{2\alpha}$ therapy. The paint was placed as a thick layer in the midline over the sacrum and base of tail (Figure 22.18). When a cow is in standing oestrus, mounting by other cows will result in the abrading and removal of the paint. In their initial study, an additional 6% of cows that were not observed in standing oestrus by the herd manager were correctly identified as being in oestrus using tail paint; incorrect diagnosis was made in 4.8% of cows and was assumed to be due to shedding of the coat. Improvements in detection have been found to be 11.2% (Ball et al., 1983). Although some false positive detections of oestrus were made in cows and heifers when between 25 and 75% of paint remained (Kerr and McCaughey, 1984), pregnancy rates of 60% were obtained following artificial insemination on the observation of the condition of the tail-paint. At the time of writing, the use of tail-paint is almost ubiquitous in New Zealand.

Water-based paints or pastes have been used in the UK. It is important that these should be applied using a brush against the line of the hair to ensure good adhesion before smoothing in the direction of the hairline. There should be regular inspection of the paint so that repainting can be done if necessary. In many British herds, the use of tail-paint was of no particular benefit to the overall oestrus detection rate. This may be because of the small SAGs that occur in non-seasonal herds, or it could be due to the choice of water-based materials. In either case, the poor results obtained in Britain demonstrate that such methods are only *aids to detection* and cannot adequately substitute for accurate observation.

More recently, radio-telemetric heat mount detectors have become available. In this system, an electronic pressure-sensing system is linked via

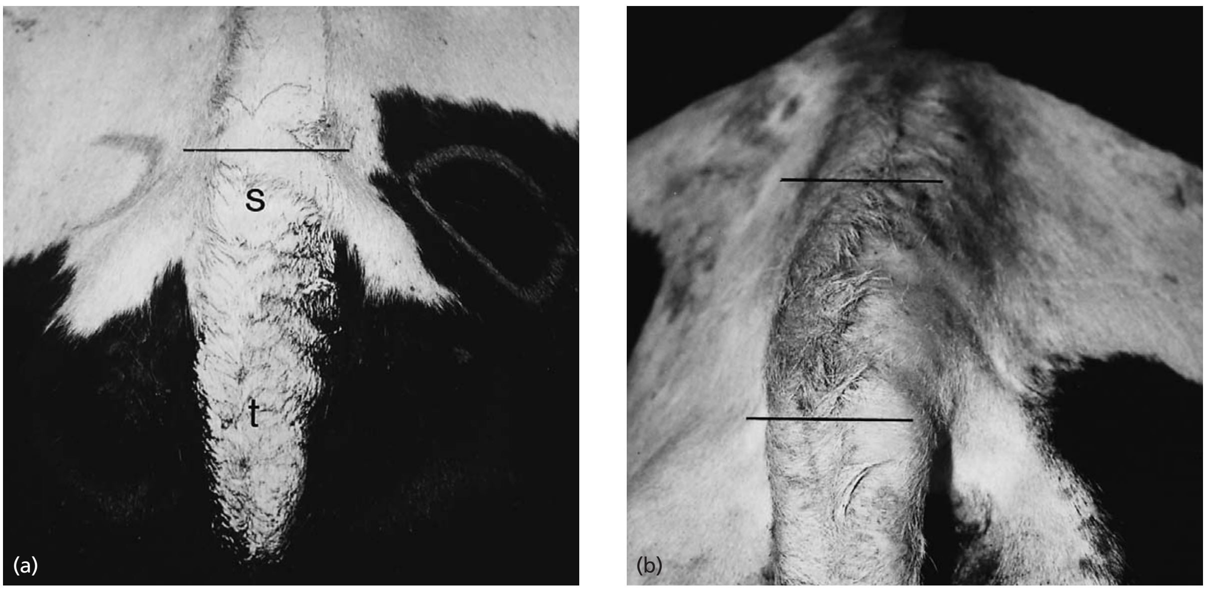


Fig. 22.18 Tail paint applied to a cow as an aid to oestrus detection. (a) After application over the caudal sacral (s) and tail-head (t). (b) When rubbed off by mounting during oestrus. The horizontal lines delineate the extent of the original application of paint. Photographs by courtesy of N. B. Williamson.

a radiotelemetric link to a computer data analysis system, which sorts the information by cow and generates activity lists (e.g. oestrus, possible oestrus, etc.) (Nebel et al., 1995; Walker et al., 1995; Dransfield et al., 1998). At the time of writing, the system is available commercially as the HeatWatch™ system. Initial studies indicate that results from the HeatWatch system are sufficiently reliable to present an alternative to visual oestrus detection (Albright and Arave, 1997; Cavaleri et al., 2000a). Further work has been undertaken to develop pressure sensors and/or transmitters that are implanted, rather than being affixed to the animal's skin. However, no such systems are presently available commercially.

Other physiological predictors of oestrus

Pedometers. During oestrus, the cow shows greater movement and activity. If pedometers are attached to individual animals, this increase in locomotor activity can be identified and used to predict the occurrence of oestrus (Kiddy, 1977). Since 1977, a number of devices have been made to record the frequency of movement, with progressive improvements in accuracy, such that Schofield (1990) concluded pedometers to be a most reliable method of oestrus detection. Computer-based 'intelligent' data interpretation systems are also proving of value in improving the accuracy of pedometers (Eradus and Braake, 1993). Thus, Varner et al. (1994) noted that the locomotor activity of individual cows not only depended upon the stage of the cycle, but also upon the size of the SAG: a factor that must be taken into consideration in the design of data handling systems. Finally, Goodrich and Sun (1994) used sonar to detect movement, finding improvements in oestrus detection efficiency over direct observation. Since this method requires no devices to be fitted to cows, it may eventually prove to be a more effective method of detection than the pedometer.

Vaginal probes to measure electrical resistance/impedance. Since the early 1970s there has been considerable interest in measuring the changes that occur in the electrical resistance of vaginal mucus during the oestrous cycle. At oestrus, the resistance falls, in association with the rise in oestrogen concentrations. Generally, results have been disappointing (Foote et al., 1979; Cavestany and Foote, 1985). The reason for the variability in the measurements may well be related to the fact that the tip of the probe, with its associated electrode, is not in contact with vaginal mucus. Kitwood et al. (1993) reported that the position of the probe within the vagina affects impedance readings. In addition, the authors have obtained aberrant results when the cow has recently urinated before the probe was inserted. An example of one such commercially available instrument is shown in Figure 22.19. When regular examination of cows can be backed up with a computer-based data logging and analysis system, results are better than when 'one-off' measurements are made, since although most cows exhibit decreases in

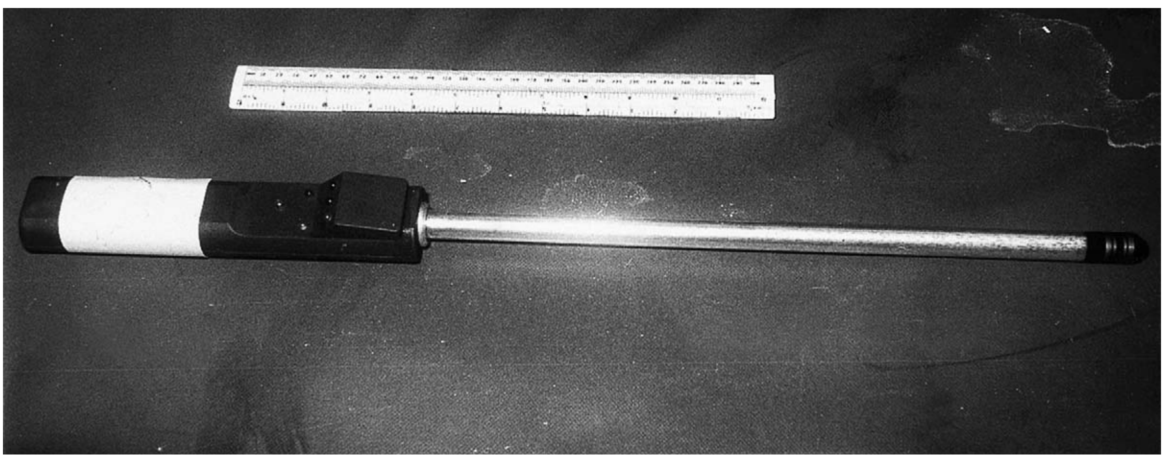


Fig. 22.19 Vaginal probe for measuring changes in electrical impedance in order to detect oestrus.

resistance at oestrus, most individuals differ in their baseline resistance during dioestrus. Hence, the measurement of relative changes in resistance may be more useful than measurement of absolute values. Nevertheless, Rezac et al. (1991) found that lowest impedance values could occur on day –1, 0 or +1 relative to the day of oestrus (day 0).

The mechanical nose. Development of gas-sensing systems and work on the identification of pheromone-secreting cells in the perineum of oestrous cows (e.g. Blazquez et al., 1994) make it possible that direct electronic sensing of the odours of oestrous pheromones, perhaps as cows pass a detector during milking, may be feasible within the foreseeable future.

Indirect detection

Use of teaser bulls, androgenised steers or cows. Vasectomised or other sterile entires or androgenised steers can be used, either equipped with some form of marking device or in association with 'heat mount' detectors. They have not been very popular in the UK, largely because teaser bulls with good libido present a major safety hazard when allowed to run loose with the herd. Furthermore, where venereal diseases are present they represent a major health hazard because of their ability to transmit such diseases. In other countries, penile deviation is used as a means of preparing sterile bulls. For this, the preputial orifice is freed from its normal attachments and is relocated some distance from the midline. Although this procedure is not permitted in the UK, it is considered to be an effective aid to the detection of oestrus in many other countries. Its disadvantages include those which are common to the use of gonad-intact bulls. Moreover, some bulls learn how to serve despite the penile deviation, while others desist from mounting at all. These, and a number of other surgical procedures for creating marker bulls, are described by Wolfe (1986).

Androgenised cows can also be effective 'teasers' (Britt, 1980). By administering testosterone propionate in oil by intramuscular injection every week for 3 weeks, a suitable teaser is prepared which can be used about 2 weeks after the last injection. Maintenance of sexual activity requires repeat treatment at intervals, but these androgenised cows have distinct advantages since they are safer and do not transmit venereal disease.

Use of dogs. Dogs can be trained to detect odours associated with oestrus in cows. The sources of the odours are widespread throughout the genital tract and also appear in milk and urine (Kiddy et al., 1984).

Use of closed-circuit television. Television cameras, recorders and monitors are now much cheaper and more reliable than before. During the night, provided that there is adequate lighting and good animal identification, a continuous video recording can be made of the 'loafing' areas of the yard where cows are housed. The herd manager can then rapidly scan the recording in the morning and identify cows that are in oestrus.

Use of milk progesterone assays. The return to oestrus in non-pregnant cows can be anticipated by the measurement of progesterone concentrations in sequential milk samples. Protocols for such measurement regimens are given in Chapter 24.

Use of oestrus synchronisation and induction programmes. Immediate improvements can be achieved by oestrus synchronisation programmes. While the most cost-effective programmes require that animals are inseminated at detected oestrus, most of the methods give tolerable results when fixed-time insemination is used. Hence, since it is possible to anticipate approximately when oestrus will occur following their use (see Chapter 1), the herd manager can be extra-vigilant at these times and can inseminate cows that are observed in oestrus (Cavaleri et al., 2000a,b). Failing this, no attempt need be made to detect oestrus and cows can be inseminated either once or twice at fixed times as outlined in Chapter 1.

Effects of incorrect timing of AI upon pregnancy rate

Oestrus is short in the cow, with ovulation occurring 10–12 hours after the end of oestrus. During the next 6 hours the oocyte travels about a third of the way down the uterine tube, during which time fertilisation occurs, about 30 hours after the onset of oestrus (Robinson, 1979). The best conception rates occur if insemination is carried out in the middle to the end of standing oestrus, i.e. 13–18 hours before ovulation. Cows may conceive if they are inseminated at the beginning of oestrus or even

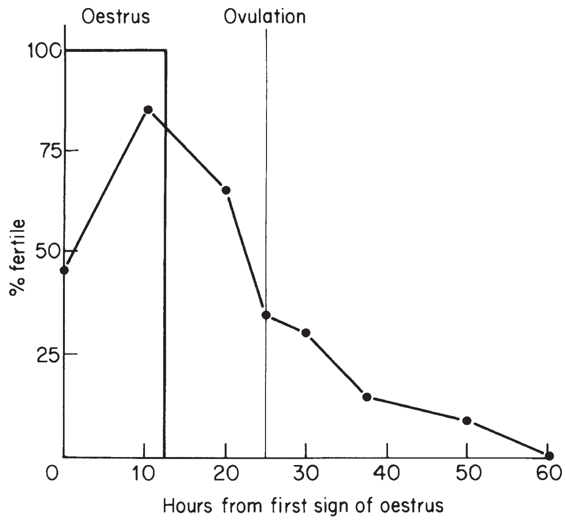


Fig. 22.20 Conception rates in the cow: the effect of the time of insemination in relation to oestrus and ovulation (after Trimberger, 1948).

36 hours after the end of oestrus but conception rates are reduced (Figure 22.20) (Trimberger, 1948).

When natural service is used there are no problems, since a cow will only stand for the bull when she is in oestrus, and under free-range conditions a cow may be served several times at each oestrus. The correct timing of artificial insemination is a dependent upon true, accurate and early identification of oestrus, the accurate identification of the individual animal and informing the artificial insemination organisation at the correct time. A cow that is first seen in oestrus in the morning is usually inseminated in the afternoon of the same day, whilst a cow that is first seen in oestrus in the afternoon is inseminated early the next day.

A number of observers (Hoffmann et al., 1974; Appleyard and Cook, 1976), have used milk progesterone concentrations to show that between 10 and 15%, or perhaps even 22%, of cows are inseminated during the luteal phase of the oestrous cycle. It is not surprising that these animals fail to conceive. However, these figures do not include those animals which are inseminated during the follicular phase of the cycle at times that are not optimum for good conception rates. Bulman and Lamming (1978) found that 15% of cows were inseminated during the luteal phase, but a further 15% were inseminated during inappropriate stages of the follicular phase.

The main reasons for these errors are incorrect identification of animals that are in oestrus, and failure to appreciate the true signs of oestrus. Frequently, where large numbers of cows are inseminated at the incorrect time, the oestrus detection rate is poor, thus generally reflecting a poor standard of herd management. In such circumstances, some of the methods described above should be used to improve the oestrus detection rate in the herd. In seasonally calving herds, the emphasis that is placed upon attaining high submission rates during the first few weeks of the mating period is such that many cows are presented for insemination that are not in oestrus. However, most of these cows are correctly identified a few days later, so the effect upon pregnancy rates is far less significant (see Chapter 24).

Cystic ovarian disease

Ovaries are said to be cystic when they contain one or more fluid-filled structures larger than a mature follicle (i.e. > 2.5 cm diameter), which are persistent for longer than 10 days and which result in aberrant reproductive function. The definition sometimes specifically excludes the presence of a corpus luteum (Youngquist, 1986); however, it is not always correct to do so (Al-Dahash and David, 1977b; Carroll et al., 1990). Details of fluid-containing structures in bovine ovaries are listed in Table 22.5.

Cysts arise as a result of anovulation of a Graafian follicle. Under normal circumstances, anovulation is followed by either atresia or luteinisation, after which the follicle undergoes regression. In cystic ovarian disease, the follicle increases in size and persists, for at least 10 days. There is degeneration of the granulosa cell layer, which results in an alteration of the normal cyclical activity of the cow, so that she becomes either acyclic or nymphomaniacal.

Many cows develop large, fluid-filled structures in the ovaries during the immediate postpartum period (see Chapter 7). It has been reported that up to 60% of cows develop cysts before the first

Table 22.5 Fluid-containing structures in bovine ovaries

Follicles	*Vacuolated corpora lutea*	*Luteinised follicles*	*Follicular cysts*	*Luteinised cysts*
Transient, dynamic, soft, fluctuant structures	Occurs after up to 25% of normal ovulations	Follow anovulation of mature follicle	Follow anovulation of mature follicle	Follow anovulation of mature follicle
Usually identifiable clinically ≤ 1.5 cm diameter at all stages of oestrous cycle	Same size as non-vacuolated corpus luteum but may feel slightly softer on palpation	No evidence of ovulation point	Soft, thin-walled, fluid-filled structure ≥ 2.5 cm diameter which persists	Thick-walled, fluid-filled structure ≥ 2.5 cm diameter which persists
1.5–2.0 cm in diameter just before, during and for 12 hours after oestrus	Evidence of ovulation point	<2.5 cm diameter	Frequently multiple in one or both ovaries	Usually single
	Central vacuole disappears during pregnancy	Larger cavity than vacuolated corpus luteum	Thickness of cyst wall <3 mm	Thickness of cyst wall >3 mm
		More likely to occur in immediate postpartum period	Associated with low peripheral blood progesterone levels	Associated with high peripheral blood progesterone levels
	Associated with normal oestrous cycle	Associated with normal or shorter length of oestrous cycle	Affected cows will be either anoestrus or nymphomaniacal	Affected cows will be anoestrous

postpartum ovulation (Morrow et al., 1966; Kesler et al., 1979). These normally go undetected, unless the cow is examined transrectally and, if they regress spontaneously without any extension in the interval to first oestrus or evidence of nymphomania, they should not be considered as true ovarian cysts. The point of distinction is that they do not cause aberrant reproductive function.

Incidence

Surveys of the incidence of cystic ovarian disease need to be interpreted with some caution, since information on the effects of the cysts upon reproductive function is usually absent from abattoir surveys, whilst in herd studies, cysts should only be regarded as pathological if they persist into the breeding period. Incidences found in abattoir surveys range from 0.5% (Australia: Summers, 1974) to 18.5% (Japan: Fujimoto, 1956). Al-Dahash and David (1977a) found an incidence of 3.8% in a British abattoir survey. Studies in the USA have reported an incidence of 5.6–47.4% (Kesler and Garverick, 1982; Peralta and Ax, 1982). Day (1991) quotes four North American references giving figures ranging from 6 to 19% per lactation. In a study of 34 dairy herds in Ontario, there was a lactational incidence of 5%, with a median time to first diagnosis postpartum of 90.5 days. Watson (1996), in a study of 3000 cows in 24 herds, found that cystic ovarian disease caused clinical signs in 7–8% of cows. Youngquist (1986) estimated the incidence at between 10 and 20%, but considered that if the cysts which occur before the start of the breeding season are also taken into account, the incidence is about 30%. Anecdotal evidence has frequently suggested that cystic ovarian disease is increasing in incidence; a 20-year retrospective study of a single dairy herd involving 923 cows and 2246 calvings showed a steady increase from 1963. In 1966 only 10% of cows were affected, whereas in 1983 the comparable figure was 57%.

Predisposing factors

Cystic ovarian disease arises as an interaction between a hereditary predisposition, stress, milk yield, age and plane of nutrition.

There is evidence to suggest a hereditary basis for cystic ovarian disease. Early studies of the condition showed that there was a significantly higher incidence in some families within a breed (Garm, 1949; Casida and Chapman, 1951). Likewise, the incidence of cystic ovaries is influenced by the cows' sire (Palsson, 1961; Menge et al., 1963; Kirk et al., 1982). Beef breeds are seldom affected. In a study of 390 000 Swedish Red and White (SRB) and Swedish Friesian (SLB) cows, the SRB cows were twice as likely to have cystic ovarian disease as those of the SLB breed (Emmanuelson and Bendixen, 1991). In the SRB but not the SLB breed, the probability of cystic ovaries increased with parity. Surprisingly, therefore, estimates of the heritability are low (Youngquist, 1986; Day, 1991). Nevertheless, the incidence of the condition can be dramatically reduced within a breed by avoiding the use of cows that have had cystic ovarian disease as bull mothers, and by avoiding the use of bulls that have bred daughters that have the disease as bull sires (Bane, 1964).

The incidence of cystic ovarian disease is greatest in animals between 4 and 6 years of age (Roberts, 1986). It is uncommon in animals in their first lactation. The association with age may simply reflect the fact that milk yields tend to be highest during these lactations, for several studies have linked the incidence of cystic ovaries to milk yield. Such a relationship was demonstrated by Hendricksson (1956) and Marion and Gier (1968), who observed that the disease was more prevalent in high-yielding cows and that it occurs at the stage in the lactation curve when yield is at its peak. Recently, a relationship between milk volume and fat output has been shown (Ashmawy et al., 1992). The same authors also demonstrated that in the two groups of cows that they studied, the repeatability of cyst development was 20% in Guernseys and 6% in Holsteins. The feeding of high-protein diets appears to be a contributory factor. However, Zulu and Penny (1998) did not identify milk yield per se as a cause of cystic ovarian disease, although they did find a higher incidence in a strain of cows that were selected for high yield than in unselected animals; but Laporte et al., (1994) found that cows with cystic ovarian disease had higher yields in the ensuing lactation than did normal cows. Finally, Gearhart et al. (1990) reported that drying off cows in an excessive condition score was associated with the disease.

Whilst the season of the year also appears to have some effect, since the disease is more prevalent in winter than at other times of the year (Bierschwal, 1966; Roine, 1973), this may reflect the fact that the majority of cows are calving in the autumn and thus will have reached peak yield at this time. Ketosis, dystocia, stress, twin births, RFM and milk fever have also been considered risk factors for the condition (Morrow et al., 1966, 1969; Hardie and Ax, 1981; Roberts, 1986; Laporte et al., 1994). Likewise postpartum uterine infection may also predispose to the disease (Bosu and Peter, 1987).

There has been some evidence that β-carotene, the plant precursor of vitamin A, may be important in reducing the incidence of ovarian cysts (Lotthammer, 1979). However, this has not been supported by the work of others (Folman et al., 1979; Marcek et al., 1985), who found that supplementation of cows' diets with β-carotene had no beneficial effect in reducing the incidence of cysts.

Aetiology

A few cases of cystic ovarian disease are caused by mechanical interference with the process of ovulation. Al-Dahash and David (1977a) found that ovarobursal adhesions were present in 7.67% of the genital tracts with cystic ovaries, compared with 1.8% of the non-cystic population. The interrelationship between ovarobursal adhesions and ovulation failure is further considered below.

More commonly, cystic ovarian disease develops as a consequence of a failure of the endocrine mechanism of ovulation. Although much progress has been made in understanding the precise endocrine abnormalities that are responsible for cystic ovarian disease, many unanswered questions remain.

Follicles grow and synthesise steroids under the trophic control of LH and follicle-stimulating hormone (FSH) (Eyestone and Ax, 1984). During the initial stages of follicular development, most steroid synthesis is under the control of FSH but, once a follicle reaches a certain stage in its development, it acquires aromatase activity within its granulosa cells, which allows conversion of androgens into oestrogens (principally oestradiol-17β). There is much variation in the ability of medium-sized follicles to secrete oestradiol. The acquisition of oestrogenic activity is coincident with the development of LH receptors in the theca interna cells of most follicles, whilst only highly oestrogenic follicles develop LH receptors in the granulosa. The possession of granulosa cell LH receptors is necessary to make follicles capable of progressing to ovulation. During preovulatory follicular growth, large amounts of oestradiol are produced by the follicle and are secreted into the peripheral circulation. As the follicles develop towards ovulation, peripheral oestradiol concentrations increase to the point at which positive feedback release of LH is triggered, resulting in the preovulatory LH surge. The positive feedback effect of oestradiol occurs primarily through changes in the pattern of GnRH secretion, but the high concentrations of oestradiol also increase the sensitivity of the pituitary to GnRH. LH then binds to its receptors in the preovulatory follicle, causing a loss of aromatase activity and decline of androgen and oestrogen synthesis, an increase in eicosanoid synthesis, an increase in follicular plasminogen activator, and down-regulation of its own receptors. Proteolytic enzymes break down the apical follicle wall and ovulation occurs.

Defects in this complex process are believed to lead to cyst formation, although the evidence regarding which particular events are critical in the development of cysts remains equivocal. Cysts have been induced by the administration of LH antiserum (Nadaraja and Hansel, 1976), or by suppressing the preovulatory LH surge through progesterone administration either just before, or at the onset, of oestrus (Lee et al., 1988). From the results of such experiments, it has been postulated that cysts occur when insufficient LH is released to cause normal ovulation. Significantly lower LH concentrations have been reported during pro-oestrus and oestrus in cows with ovarian cysts than in normal cows (Kesler et al., 1979). Conversely, others have found LH concentrations in cystic cows to be similar to, or higher than, those of normal animals (Brown et al., 1986; Cook et al., 1991). Moreover, the pituitaries of cows with follicular cysts contain a similar number of LH receptors to normal cows, and the pituitaries of both respond to exogenous GnRH by secreting LH (Kittock et al., 1973). Farin and Estill (1993) considered that it was the presence of higher than normal concentrations of LH during the follicular phase which was associated with the development of cysts.

An alternative but closely related explanation might be an alteration of the positive feedback LH response to the preovulatory oestrogen peak. Zaied et al. (1981) administered exogenous oestradiol benzoate to normal and cystic cows, but observed no difference in the magnitude of responses. They did, however, note that there was a temporal delay between the oestrogen peak and the LH peak in cows with follicular cysts. However, Nanda et al. (1991) found a significant attenuation of the LH response to oestradiol in cystic compared to normal cows. An inhibition of ovulation, followed by cyst formation, has been produced experimentally with injections of adrenocorticotrophic hormone (ACTH) (Liptrap and McNally, 1976). The authors were unable to produce a similar response with hydrocortisone treatment, suggesting a direct effect of ACTH upon the ovulatory mechanism. Studies of ewes have shown that ACTH administration reduced the LH response to GnRH, reduced the self-priming effect of GnRH upon the pituitary and caused a delay in the LH response to oestradiol (Phogat et al., 1999). These changes in the LH surge mechanism closely resemble those occurring in cystic ovarian disease, suggesting that stress, such as is mediated by the ACTH–adrenal cortex axis, may be a significant contributor to the development of ovarian cysts. The importance of this mechanism in the generation of cysts was emphasised by Nanda and Dobson (1990), who found abnormal LH surge characteristics in cows that had stress-induced elevations of corticosteroid concentrations. Other work has examined the role of endogenous opioid peptides in the aetiology of cystic ovaries. These peptides, which affect both GnRH secretion and the LH response of the pituitary, are also produced during stress and may, therefore, be involved in the generation of cysts.

Thus, whilst most evidence indicates that the development of ovarian cysts may be primarily due to deficiencies of LH secretion during the preovulatory surge, it is also feasible that asynchrony of the hormonal events of the perovulatory period also contributes significantly to their development.

Finally, there is evidence that the prolactin–thyroid system may be involved in the development of ovarian cysts. Hypothyroidism has been associated with cystic follicles in a number of species, and thyroxine concentrations are negatively correlated with milk production, such that high-yielding cows have lower concentrations than low-yielding animals. Hence, an association between hypothyroidism and cystic ovarian disease in cattle has been postulated (Eyestone and Ax, 1984). Hafez (1975) suggested that ovulatory failure and cyst formation are related to high prolactin secretion (i.e. as associated with high yield) and, although McNeilly (1980) considered that prolactin is not involved in regulating FSH and LH release at ovulation, the administration of bromocriptine (a prolactin inhibitor) blocked cyst formation in hypothyroid rats (Copmann and Adams, 1981).

There is also some evidence that the problem of ovulatory failure and cyst formation may be due to defects within the ovary. The ability of follicles to respond to the preovulatory LH surge is dependent upon the timely induction of LH receptors during follicular maturation; if too few receptors are available then ovulatory failure may occur. Studies in women have shown that in cells from cystic follicles the number of LH receptors was only 51% of that found in normal preovulatory follicles (Rajaniemi et al., 1980). Yet, it is important to stress that follicular cysts will respond to hCG and GnRH therapy by either ovulating or luteinisation (Berchtold et al., 1980; Kesler et al., 1981), thus confirming the presence of adequate LH receptors in such structures. It is interesting to note that Bartosik et al. (1967) thought that prolactin may reduce the sensitivity of the ovary to normal concentrations of LH, suggesting that effects of prolactin could be mediated either centrally or directly upon the ovary.

Distribution, classification, diagnosis and clinical signs

Distribution and classification. Cysts may be present in one or both ovaries. More cysts are identified in the right ovary than in the left, reflecting the relative activities of the two ovaries. Garm (1949) found that multiple cysts were more frequent than single cysts, but Elmore et al. (1975) found that 75% were solitary cysts. Al-Dahash and David (1977b) found, in a survey of over 8000 genital tracts, that 53.5% of the 307 tracts which had cystic ovaries had a single cyst and 46.2% had multiple cysts. The majority of cysts found in this study were between 2.5 and 3.0 cm in diameter, with very few larger than 5 or 6 cm (Table 22.6).

Diagnosis. Traditionally, ovarian cysts have been classified as either follicular or luteal cysts. Follicular cysts (Figures 22.21, 22.22 and 22.23a) are thin-walled and have little or no luteal tissue in the cyst wall. It is common to find multiple follicular cysts. Luteal, or luteinised, cysts (Figures 22.23b, 22.24 and 22.25) are thick-walled and more usually single and have a large quantity of luteal tissue in the cyst wall. However, the accurate classification of cysts is difficult by palpation per rectum and there are a number of other structures that can be confused with them. Farin and Estill (1993) suggest that the corpus

Table 22.6 Distribution of ovarian cysts and their association with corpora lutea (from Al-Dahash and David, 1977b)

Type of cyst	*Number present*	*Percentage of cysts*		
		No corpus luteum present	*Corpus luteum present*	*Total*
Thin-walled	Single	16.93	18.85	80.19
	Multiple	40.26	4.15	
Thick-walled	Single	10.22	7.67	19.81
	Multiple	1.92	0	
Total		69.33	30.67	

Fig. 22.21 Genital tract with large 10 cm thin-walled cyst in right (r) ovary and left (l) ovary with corpus luteum.

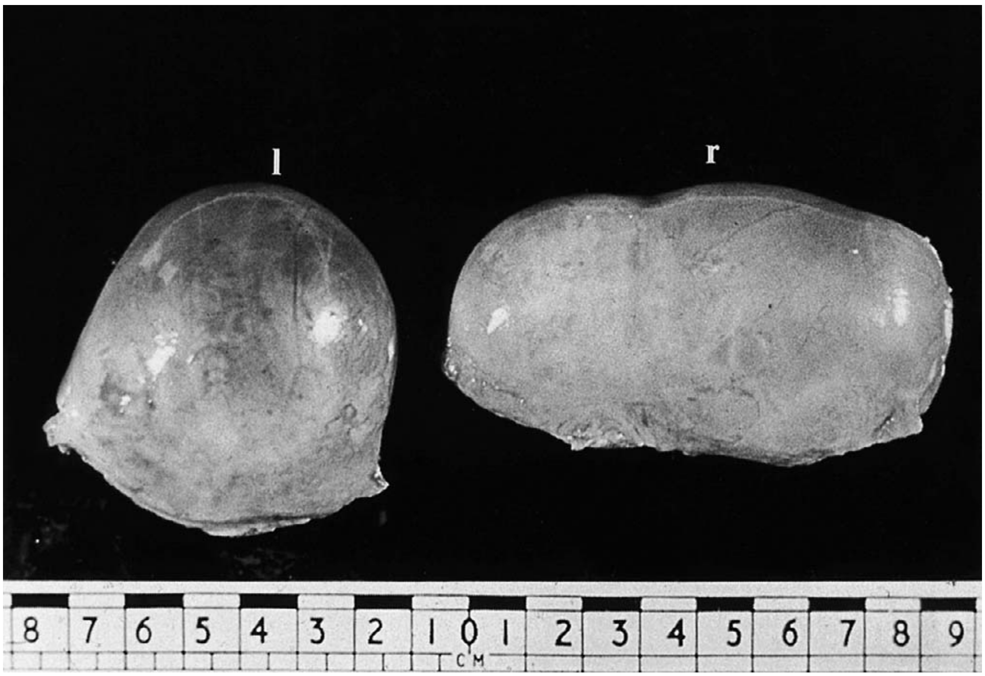


Fig. 22.22 Ovaries of a cow with two thin-walled cysts (4–5 cm in diameter) in the right (r) ovary and a single thin-walled cyst (5 cm in diameter) in the left (l) ovary.

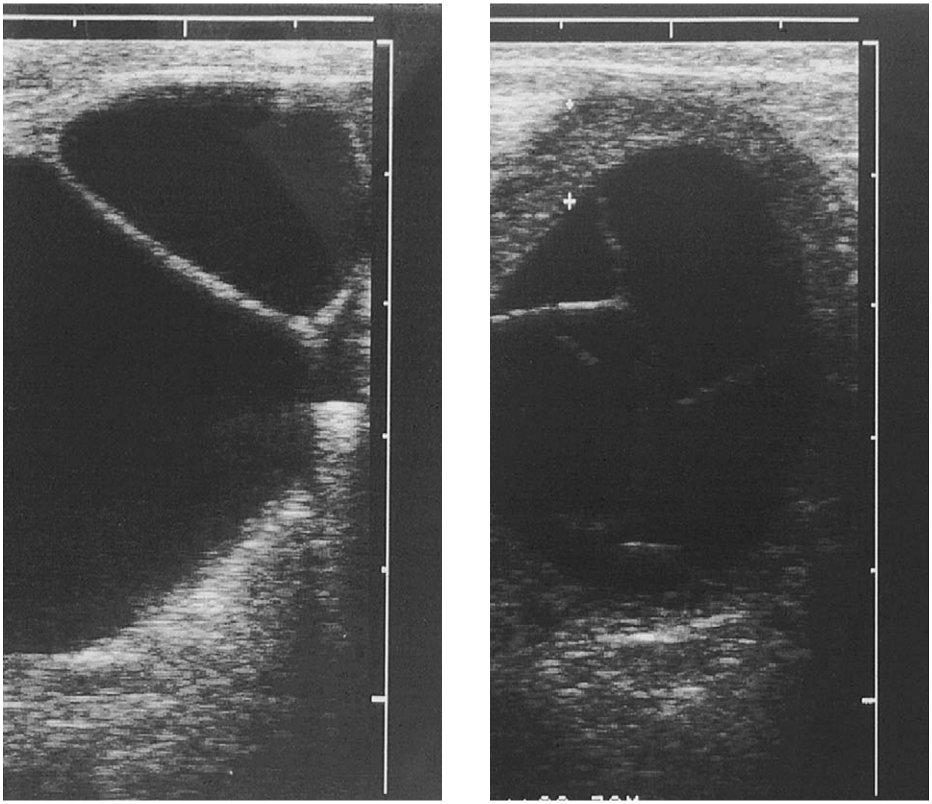
(a)  (b)

Fig. 22.23 (a) Ultrasound image of the ovary of a cow with a thin-walled cyst. (b) Ultrasound image of the ovary of a cow with a luteal cyst. Note wall of luteal tissue which is > 3 mm in thickness (by courtesy of W. R. Ward).

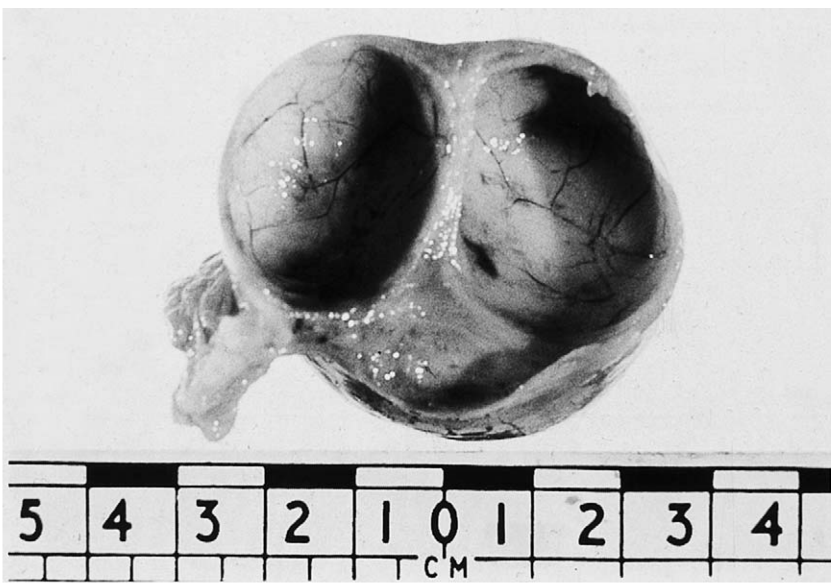


Fig. 22.24 Cross-section of an ovary of a cow showing three cysts with some degree of luteinisation.

Fig. 22.25 Cross-section of an ovary of a cow showing a typical single, thick-walled, luteal cyst. Note that the wall comparises at least 3 mm of luteal tissue.

haemorrhagicum, preovulatory follicle, adjacent luteal and follicular structures, non-ovarian cysts, abscesses and tumours can all be confused with cysts.

More recently, it has become generally accepted that a definitive diagnostic test is the measurement of progesterone concentrations in blood plasma/serum or milk. In this method, a discriminatory value of 2 ng/ml for milk (Booth, 1988) or 0.5–1.0 ng/ml for plasma/serum (Carroll et al., 1990; Farin et al., 1992) is used to determine the type of cyst. Thus, progesterone concentrations greater than or equal to the discriminatory value denote a luteinised cyst, less than the value denotes a follicular cyst. Based upon progesterone determinations, follicular cysts are between two and three times more common than luteal cysts (Kesler and Garverick, 1982; Leslie and Bosu, 1983; Booth, 1988).

However, cysts have been identified in association with a corpus luteum (Al-Dahash and David, 1977b; Roy et al., 1985; Carroll et al., 1990) (see Figure 22.21), which could lead to some discrepancies in the use of progesterone determinations to diagnose which type of cyst is present. For example, a cow with a true follicular cyst (i.e. containing very little luteal tissue), but with a corpus luteum present in one of its ovaries, would have a plasma or milk progesterone concentration above the discriminatory value, indicative of a luteal cyst.

Moreover, cysts are not static structures but are dynamic; cysts regressing spontaneously and being replaced by others (Kesler and Garverick, 1982; Cook et al., 1990). There are also changes in the type of cyst, as determined by the degree of luteinisation. Carroll et al. (1990), who used transrectal ultrasound imaging to demonstrate the dynamic state of ovarian structures, the fluctuations in plasma progesterone concentrations with time and the occurrence of ovulation with corpus luteum formation in the presence of cysts, therefore questioned the accuracy of progesterone assays as a method of diagnosing the type of ovarian cysts.

The accuracy of diagnosis can be improved by the use of transrectal ultrasound imaging (Jeffcoate and Ayliffe, 1995). The thicker wall of the luteal cysts allows differentiation from follicular cysts; a thickness of 3 mm is generally considered to be the point of differentiation between the two types (Figure 22.23b).

Clinical signs. The main clinical signs of cystic ovarian disease in cattle are nymphomania, anoestrus or masculinisation.

Cystic ovarian disease was first discovered as a result of investigations of nymphomaniac behav-

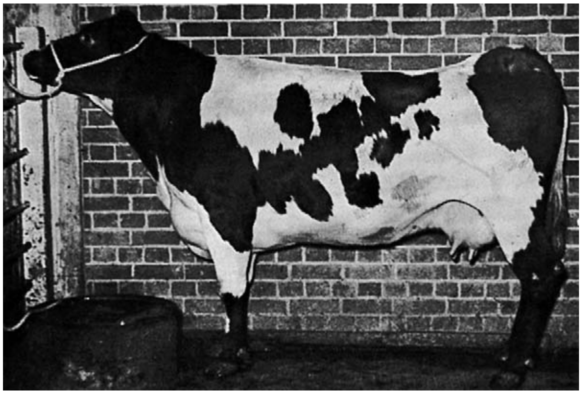


Fig. 22.26 An Ayrshire cow with the typical nymphomaniac configuration.

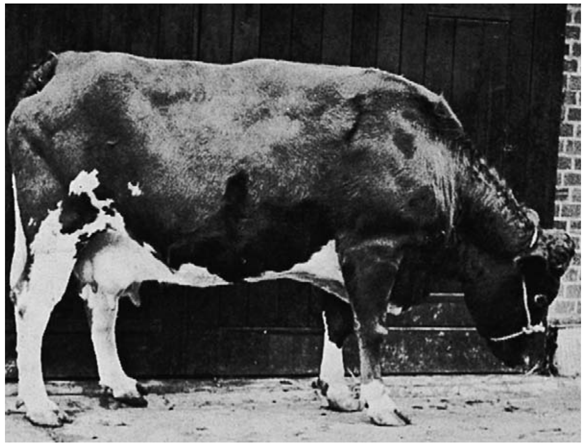


Fig. 22.27 Cow with masculine configuration and behaviour (virilism) associated with a long-standing luteal cyst.

iour in cows. Roberts (1955), in a survey of 352 cows with cystic ovaries, found that 73.6% were nymphomaniac and 26.4% acyclical. Cows with follicular cysts are often nymphomaniacal, i.e. displaying excessive, prolonged signs of oestrus and a shortened interval between successive heats. There is oedematous swelling of the vulva, frequent and copious discharge of clear mucus, sinking of the sacrosciatic ligaments and upward displacement of the coccyx (Figure 22.26). Affected cows may have a nervous disposition, with depressed milk yield and loss of bodily condition. They will attempt to ride other cows and, as with cows in oestrus, will stand to be mounted by other cows. Because of their excessive sexual activity they have a general disruptive effect upon the rest of the herd, making accurate oestrus detection difficult. Furthermore, owing to the relaxation of the pelvic ligaments, they are prone to pelvic and hip fractures.

The luteal or luteinised cyst usually results in a cessation of cyclical activity; the structure functions as a persistent corpus luteum. It is difficult to understand why it does not regress under the influence of endogenous luteolysin, since it will regress under the influence of exogenous prostaglandin (see later). If cows with luteinised cysts are left untreated then a proportion of them will become virilised (Arthur, 1959). These individuals will develop a masculine conformation and will attempt to mount other cows, but unlike the nymphomaniacal cow they will not stand to be mounted by other cows (Figure 22.27).

An interesting effect of the presence of a cyst and an associated corpus luteum is that there is a greater tendency for the granulosa layer of the cysts to be absent (Al-Dahash and David, 1977c), which may have been due to the effect of progesterone from the corpus luteum or the age of the cyst. In the same survey, 22.8% of the cysts which were examined showed evidence of luteinisation, varying from small isolated patches to a continuous thick layer below the theca; usually it was seen as a thick crescentic layer at the base of the cyst. Luteinisation was most frequently seen in the single thick-walled cyst.

Cows with follicular cysts have blood oestrogen concentrations that are not greatly elevated above those of normal cows (Kittock et al., 1973; Dobson et al., 1977). Likewise, testosterone concentrations in cows with follicular cysts and nymphomania are no different from those for normal cows, nor can they be correlated with the intensity of nymphomaniacal behaviour (Eyestone and Ax, 1984). Roberts (1986) concluded that it is not possible to correlate the concentrations of oestrogens, androgens or progesterone in cows with cystic ovaries with their behavioural signs, probably because both progesterone and testosterone modulate and potentiate the effects of oestrogens in the development of oestrous behaviour, and the effect of each steroid is related to the duration of its presence as well as its absolute concentration.

Most recent surveys of behaviour patterns show anoestrus to be the dominant sign. Bierschwal (1966) reported 60% as acyclical, Anttila and Roine (1972) found 57% to be anoestrus, and Dobson et al. (1977) found 73% acyclical and 27% nymphomaniacal. Roberts (1986) reviewed series of cases in which 62.5 to 85% of animals were anoestrus. Conversely, Booth (1988), in a survey of 200 cows with cystic ovarian disease, reported that 38% of the 141 cows with follicular cysts (72.5% of the total) showed signs of nymphomania, but Carroll et al. (1990) identified nymphomania in only 12.5% of 16 cows that developed ovarian cysts.

Treatment and consequences

Treatment. Spontaneous recovery from cystic ovarian disease occurs frequently in the early post-calving period. Self-cure rates within 45 days of calving vary from 13–29% of cases (Beck and Ellis, 1960; Whitmore et al., 1974; Bierschwal et al., 1975; Garverick, 1997) to as much as 50% (Morrow et al., 1966).

The treatment of cystic ovarian disease in cattle has been reviewed in detail by Nanda et al. (1989). The earliest method of treating cysts was by manual rupture per rectum. Although rupture sometimes occurs inadvertently, it should not be done intentionally as it can cause trauma or haemorrhage, which might result in ovarobursal adhesions. Surgical removal of one chronically affected ovary, or paracentesis using a long hypodermic needle through the sacrosciatic ligament might be worth considering in a limited number of cases where other treatments have failed.

Most cysts are now treated using reproductive hormones. The choice of hormonal treatment regimen depends largely upon the type of cyst that is present; follicular cysts are usually treated with either gonadotrophic hormones (i.e. hCG or GnRH) or progesterone, whereas luteinised cysts are normally treated with luteolytic substances.

The first successful treatment of follicular cysts was with unfractionated sheep pituitary extract (Casida et al., 1944). Subsequently, intravenous hCG has been used, as first described by Roberts (1955). The hCG is usually given by the intravenous route, at doses of between 3000–4500 i.u. (UK practice) to 10 000 i.u. (USA practice). Others have given small doses of hCG directly into the cyst, although this method has never gained widespread acceptance. GnRH has also been used successfully to treat follicular cysts. It was thought at first that GnRH or hCG administration caused luteinisation of the cyst either by inducing an increase in endogenous LH secretion, or by causing luteinisation directly. However, it is increasingly well recognised that GnRH has little direct effect upon the cyst itself but, instead, it causes ovulation of new follicles (Ribadu et al., 1994; Jeffcote and Ayliffe, 1995). These follicles develop into corpora lutea. Thus, whether GnRH induces luteinisation of the cyst or the formation of new corpora lutea, the result is an increase in progesterone concentrations, usually within 10 days of treatment. Elevated progesterone concentrations cause a negative feedback-induced decline in endogenous LH secretion. A consequential decline in follicular steroid synthesis occurs, leading to declining oestradiol-17β concentrations. This is considered to be the most important factor in restoring normal cyclical activity (Kesler and Garverick, 1982). Doses of 100–250 μg of GnRH probably cause luteinisation of the cyst (Kesler et al., 1981). The use of GnRH analogues (e.g. buserelin, 10 μg dose) or larger doses of GnRH (0.5–1.0 mg) has been associated with ovulation of follicles and formation of new corpora lutea (Berchtold et al., 1980).

Results with GnRH and hCG treatment have generally been good. Dobson et al. (1977) reported a 90% response to GnRH and a 76% response to hCG, while 50% and 27% of animals conceived at 1.4 and 2.25 services, respectively. Over 80% of cows treated with GnRH had resumed normal cyclical activity within 18–23 days of treatment (Kesler et al., 1978). In a large and detailed survey involving 225 cows with ovarian cysts and irregular oestrous cycles (Whitmore et al., 1979), 76% responded to a single injection of 100 μg of GnRH and only four failed to respond to up to three injections; 83% of the treated cows became pregnant, with a 49% pregnancy rate to first service. In the study by Kesler et al. (1979) those cows that failed to respond to GnRH treatment had mean pretreatment peripheral progesterone concentrations of

0.4 ± SE (standard error) 0.2 ng/ml; perhaps the degeneration of the cyst wall prevented the thecal cells from responding to LH stimulation.

In a study involving 116 cases of follicular cysts which were treated with either 500 μg of gonadorelin or 20 μg of buserelin intramuscularly, 52.6% recovered 3–15 days after treatment and 93.4% conceived within 1.55 inseminations. Only 7.8% had recurrent cysts. Some were treated with a second dose of GnRH (Nanda et al., 1988). Ijaz et al. (1987) reviewed an extensive series of reports of cystic cows that had been treated with GnRH, concluding that 62–97% recovery rates were achieved, with an interval to oestrus of 18 to 23 days and a conception rate to that oestrus of 37–55%.

Alternatively, follicular cysts can be treated with progesterone. Very early trials used parenteral injections of progesterone, with considerable success (see Roberts, 1986), but the difficulties of parenteral administration have led to the use of alternative routes. Intravaginal progesterone-releasing devices are now the most widely used route of administration. In a study in which PRIDs were used in 25 cows (18 of which had been treated unsuccessfully with other hormones), 68% recovered within 13–18 days after the insertion of the PRID and 88% of these conceived within three inseminations (Nanda et al., 1988). Signs of nymphomania abate within 24 hours of PRID insertion, the cysts gradually regress and, following removal after 10–12 days, there is oestrus with ovulation and corpus luteum formation. It is believed that progesterone absorbed from the PRID suppresses the gonadotrophin support that is required for the maintenance of the cyst, resulting in its demise. Following progesterone withdrawal, there is a surge of gonadotrophin with ovulation and corpus luteum formation. The authors' experience of this method of treatment of follicular cysts is that it is very effective.

Luteal cysts have been treated with progesterone, hCG, GnRH and $PGF_{2\alpha}$ (or its analogues). Results with progesterone have been variable; thus, Trainin (1964) reported that only 10% of cows showed regression of the cyst with only one cow conceiving, but Dobson et al. (1977) had a good response with eight of nine cows showing regression of the cyst, of which five conceived with a mean of 1.5 services per conception. They used a treatment regimen of 100 mg of progesterone in oil by intramuscular injection on three successive occasions at intervals of 48 hours. A PRID is another effective method of administering progesterone. Treatment with hCG was fairly successful, according to Bierschwal (1966), with 50% of acyclic cows conceiving to the first oestrus after treatment and the average interval to first oestrus being 24.5 days. Presumably, hCG stimulates further luteinisation and the heavily luteinised cyst then perhaps becomes susceptible to the action of endogenous luteolysin. Alternatively, since secondary ovulations with corpus luteum formation frequently occur, the subsequent release of luteolysin which causes regression of the corpus luteum might have a similar effect on the cyst. This is also probably the response after the injection of GnRH, since good results have also been reported following its use; 65% of cysts regressed and 50% of the cows thus treated conceived at a mean interval of 37 days after treatment (Dobson et al., 1977).

The most logical way to treat a luteal cyst is the use of $PGF_{2\alpha}$, although there is still no explanation for the failure of cows to respond to their endogenous luteolysin. A predictable response was obtained by Dobson et al. (1977); 26 of 27 cows showed regression of the cyst, the majority came into oestrus in 3–5 days, and 56% of the cows conceived, at a mean treatment-to-conception interval of 27 days. Jackson (1981), in a survey involving several countries, reported over 80% response with disappearance of the cyst and oestrus within 3–5 days, with at least 60% and in most cases over 90% of these cows conceiving. Many similar reports exist in the literature (see Ijaz et al., 1987). Indeed, failure of cows with supposed luteal cysts to respond to $PGF_{2\alpha}$ therapy is almost invariably due to misdiagnosis.

In an attempt to reduce the interval between treatment and first service, the suggestion has been made that routine treatment of cysts could be managed by giving GnRH when the cyst is first diagnosed, followed by $PGF_{2\alpha}$ 9 days later (Kesler et al., 1978; Garverick, 1997). However, in a study in which comparisons of subsequent fertility were made with GnRH therapy alone, the results were worse; in addition, such a treatment regimen is more expensive (Archibald et al., 1991). Similar disappointing results were reported by Nanda et al. (1988). Nevertheless, in situations in which progesterone is not licensed for use in lactating dairy cows, combinations of GnRH and $PGF_{2\alpha}$ constitute the only practical way in which progesterone concentrations can be manipulated for the treatment of cysts (Garverick, 1997).

The study of Watson and Cliff (1996) found that of those cows with cystic ovaries that were untreated, 54% eventually conceived; of those that were treated, 79–87% conceived. Surprisingly, however, there was no difference between treatments in the overall success rate, although fewer cows needed retreatment after an initial progesterone treatment than with other therapies. Moreover, in the aforegoing discussion of ovarian cysts, emphasis has been placed on the differentiation of the type of cyst, based on clinical history, rectal palpation, progesterone assay or ultrasound imaging. Perhaps such differentiation is not necessary since, in a survey of 84 cows with cystic ovaries, Elmore et al. (1975) treated the cows with GnRH or hCG irrespective of the type of cyst that was present. Excellent results were obtained. The majority of cows had luteal cysts, with first service conception rates of 55 and 46%, overall conception rates of 97 and 100% and mean treatment-to-conception intervals of 37 and 48 days following GnRH and hCG, respectively.

Consequences of cystic ovarian disease. Cystic ovarian disease depresses fertility in a number of ways; it extends the calving interval, decreases lifetime milk yields and increases the involuntary culling rate. The cost has been calculated to be $137 per lactation per cow (Bartlett et al., 1986). Interestingly, both the field study of Watson and Cliff (1996) and the computer modelling of Scholl (1992) found that cystic ovarian disease has a major effect upon reproductive outcomes of individual animals, although the condition was not of major significance in determining the reproductive performance at the herd level.

However, one further consequence of cystic ovarian disease is the development of mucometra (Figure 22.28), in which there is distension of the uterus with mucoid fluid and thinning of the uterine wall. In the survey conducted by Al-Dahash and David (1977a), these uterine features were all associated with ovaries which contained thin-walled, follicular cysts and no corpora lutea. In the same survey, several specimens showed marked dilatation of the uterine glands that was associated with thick-walled luteal cysts with or without a corpus luteum.

Fig. 22.28 Cow with cystic ovarian disease and consequent mucometra. Note the thin-walled distension of the uterus that is almost symmetrical between the two uterine horns; o = ovaries.

Prevention

By careful genetic selection, improvements have been made by eliminating bulls that have sired daughters which have subsequently suffered from cystic ovarian disease. Ideally, cows should not be treated for cystic ovaries, and certainly their progeny should not be used for breeding. Unfortunately, this places the herd manager and the veterinarian in a dilemma since, frequently, those cows that are affected are the best producers.

Prophylactic use of GnRH has shown some success in reducing the prevalence of cysts in herds. It has been recommended that all cows should be treated with 100–200 μg of GnRH 12–14 days postpartum (Kesler and Garverick, 1982). Whether it is cost-effective has not been calculated.

Miscellaneous conditions

Anovulation

A syndrome that is associated with those conditions that lead to both true anoestrus or to cystic ovarian disease is that of ovulation failure. Sometimes anovulation is observed before the onset of a period of anovulatory anoestrus, with the follicle regressing and becoming atretic. Similarly, during the puerperium, before the onset of normal cyclical ovarian activity, a similar situation may arise, which is comparable with that observed in seasonal polyoestrous species at the start of the breeding season. If cows are examined per rectum during the first few weeks after calving, a number of enlarged anovulatory follicles can often be detected; they are incorrectly described as being cysts (see later) but they are transient and do not persist even if no treatment is given.

Sometimes, however, a follicle does not regress but, having reached its maximum size of 2–2.5 cm in diameter, the wall becomes luteinised. This structure functions in the same way as a corpus luteum, either regressing after 17–18 days or, frequently, much earlier so that the cow returns to oestrus at a shorter than normal interval. After the demise of the luteinised follicle, the subsequent oestrus will probably be followed by a normal ovulation. Such a structure will be <2.5 cm in diameter and fluid-filled, with a rim of luteal tissue lining the follicle and with no evidence of a point of ovulation.

Evidence of this occurring in a living animal comes from a report by Watson and Harwood (1984). They found that, in a Friesian cow, ovulation failed to occur after the second oestrus, 68 days after calving. Sequential blood samples demonstrated a normal LH peak, lasting about 10 hours and reaching a maximum concentration of 24 ng/ml, which was preceded by a normal preovulatory oestradiol-17β peak the day before oestrus. Thus, there was apparently no extraovarian endocrine abnormality and the cow ovulated and conceived at the subsequent oestrus 22 days after the previous anovulatory one. Obviously it could have been confused with a luteal cyst (see above), but it was not larger than a mature follicle and did not persist. Others may also confuse it with a 'cystic' corpus luteum (see Chapter 1), which is a normal corpus luteum containing a central fluid lacuna, and hence has an ovulation papilla. 'Cystic' corpora lutea are not abnormal; they account for 25% of the corpora lutea examined in ovaries obtained from the abattoir. They contain the same amount of progesterone as non-cystic corpora lutea (Donaldson and Hansel, 1968). Perhaps, to avoid confusion, the term 'vacuolated' corpus luteum would be preferable. Such structures can often be identified using transrectal ultrasound imaging.

Diagnosis of anovulation can only be made retrospectively, by noting on transrectal palpation or ultrasonography that a follicle persists longer than one would have suspected. In the case of the luteinised follicle it will remain for 17–18 days before regressing; the ovary containing it will be rounded, smooth and fluctuating, rather than irregular and solid as it is with a corpus luteum. There is no information on the incidence of these conditions.

Treatment is directed towards ensuring that ovulation occurs at the next oestrus; hence hCG or GnRH administered as described for delayed ovulation are indicated. If ovarobursal adhesions are present no treatment is possible.

Suboestrus or silent heat

A number of authors (Casida and Wisnicky, 1950; Morrow et al, 1966; King et al., 1976) have shown that the first and second ovulations postpartum frequently are not accompanied by behavioural signs of oestrus, and are thus truly 'silent heats'. It is, however, unlikely that many true 'silent heats' occur after the second cycle. When ovulation occurs in the absence of observed oestrus it is more likely to be the result of a failure of observation than of an impaired expression of oestrous behaviour. However, Hall et al. (1959) reported an incidence of 10.6% of silent heats, even when cows were examined four times in 24 hours, with no improvement in the detection rate when the frequency of observation was increased to every 2 hours. Labhestwar et al. (1963) reported a figure of 23.7% in 3076 ovulations, and a similar figure of 27.3% was quoted by Kidder et al. (1952) when the herd was inspected every 12 hours; it was especially high (44%) during the first 60 days after calving but even during the subsequent 60 days it was 11%.

A genetic predisposition to silent heat has been identified (Labhestwar et al, 1963), with certain sire lines showing a statistically significant effect. The same authors found that it was more common in the hotter months of the year, although in temperate climates it has been shown to be more common in the winter than in the summer months (Hammond, 1927). Suboestrus has undoubtedly been associated with heat stress, whilst cows suffering from 'fescue toxicity' (ergotism) may also become suboestrous. A number of nutritional deficiencies are also said to cause suboestrus, including β-carotene, phosphorus, copper and cobalt. Attempts have been made to identify an endocrinological reason for a cow failing to show behavioural signs but to date none has been identified.

Diagnosis of the condition is made on the clinical history and rectal palpation of the genital system. No differentiation can be made from non-observed oestrus, since the clinician will be checking for evidence of cyclical ovarian activity as demonstrated by the presence of a corpus luteum or (if the cow is in late dioestrus, early dioestrus or oestrus), by the presence of good uterine tone. The corpus luteum must be differentiated from a cyst; it may be persistent or the cow may be pregnant. If there is any doubt, then a re-examination should be made in 10 days. Since the accuracy of identifying a corpus luteum by rectal palpation has been reported as 89% (Dawson, 1975) and 77% (Boyd and Munro, 1979), the determination of progesterone in milk or blood, or the use of transrectal ultrasonography, should confirm its presence.

Treatment is straightforward. If a mature corpus luteum is present and the cow is not pregnant, $PGF_{2\alpha}$ or an analogue can be given, followed by fixed-time insemination or breeding at the time of observed oestrus (see Chapter 1). If the corpus luteum is at a refractory stage (see Chapter 1), a double injection prostaglandin regimen at an 11-day interval could be used. Alternatively, a PRID or other progestogen implant could be used followed by fixed-time insemination.

The perception of some herd managers that their poor oestrus detection is due to some kind of pathalogical 'sub-oestrus' can, however, be used to improve detection efficiency on such farms. First the veterinarian agrees with the herd manager that the problem is not due to his/her inability to detect oestrus, but due to 'quiet or shy bullers', which then provides the opportunity to instigate all kinds of 'oestrus detection drives' to improve oestrus detection which are entirely standard, but which would be totally resisted if the herd manager felt that he/she was 'at fault'. On one large farm, the author organised a meeting of all the farm staff, gave them all little notebooks in which to record oestrus observations and, eventually, found that one tractor-driver was the best detector on the premises. In another situation, a recalcitrant herdsman was persuaded to do a 9.30 p.m. observation, because it was the best time to identify 'problem' cows. In both cases, oestrus detection efficiency was dramatically improved. Indeed, the authors have found that simply taking the pressure of 'fault' off the herd manager can produce a tremendous response in terms of efficiency of detection.

Persistent corpus luteum

The mechanisms involved in the control of the life span of the corpus luteum are described in Chapter 1. Anything which interferes with the production or release of $PGF_{2\alpha}$ will result in a persistent corpus luteum. Pregnancy is the condition which most frequently results in persistence of the corpus luteum. However, as described previously in the sections on uterine pathology, in the presence of uterine infection and inflammation of the tissues, there is interference with the production or the release of $PGF_{2\alpha}$ (Ginther, 1968; Seguin et al, 1974). This condition can be self-perpetuating since progesterone domination of the uterus reduces its resistance to infection and prevents recurrent periods of oestrus when the uterus is more resistant (Rowson et al, 1953). One consequence of this is pyometra which, if untreated, can persist for several months.

By the sequential measurement of milk progesterone concentrations, there is firm evidence that the life span of the corpus luteum can be extended in the absence of uterine lesions and in apparently normal cows. For example, Lamming and Bulman (1976) identified 2% of the cows with elevated milk progesterone levels for more than 30 days. Recently, Taylor (2000) found extended luteal phases in cows, especially those of high genetic merit, in the immediate postpartum period. Neither study ascertained whether uterine lesions were present in such animals. It is the authors' opinion that most cases of 'persistent corpus luteum', in the absence of uterine lesions, are incorrectly diagnosed and are due to non-detected oestrus. However, if it is considered that a persistent corpus luteum is present, it can be readily treated with $PGF_{2\alpha}$ or a synthetic analogue, provided, of course, that the clinician is confident that the cow is not pregnant.

Ovulatory defects

Ovulation in the cow occurs 10–12 hours after the end of behavioural oestrus and 18–26 hours after the ovulatory LH peak (see Chapter 1). During oestrus and after the end of oestrus, several follicles undergo development but usually only one, or occasionally two, ovulate; the other follicles regress and become atretic. A number of defects associated with ovulation can occur (Figure 22.29); these are outlined below. The consequences for fertility of an ovulatory defect are two-fold; either the oocyte is not liberated and hence cannot be fertilised, or it is liberated too late so that the spermatozoa are now incapable of fertilisation, or else the oocyte has aged and is not capable of normal development.

Ovulatory defects occur due to endocrine deficiency or imbalance, failure of the development of hormone receptors at the target tissue or mechanical factors. If the quantity of pituitary hormone released is insufficient, or its timing is incorrect (this is particularly true of LH), then ovulation is delayed or fails to occur. In a minority of cases, because of extensive lesions involving adhesion of the ovarian bursa to the surface of the ovary, the physical process of ovulation is prevented.

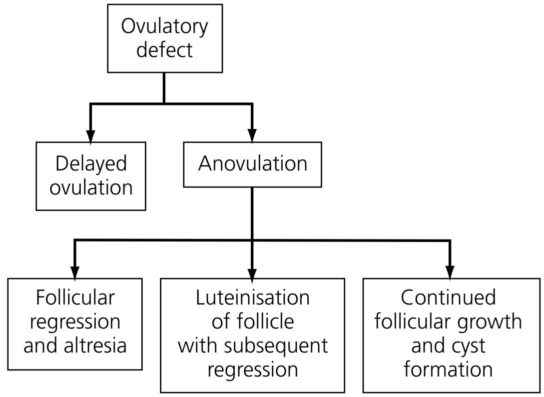


Fig. 22.29 Ovulatory defects in the cow.

Delayed ovulation. There is little information on the incidence of delayed ovulation as a cause of infertility. Hancock (1948) found that only 36% of cows that had ovulated by the second day after oestrus conceived to service during oestrus, compared with 65% conception in the majority (69%) which had ovulated by the first day after oestrus. An incidence of 18% delayed ovulation has been reported in South Africa (Van Rensburg and de Vos, 1962); the delay was less than 48 hours in 85% of the cases and more than 48 hours in 15%. These authors recommended that, if ovulation had not occurred by 24 hours after service, the cow should be reinseminated; and of 51 cases so-treated 32 conceived, compared with no conceptions in 18 cows in which the follicle had failed to rupture within 24 hours of a single service. A much lower incidence of less than 2% in 'Repeat Breeder' cows has been reported by Zemjanis (1980). In a group of Holstein cows, Erb et al. (1976) (who assumed that ovulation normally occurred within 2 days of the LH peak) found that, of the seven cows that ovulated later than 2 days after this surge, none conceived.

Several authors have noted that certain cows have an apparent prolonged follicular phase of the oestrous cycle as determined by the presence of low progesterone concentrations in blood and milk (Erb et al. 1976; Bulman and Lamming, 1978; Jackson et al., 1979). However, there is no evidence that this is because of delayed ovulation;

rather it is related to a delay in the corpus luteum assuming normal steroidogenesis.

Diagnosis of the condition is difficult. Perhaps it is possible to diagnose the condition in the way suggested by Zemjanis (1980) by the identification of the same follicle in the same ovary on two successive examinations, one at peak oestrus and another 24–36 hours later. The occasions on which such examination would be undertaken in routine practice are truly rare and, given the dynamic state of ovarian follicles, accurate diagnosis would be extraordinarily difficult to achieve.

Hormonal imbalance. There is much conflicting evidence concerning the relationships between hormonal imbalance and infertility, probably influenced to some extent by the errors inherent in measuring hormone concentrations in a limited number of peripheral blood samples.

It is known that the rate of transport of the oocyte and zygote along the uterine tube is under the influence of oestrogens and progesterone (Whitney and Burdick, 1936, 1938). Thus, if there is an incorrect balance of these hormones there may be accelerated or retarded passage of the zygote, so that it reaches the uterus at a time when the environment is hostile to its survival. It has also been known for some time that in order to have good embryo survival after embryo transfer the recipient's and donor's oestrous cycles must be synchronised within one day of each other (Rowson et al., 1969; Newcombe and Rowson, 1975).

In practice, it is possible neither to diagnose nor to treat this condition. However, attention should be drawn to a situation in which hormone imbalance is certain to occur: namely, the superovulated animal. Superovulated cattle have grossly abnormal patterns of steroid production after an eCG-induced poly-follicular oestrus, their patterns of steroid concentrations are often abnormal during the first and second cycle after superovulation, and several weeks elapse before the ovarian follicular hierarchy is restored to normal. Secondly, the use of progesterone-based synchronisation regimens in cyclical cattle can lead to abnormally prolonged periods of elevated progesterone concentrations. In sheep, long periods of high progesterone concentrations are associated with poor fertility, a situation which also pertains in cattle.

EFFECTS OF NUTRITION UPON REPRODUCTION

The interrelationships between nutrition and reproduction are amongst the most important, and probably the least understood, of the factors that control reproductive performance. Anovulatory anoestrus is a well-recognised consequence of deficiencies of both macro- and micronutrients, even though, as previously discussed, little is known about the physiological mechanisms that are involved in linking the metabolic and reproductive systems. Much the same situation pertains in relation to other aspects of the relationship between nutrition and reproduction, especially in regard to pregnancy rate. Whilst there is very good evidence that nutrition is inextricably linked with pregnancy rates, there are few data which explain the mechanisms involved.

In this section, the effects of energy and protein status upon pregnancy rate will be considered. Consideration will be given to the many facets of impaired reproductive performance that occur with the 'fatty liver' syndrome. Finally, the effects of micro nutrient deficiencies upon both oestrous cyclicity and (where it is possible to separate the effects) pregnancy rate will be considered.

Puberty and the rearing period

Puberty, as determined by the appearance of the first oestrus, occurs between 5 and 20 months of age. It is considered that body size, rather than age, is the main stimulus (Joubert, 1954; Asdell, 1955; Reid et al, 1964), although condition score and plane of nutrition (Sorensen et al., 1959; Leaver, 1977; Ducker et al, 1985; Spitzer, 1986) and, possibly, day length, all affect the timing. In order to attain puberty, beef heifers should have reached 50%, and dairy heifers 35–45%, of their mature weight. Thus, typical weights at puberty are 240–260 kg for Friesians, 270–300 kg for Herefords and 230–250 kg for Aberdeen Angus. Within a group of cattle, however, puberty occurs at different body weights, so one should consider body weight as an indicator of the probability of an animal being pubertal, rather than as its absolute determinant. Thus, Spitzer (1986) showed that as body weight increases, so does the proportion of beef heifers that exhibit oestrus; 50% of Hereford heifers exhibit oestrus at 272 kg bodyweight, whereas Charolais have to reach 308 kg for the same proportion of animals to be pubertal. For 90% of animals to be pubertal, the weights are 318 kg and 352 kg body weight, respectively.

The effect of dietary intake on the time of onset of puberty was clearly demonstrated by Asdell (1955), who found that when he fed Friesian heifers on high, medium and low planes of nutrition, puberty occurred at 9, 11 and 15 months of age, respectively. As the feeding level increases, the age at puberty decreases; even the feeding level before weaning in beef heifers has a significant effect (Wiltbank et al., 1966). In Friesian heifers, the age at puberty decreases by 0.77 days for each additional 0.45 kg body weight at 6 months, and by 0.36 days for each additional 0.45 kg body weight between 6 and 12 months of age. Maintaining dairy heifers on only 62% of their energy requirements delays puberty until 20 months of age (Gerloff and Morrow, 1986).

These results demonstrate the importance of adequate feeding during calfhood to ensure the early onset of puberty. In most farming systems, it is preferable for heifers to calve for the first time at close to 2 years of age. For beef heifers, many studies from both the US and UK have found that heifers which first calve at 2 years of age are more profitable than those calving at 3 years (Pinney et al., 1962; Nunez-Dominguez et al., 1991, and see Spitzer, 1986). In the study of Pinney et al. (1962), 2-year-old calvers produced 0.8 more calves per cow at 10% less cost over their entire production life than 3-year-old calvers. For seasonally calving dairy cows, both the age of first calving and the date of first calving are critical. Animals must calve right at the start of the season, which may mean that some calve at 23 or even 22 months of age. Attaining maximal growth rates during the rearing period is critical to achieving this (MacDiarmid 1999), in terms both of attaining adequate body size and of ensuring that the youngest heifers have attained puberty at the start of the breeding season. For non-seasonal dairy herds, it may be preferable to allow animals to calve for the first time at 27 or even 30 months of age, in which case, early attainment of puberty is less important.

Although heifers can conceive at the onset of puberty, it is not advisable for them to do so because, since they are not fully grown, dystocia will be common at their first calving due tos feto-maternal disproportion (see Chapter 11). Generally, animals that have reached 65–70% of their adult body weight a month before the start of the mating season are likely to be pubertal, to be exhibiting regular oestrous cycles and to have attained adequate body weight by the time of their first calving. As a guide, target weights are that Friesian-Holstein heifers should be at least 330 kg and Jersey heifers 280 kg, a month before mating commences (Fielden et al., 1977). In practice, the weights of Friesian heifers at first service range between 325 and 440 kg. Heifers which have reached these targets should be able to reach their genetic potential for milk production in their first lactation, provided, of course, that their subsequent metabolic needs throughout pregnancy are not neglected.

Once cyclical activity has commenced at puberty it should continue uninterrupted, apart from pregnancy and the immediate postpartum period, throughout the life of the animal. There is no evidence that growth restrictions in early life will influence reproductive performance once the feeding of a normal diet has been implemented (Alden, 1970; Choi et al., 1997; Troccon et al., 1997). However, dietary insufficiency or, in some circumstances, dietary excess can have profound effects upon reproductive function. During the immediate post-conception period the heifer is under considerable stress, since she is continuing to grow to physical maturity whilst conceiving and maintaining a pregnancy to term.

Effects of energy and protein intake upon conception rates

Energy

The most severe effect of inadequate nutrition is the cessation of cyclical activity (see below), although other less severe manifestations are silent oestrus, ovulatory defects, fertilisation failure and embryonic or fetal death. Most studies do not differentiate between fertilisation failure and embryonic death within the first 21 days of gestation.

Maiden heifers. Some of the earliest studies on the effect of feeding levels on heifer fertility were reported by Asdell (1955), who demonstrated that Holstein heifers which were bred at 15 months of age and had been fed during the period before service on low, medium and high planes of nutrition required 1.89, 1.64 and 1.33 services per conception, respectively. Likewise, Leaver (1977) fed Friesian heifers from 6 weeks before, to 6 weeks after, artificial insemination on three different levels of nutrition. These gave growth rates of 0.68 (high), 0.50 (medium) and 0.34 (low) kg/day, respectively, producing a wide range of body condition scores at the time of mating. Pregnancy rates were 42, 72, 70 and 63% for poor, moderate, good and very good bodily condition, respectively. Thus, heifers that are in poor or moderate condition achieve improved pregnancy rates when dietary intake is increased but those in good condition show no response. This study also suggests that heifers that are overfed and fat have impaired fertility.

Additional feeding after insemination improved conception rates only in those heifers growing at less than 750 g/day before insemination (Rochet, 1973). Drew (1978) found no effect of live weight or condition score at the time of service on pregnancy rate. However, when the energy intake was increased by supplementary feeding with rolled barley (equivalent to 20 MJ/head/day) for 6 weeks before the service date, so that live weight gain increased from 0.23 kg/day to 0.43 kg/day, the conception rates increased from 50 to 60%. It was therefore recommended that Holstein–Friesian dairy heifers should be growing at a rate of 0.7 kg/day to achieve optimum fertility.

Young (1965, 1967, 1968) demonstrated that range heifers are particularly sensitive to the effects of malnutrition during the later stages of their first pregnancy, if they have not reached physical maturity. This was evidenced by delays in returning to oestrus after calving and poor pregnancy rates at first service after calving.

Conversely, long-term feeding at high levels has been shown to reduce pregnancy rates, increase the proportion of barren animals and also increase the prevalence of dystocia (Wickersham and Schultz, 1963; Reid et al, 1964). The risk of dystocia can be reduced by restricting food intake in late gestation in beef heifers, so reducing the birth weight of the calf (Young, 1970), but this is obviously a risky approach. Moreover, in the previously mentioned study of Ducker et al. (1985), heifers which were on a high (83.6 MJ/day) plane of nutrition for both the last 10 weeks of pregnancy and during weeks 6–18 of lactation (146.8 MJ/day) suffered a greater level of embryonic death than animals which were on low levels of feeding during either pregnancy (64.6 MJ/day) or lactation (119.8 MJ/day).

Adult cattle. First-calving heifers are probably at the greatest risk of any class of cattle of suffering from nutritionally induced impairment of reproductive function, with many becoming anoestrous. However, both first calvers and adult cattle experience a significant reduction in pregnancy rates when they are under nutritional stress. Separating the effects upon pregnancy rates per se and those which involve some effect upon cyclicity and some upon pregnancy rate is not easy and, indeed, may not be appropriate; yet whereas the effects of energy balance upon oestrous cyclicity are reasonably well characterised, those upon pregnancy rate are less clear-cut.

A great many studies have shown that 'fertility' is impaired in animals that are underfed. Ward (1968) and Lamond (1968) demonstrated that there was a body weight below which fertility was lowered; the highest rates of infertility were found in the cows which were in the poorest condition. Warnick et al. (1967) found that beef cows were infertile when losing weight, whilst a 2% increase in live weight in the 3 weeks before service improved conception rates (Moller and Shannon, 1972). Morris (1976) considered that, if the weight loss between calving and 60 days postpartum is ≥5%, there should be concern; if it exceeds 10%, there is likely to be poor fertility. Hence, Morris (1976) advised that positive action needs to be taken before such problems occur; this means regular weighing, accurate body scoring or the use of a girth band measure regularly after calving. Some confirmation can be obtained by noting the response of the cows to the introduction of an energy supplement diet, such as barley, or of feeding above yield.

Looking at effects upon pregnancy rate itself, McClure (1961) showed that cows which lost the least weight after calving and were gaining weight at the time of service had a higher chance of conceiving to first service than those cows which exhibited a lower recovery of body weight during early lactation. In a group of Ayrshire cows, those which gained weight over the service period had a 77.6% conception rate to first service compared with 16% for those which lost weight (King, 1968). The same author suggested that there is a change of 1% in conception rate to first service for every 1% change in live weight. McClure (1970) reported that a 10% fall in live weight postpartum was associated with low fertility.

Klug et al. (1989) gave freshly calved heifers and cows low and high energy diets. For heifers on the low energy diet, the pregnancy rate to first insemination was 59%, whereas the value in animals on the high energy diet was 68%. For cows, the pregnancy rates to first insemination were 46 and 60%, and the number of inseminations per conception 2.0 and 1.6, respectively. Hegazy et al. (1997) reported that Egyptian Holstein cows, which calved in a condition score of 1.5 (0–5 scale), required 4.75 ± 0.24 services per conception, compared with 2.11 ± 0.27 or 1.53 ± 0.36 serves per conception for cows calving at condition scores of 2.5 and 3.5, respectively. This effect upon pregnancy rate was in addition to a significant extension of the interval between calving and first oestrus in the thinner cows. Pehrson et al. (1992) found that provision of a high energy feed additive that contained high levels of gluconeogenic substances to high yielding cows reduced the calving to conception interval without affecting the calving to first service interval.

However, not all studies have agreed that there is a simple relationship between energy intake and pregnancy rate. For example, Garnsworthy and Haresign (1989) concluded that, while cows calving in a condition score of 2 had lower pregnancy rates than those calving at condition score 3, it was more likely that the apparent effect upon pregnancy rate occurred due to other disease problems or poor oestrus detection than to pregancy failure per se. Ducker et al. (1984) found that increasing the energy intake in Friesian cows during a 9-week period around the time of insemination did not improve pregnancy rates when compared with controls. However, these same authors identified an effect of milk yield, since those cows that were giving high yields at 21 days of lactation had poorer fertility when compared with those with more modest values. Conversely, those with high cumulative yields by day 21 of lactation became pregnant more readily.

Ferguson (1991) reviewed literature which showed that feed mismanagement, especially in terms of dry cow rations and of total feed delivery to the high-yielding cow, had markedly detrimental effects upon reproductive performance. Hence, carry-over effects from the dry and transition periods are as likely to affect pregnancy rates as they are to affect anoestrus rates.

There are two main ways in which energy deficiency is believed to affect pregnancy rates, the first via the GnRH system and the second via metabolic regulators of ovarian function. Webb et al. (1997) recently reviewed the literature concerning these possibilities. FSH secretion is largely unaffected by nutrition (Rhodes et al., 1995), but LH secretion is impaired in animals that are in negative energy balance (Canfield and Bulter, 1990; Wright et al, 1992; Rhodes et al., 1999). Effects of nutrition upon the LH response to GnRH are also evident. Webb et al. (1997) concluded that, although the response is unaffected by short-term changes in body weight, it is reduced when the animal is below a critical body condition score.

Circulating concentrations of glucose, insulin and insulin growth factor 1 (IGF-1) are lower in cows in negative energy balance than in fully fed animals, whilst concentrations of non-esterified fatty acids (NEFA) are higher. All of these might be expected to affect follicle development, independently of effects upon gonadotrophins. Some evidence exists to support this concept and Estill (1993) suggested that the effects of negative energy balance upon pregnancy rate are due to impairment of follicle development and/or luteal function. Several studies have shown smaller preovulatory follicles in cows in negative energy balance (Murphy et al., 1991; Rhodes et al., 1995, cited by Webb et al., 1997). On the other hand, it has also been shown (Lucy et al., 1991, 1992; Murphy et al., 1991) that a low plane of nutrition in the post-calving period increases the number of smaller follicles.

Nevertheless, whatever effects underfeeding has upon follicular growth, there is evidence that it affects oocyte function. McEvoy et al. (1997) showed that oocytes which were derived from underfed cows had a reduced capacity to develop to blastocysts in vitro. Moreover, Kruip et al. (1998) associated low pregnancy rates with high levels of tissue mobilisation in cows that had been overfed during the dry period. They considered that alterations in gonadotrophin secretion were not the cause, but that the low pregnancy rates might be caused by direct toxic effects of non-esterified fatty acids upon follicles and oocytes.

Fatty liver syndrome. A number of reports (Morrow, 1976; McCormack, 1978; Reid, 1980) have described the so-called 'fatty liver syndrome' of high-yielding dairy cows. This condition occurs in animals that are overweight at calving, have poor appetites and consequentially mobilise body fat reserves to meet their energy deficit for lactation. Inadequate supplies of endogenous and exogenous protein exacerbate the syndrome (Roberts et al., 1981). The liver becomes infiltrated with fat and the cow often develops ketosis.

Fatty liver predisposes to postpartum metritis and RFM (Sommer 1975; Morrow et al., 1979; Filar et al., 1994). Morrow et al. (1979) also described a high incidence of metabolic and infectious diseases in one herd of 600 cows. There is also evidence of depressed fertility (Reid et al., 1979). In a study of two groups of cows, one with severe fatty liver (>30% fat in liver parenchyma) and one with mild fatty liver (<20% fat in the liver parenchyma), the mean calving intervals and services per conception were 395.5 days and 2.39 services and 359 days and 1.73 services, respectively. Five of 10 cows with severe fatty liver had calving intervals greater than 400 days, averaged over all the previous lactations, whilst more of the cows in the mild fatty groups had an average calving interval greater than 400 days. Calving to first service interval is prolonged in cows with fatty liver (Higgins and Anderson, 1983), mainly due to a delay in the time to first postpartum ovulation which, in cows with moderate and severe fatty liver, has been shown to be delayed (Reid et al., 1983). Other evidence of impaired reproductive function in cows with mild and moderate fatty liver is a shorter interval between the first and second ovulations. For the cows with fatty liver, the average interval was 16 days, compared with 21 days in the other cows (Watson, 1985).

The presence of fatty liver can be shown by biopsy. Various blood parameters can also be used as evidence of impaired liver function. Non-esterified fatty acids, bilirubin, aspartate aminotransferase and β-hydroxybutyrate concentrations are increased, while those of glucose, cholesterol, albumen, magnesium and insulin are lowered (Lotthammer, 1975; Sommer, 1975; Reid, 1984) in cows 8 weeks before calving. Albumen values normally decline in cows after calving, eventually returning to precalving values at 1–9 weeks (Rowlands et al., 1980); depressed values are associated with fatty liver (Reid et al., 1979). Since albumen is synthesised in the liver, impaired liver functions will influence its production, whilst if fat has replaced glycogen in the liver parenchyma total glycogen reserves will be reduced. The evidence of an inverse correlation between serum albumen levels and fertility is conflicting; early work (Rowlands et al., 1977) demonstrated one, but subsequently this has not been substantiated (Rowlands et al., 1980), although it is likely that cows that are able to regulate their serum albumen levels should have better fertility.

There are also endocrine changes in cows with fatty liver. Basal concentrations of LH are lower and there are fewer pulses of LH in affected than in normal cows. Likewise, preovulatory concentrations of LH are lower in cows with fatty liver, as is the LH response to administered GnRH. Luteal progesterone concentrations are lower than in normal cows (see Reid, 1984). These changes may result from hypoglycaemic impairment of GnRH activity, but may result from NEFA-induced damage to endocrine cell membranes. Kruip et al. (1998) also postulated a toxic effect of NEFA upon follicles and oocytes. The low insulin concentrations that are associated with the fatty liver syndrome might also affect oocyte functionality.

Treatment is not possible, and usually there will be eventual recovery. Attempts to prevent the disease can be made by ensuring that cows are not excessively fat at calving and receive adequate energy thereafter to exclude the need for excess fat mobilisation. Morrow et al. (1979) stressed the importance of preventing excess energy intake during the end of the previous lactation and the dry period.

Protein

Deficiency. Evaluation of the protein requirements of cattle in relation to reproductive function are subject to the same limitations previously described for nutrition studies in general. Many early experiments failed to ensure that diets containing different levels of digestible crude protein were isocaloric (Tassell, 1967a). In addition, due to ruminal metabolism, crude protein (CP) dry matter (DM) intake alone does not adequately describe the protein requirements of a dairy cow. The assessment of the supply of rumen degradable and undegradable protein probably is a more meaningful measurement with regard to fertility (Ferguson and Chalupra, 1989); for the high-yielding cow, even estimates of the metabolisable value of undegradable protein are needed to match demands and intake.

Nevertheless, in most of the older literature and in many situations other than that of the very high-yielding dairy cow, CPDM is the primary measure of protein intake. It is generally recommended that for a dairy cow producing more than 30 kg of milk per day, 16% crude protein per dry matter is the optimum. In a study involving high-yielding Friesian cows (Treacher et al., 1976), it was found that if cows were fed 75% of the recommended CP intake, the mean calving to first oestrus interval was extended to 46 days compared with 35 days for the control group on a normal intake; however, the calving interval was shorter in the low protein group. In a study involving high-yielding cows producing more than 30 kg of milk per day at peak lactation, a definite influence of different protein intake was demonstrated (Jordan and Swanson, 1979). Between days 4 and 95 postpartum, groups of cows were fed isocaloric diets containing three different levels of CP: 12.7%, 16.3% and 19.3%. Cows on the highest level had the shortest interval to first oestrus, but in all other aspects (services per conception, calving interval) the best results were obtained with the lowest level of protein intake. Similar results have been reported by Hagermeister (1980) who showed that if two levels of crude protein (16% and 19%) were given, pregnancy rates were 56 and 44% and services per conception 1.79 and 2.25, respectively.

By contrast, in an experiment in which isocaloric diets containing 80 and 100% of the US National Research Council recommended levels of crude protein were fed during the last 60 days of gestation, there was no difference in the incidence of reproductive problems or performance, although pregnancy rates in both groups were poor (Chew et al., 1984). No evidence of impaired reproductive performance was detected in a small number of cows fed for three successive lactations on crude protein levels of 13, 15 and 17% (Edwards et al., 1980).

Using logistic regression analysis, Ferguson and Chalupa (1989) were able to show that the age of the cow, as well as dietary energy intake, modified the impact of protein intake on reproduction. For example, fertility was reduced in mature cows (4+ lactations) fed diets containing 19% compared with 16% CPDM and rumen digestible crude protein levels of 72 versus 62%. The fertility of cows in their second or third lactations was not affected greatly. First-lactation cows had better conception rates (65 versus 36%) when fed diets of 16% CPDM that contained more rumen degradable protein.

Moreover, there are probably interrelationships with protein and the physical form of the diet. Bertoni et al. (1998) reported higher pregnancy rates in high-yielding cows receiving high energy supplementation than in animals receiving commercial or energy + protein supplements. Fekete et al. (1996) studied energy and protein deficiency in Holstein cows, concluding that with marginal energy supply, moderate (13%) protein (RDP) deficiency during the early part of lactation was more detrimental to reproductive performance than a severe (27%) deficiency of undegradable protein.

Excess. The effect of high levels of protein in the diet upon conception rate have been the subject of long-standing controversy. Lean et al. (1998) summarise literature in which many studies show adverse effects of high protein levels upon fertility, while almost as many others show no effect. However, Gerloff and Morrow

(1986) and Lean et al. (1998) concluded from various meta-analyses of field trials reported in the literature, that pregnancy rate is adversely affected at higher CP inclusion rates. Thus, Gerloff and Morrow (1986) concluded that a marginal depression of conception rate occurred at CP levels of 16–18%, but the reduction was significant at ≥19% CP.

Many authors consider that the toxicity of high levels of CP occurs as a consequence of degradation of excess RDP, leading to increased circulating concentrations of ammonia and urea. In consequence, abnormally high concentrations of urea and ammonia are present in the uterus (Jordan et al., 1983), where they may be toxic to spermatozoa (Jordan and Swanson, 1979; Hossain, 1993), oocytes or embryos (Ferguson, 1990; Elrod and Butler, 1993; Robinson and McEvoy, 1996), or adversely affect aspects of uterine function (Elrod, 1992; Butler, 1998).

In addition, abnormally high circulating concentrations of urea may also have an effect upon the hypothalamic–pituitary axis. Jordan and Swanson (1979) reported that cows fed diets of 19% CPDM had increased basal blood LH concentrations, and an exaggerated LH response to GnRH stimulation. Some effect on basal LH concentrations was also found in non-lactating ovariectomised cows (Blauwiekel et al., 1986). Excesses of dietary protein also affect blood progesterone concentrations and luteal progesterone synthesis is reduced in cows on high CP diets (Garverick et al., 1972; Larson et al., 1997), while there are also adverse effects of urea upon hepatic clearance rates of reproductive steroids (see Lean et al., 1998).

Others have reported that 20% dietary CPDM increased the incidence of RFM, dystocia and postpartum metritis compared with a 13% level. It had been suggested that there was also impaired intrauterine leukocyte function in cows receiving the higher level of protein (Anderson and Barton, 1987).

Nevertheless, the view of a negative interaction between high protein levels and fertility is by no means universally accepted. Studies of milk urea concentrations have shown only weak associations with pregnancy rates (Pacheo-Navarro, 2000; Smith et al., 2000a, b; Verkerk, 2000). Moreover, McClure (1994) has argued that the effects of high CP were mediated primarily through effects on carbohydrate fluxes. In pasture, he suggested, carbohydrate levels were depressed in circumstances in which high levels of nitrogen-containing substances were present in the plants. Moreover, he argued that high levels of nitrogen within the rumen are associated with the production of acetate rather than gluconeogenic volatile fatty acids (VFAs), resulting in relative hypoglycaemia. McClure (1994) also noted that the effects of excessive levels of rumen-degradable protein are exacerbated by feeding inadequate dietary energy, whereby the rumen flora are unable to utilise the available protein. Interestingly, a number of studies have shown that small improvements in the undegraded protein (UDP) content of the diet improve fertility or, at least, mitigate the effects of high levels of RDP (Armstrong et al., 1990; Staples et al., 1998a). This may, as suggested by McClure (1994) and Webb et al. (1997), improve the availability of gluconeogenic substrates. However, the most common source of high-grade UDP for such trials is fish meal, a feedstuff that also contains unique fatty acids that not only can affect carbohydrate metabolism, but also can affect prostaglandin and steroid metabolism (see Staples, 1998b; Meier 2000a, b; Verkerk, 2000).

Investigation of nutritional factors as a cause of infertility

It is frequently impossible to determine accurately a specific nutritional cause of infertility, because the clinical signs appear some time after the deficiency has occurred. Methods such as the use of cumulative frequency graphs of pregnancy rate (see Chapter 24), or monitoring daily bulk milk protein concentrations, can help to pinpoint the times at which the management changes took place that have adversely affected nutrition. Effects of improving nutrition are more difficult to determine, since changes in the season of the year and management can also have an effect that can obfuscate the effects of changes in diet.

In most cases, the most important factor responsible for poor fertility is underfeeding. This is due to:

- Overestimation of the feeding value of forages. For conserved forage, it is important to obtain accurate analysis of the major food components from truly representative samples. For pasture, it is important to estimate accurately both the amount and the digestibility of the material that is present.
- Overestimation of feed intake under self-feed conditions. Self-fed silage is especially susceptible to overestimation. However, it should be noted that, in most situations where self-feeding is practised, especially where there is inadequate feeding space for all cows to feed simultaneously, vulnerable cows (i.e. first calvers, smaller cows, etc.) will be at a significant disadvantage, in terms of feed intake, than their herd-mates.
- Failure to appreciate the reduction of forage intake caused by high concentrate intake (Alderman, 1970).
- Underfeeding of concentrates, due to automatic dispensers giving short measure. Parker and Blowey (1976) found errors greater than 50% in some cases. Manual dispensers in the milking shed are more commonly inaccurate than accurate.

It is necessary to calculate the requirements of the cows for maintenance and production and then obtain accurate information about the precise quantities fed. Contributions from mineral licks and other free access sources are difficult to quantify. Weighing, the use of a girth band measure or condition scoring of a representative number of animals are also useful.

Metabolic profiles

Since the introduction of metabolic profile tests in 1970 (Payne et al., 1970), they have frequently been used to help in the evaluation of the nutritional status of a herd, particularly in relation to fertility. Although some are enthusiastic about them (Morris, 1976), others (Parker and Blowey, 1976) point to the importance of using them in conjunction with other more direct methods, particularly since there are dangers associated with the use of single blood concentrations to assess the metabolic status of an animal.

Details of the tests and their evaluation are available elsewhere (Payne et al., 1970; Parker and Blowey, 1976; Morris, 1976). In general terms, metabolic profiles attempt to assess the energy balance of lactating cows by estimating blood metabolite concentrations. The most commonly measured metabolites are glucose, urea and albumen/globulin. Non-esterified fatty acids, β-hydroxybutyrate and bile acids are also measured in some protocols.

A relationship between reduced blood glucose levels, excessive weight loss at the time of mating and depressed pregnancy rates was demonstrated by McClure (1968). He found that blood glucose values less than 30 mg/dl were associated with reduced fertility. Morris (1976) also recommended this as a method of identifying an energy deficit, using either the above value or one less than twice the standard deviation below the mean for dry cows in the herd. However, the measurement of non-esterified fatty acids is a more valuable method of assessing energy status, since it directly reflects tissue mobilisation. Measurement of β-hydroxybutyrate is easier than NEFA, since samples for NEFA analysis require some care in collection and handling. Although concentrations of β-hydroxybutyrate are high in many cows in early lactation (Ward et al., 1996), the authors find this to be a less valuable measure of cows' energy status than NEFA. As mentioned previously, urea concentrations reflect protein deamination and/or the ratio of RDP to FME. Bile acids may provide a useful indication of fatty livers (West, 1991).

Perhaps a further method of relating energy intake and fertility may arise from changes in milk protein concentrations; these are affected primarily by the energy intake, rather than the protein intake, of the cows. Hagermeister (1978) demonstrated a significant inverse relationship between the calving–conception interval and milk protein concentration; thus mean values of 2.6% were related to a 105-day calving–conception interval compared with 3.4% for an interval of 94 days. The authors find changes in milk protein concentration one of the most valuable tools for the retrospective identification of changes in nutrition that have adversely affected cows' energy balance.

Effects of micronutrients upon fertility

While it is generally agreed that micronutrients (minerals and vitamins) have an effect upon fertility of cattle, there are conflicting opinions about the significance of apparent deficiencies. This is because of the inherent difficulties of accurately determining nutrient requirements; our lack of knowledge of the interaction of micronutrients in the alimentary tract; and because many of the studies that determined nutrient requirements were done 40 or more years ago when yields were much lower. McClure (1994) also points out that many older studies attributed effects of micronutrients upon fertility to studies in which reproductive performance was compared before and after supplementations, a technique which is now considered to be invalid.

McClure (1994) suggests that most micronutrient deficiencies exert their effects upon reproduction through depression of the activity of rumen microflora; reduction in enzyme activity affecting energy and protein metabolism and the synthesis of hormones; and the integrity of rapidly dividing cells within the reproductive system. To this list, Lean et al. (1998) add the role of micronutrients as antioxidants, which are responsible for protecting cells from the effects of free radicals.

Cobalt

Cobalt deficiency occurs in pastures of Australia, New Zealand, Florida, Kenya and Scotland (McClure, 1994). Deficiency usually causes anaemia, inappetance, poor bodily condition, ill thrift and loss of condition. Poor fertility may be present at the same time as these obvious signs of deficiency. As with many supposed trace element deficiencies, it exerts its effect upon fertility in a number of different ways, viz. increased number of 'silent' oestruses, poor pregnancy rates and irregular interoestrus intervals. Sometimes poor fertility in apparently normal cows can be corrected following cobalt supplementation. Deficiency occurs when diets contain < 0.07 mg/kg D.M. cobalt and is due to failure of vitamin B_{12} synthesis, which is an essential cofactor for carbohydrate metabolism. The only accurate diagnostic procedure is the estimation of liver vitamin B_{12} (Morris, 1976).

Copper

Copper deficiency has been said to cause delayed puberty, anoestrus, suboestrus or poor pregnancy rates. When this occurs in association with other signs of hypocuprosis, such as anaemia, poor growth, bleached coat colour and diarrhoea, a diagnosis is likely. However, opinions differ as to the relationship between copper status and reproduction. A number of studies have demonstrated poor fertility associated with low blood copper concentrations followed by improvements after copper supplementation (Bennets et al., 1948; Munro, 1957; Pickering, 1975). On the other hand, there are an equal number of studies suggesting that fertility is not related to blood copper concentrations (Littlejohn and Lewis, 1960; Larson et al., 1980), or that copper supplementation has no positive effect (Whitaker, 1980). Furthermore, blood copper concentrations are not a particularly good indicator of an animal's copper status; liver samples (collected by biopsy) are generally regarded as being more accurate. There is also debate concerning the point at which blood copper concentrations become indicative of clinical deficiency. Suttle (1993) emphasised the need to use a threshold value of 9.4 μmol/l (0.6 mg/l) and stated that values below 4 μmol/l are probably required before health or fertility is compromised.

Hypocuprosis can be either direct or indirect. Indirect deficiency occurs due to excessive molybdenum, iron or sulphur intake and, possibly, calcium or zinc. McClure (1994) summarised the relationship between the two. Copper deficiency occurs when cattle are fed diets containing < 3 mg/kg copper, if the molybdenum content is < 3 mg/kg; 3 to 10 mg/kg copper, if the molybdenum content is 3 to 10 mg/kg; or > 10 mg/kg if the molybdenum content is > 10 mg/kg. Hypocuprosis induced by high molybdenum intake has been recognised for many years in the so-called 'teart' pastures in south-west England. Liming of pastures to maintain the correct pH for the growth of grass and other forage crops can affect the uptake of molybdenum by plants, so that even at normal rates of application it is possible to change the pH sufficiently to increase the uptake of molybdenum (Phillipo, 1983).

Whilst the effect of high molybdenum has always been assumed to be due to hypocuprosis, a study by Phillipo et al. (1982) has provided evidence that molybdenum may have a direct effect upon reproduction. In this study, prepubertal heifer calves subjected to diets with molybdenum and iron supplementation (both of which produced comparable levels of hypocuprosis) were compared with a normal control group without supplementation, and a reduced food intake group. Neither growth rate nor time interval to first oestrus nor pregnancy rates at the fourth oestrus were affected in the iron-induced hypocuprosis group. However, in the molybdenum-supplemented group (+5 mg Mo/kg dry matter) the interval to first oestrus was extended, and the pregnancy rates by the fourth oestrus were reduced. Furthermore, there was evidence of a direct effect of molybdenum on the hypothalamus–pituitary, since plasma LH pulse frequencies were reduced. Molybdates have also been shown to interact with steroid hormone receptors (Dahmer et al., 1984).

Perhaps in the light of recent studies we need to reconsider the role of copper deficiency per se in causing infertility. In a study involving 17 beef suckler herds, in which average herd plasma copper concentrations ranged from 0.16 to 0.92 mg/l within 1 month of mating, average pregnancy rates for the herds ranged from 37 to 65%, and showed no correlation with copper concentrations. In fact, the herd with the lowest plasma copper value had a pregnancy rate of 63% (Phillipo et al., 1982). Furthermore, in four farms with low copper status, supplementation with 100 mg of copper before mating did not improve the pregnancy rates compared with untreated controls. Thus, copper per se does not appear to be a major factor in influencing the fertility of beef suckler herds (Phillipo et al., 1982).

McClure (1994) considered that the main effect of copper deficiency is upon the efficiency of food utilisation, because of effects upon the integrity of the small intestine. However, it also has a role as an antioxidant, which may be its primary importance in maintaining reproductive performance (Lean et al., 1998).

Iodine and goitrogens

Reproductive failure resulting from iodine deficiency is invariably related to impaired thyroid function in the dam, embryo or fetus, which in the last two can cause embryonic death, abortion, stillbirth or weak goitrous calves. A high level of stillbirths, sometimes associated with a delayed second stage of parturition, has been observed in herds fed high-quality succulent grass, heavily treated with nitrogen but was low in iodine. There is good evidence that treatment with iodised oil injection can improve the deficient status (Logan et al., 1991; Mee, 1991). Simple iodine deficiency can occur because of an intake below 0.8 mg/kg D.M. (Alderman, 1970), although McClure (1994) considered a level of 2.0 mg/kg D.M. to be the threshold for deficiency. Lean et al. (1998) suggested that percutaneous absorption of iodine-based teat dips may be enough to prevent deficiency in milking cows.

Disturbance of thyroid function can also be due to goitrogenic substance present in kale, lentils, soya bean, linseed and certain strains of white clover (Boyd and Reid, 1961; Tassell, 1967b). High levels of goitrogenic substance can produce anoestrus in heifers (David, 1965). Since iodine is needed for thyroxine synthesis, iodine deficiency is largely manifested through the effects of a lack of thyroxine. Thyroxine is a general metabolic regulator and, in particular, a regulator of mitochondrial activity (McDonald and Pineda, 1989). Thyroxine deficiency is associated with non-specific signs of poor growth and poor 'doing', together with loss of libido and inhibition of oestrous behaviour (although not necessarily of ovarian cycles) (Spielman et al., 1945; Williams and Stott, 1966; McDonald, 1980).

Manganese

Manganese has a ubiquitous role in reproductive function, being involved in steroid synthesis. Both the pituitary gland and ovaries are relatively rich in this trace element. A variety of reproductive disorders which depress fertility in cows have been blamed on manganese deficiency; these include anoestrus, poor follicular development, delayed ovulation, silent oestrus and reduced conception rates (Lean, 1987; Hurley and Doane, 1989). It also causes joint and limb deformities in calves. Under normal circumstances it is likely that normal pasture will provide the necessary requirement of 80 mg/kg D.M. in the food (Alderman and Stranks, 1967), although some foods (e.g. maize silage) are low in manganese. In addition, there is an interaction with the calcium:phosphorus ratio in the diet, with some evidence that high liming of pasture can cause manganese deficiency.

Manganese is a cofactor in a number of enzymes that are responsible for gluconeogenesis (see McClure, 1994) and has a significant role as an antioxidant (Lean et al., 1998). Manganese is also involved in cholesterol synthesis and, hence, affects steroidogenesis.

Phosphorus

It has been estimated that the normal requirements for phosphorus in the cow for the maintenance of pregnancy are about 13 g/day, with about 7 g extra for each 4.5 litres (1 gallon) of milk (Deas et al., 1979). Providing that forage contains adequate levels of phosphorus, normal diets should contain adequate phosphorus to ensure normal fertility. However, deficiencies can occur where forages have inadequate levels (McClure, 1975) and, perhaps, because of the interaction between calcium and phosphorus. However, phosphorus-deficient pastures are often deficient in many other micronutrients (McClure, 1994), making assessment of the role of phosphorus difficult.

The evidence for the importance of hypophosphataemia as a cause of infertility is conflicting. The provision of supplementary phosphorus has been shown to improve the breeding performance of grazing cattle (Sheehy, 1946; Hart and Mitchell, 1965; Tassell, 1967b). A number of authors have described infertility, which was characterised by anoestrus, suboestrus, irregular cycles and low conception rates (Hignett and Hignett, 1951; Morrow, 1969; Morris, 1976), in the absence of other clinical signs of phosphorus deficiency. However, not all studies have come to this conclusion. For example, in a controlled experiment with Ayrshire and Friesian heifers, Littlejohn and Lewis (1960) found no evidence of reduced fertility associated with an imbalance of calcium and phosphorus. Cohen (1975) and Carstairs et al. (1980) also failed to find a relationship between phosphorus intake and reproductive failure. McClure (1994) suggested that any effect of phosphorus deficiency may be mediated through the depression of energy intake that it causes. Lean et al. (1998) also speculated that it may affect reproduction through impairment of phosphate-dependent biochemical reactions.

Morris (1976) suggested that a blood phosphorus level of less than 4 mg/dl in affected or susceptible animals, i.e. those at peak production, confirms the diagnosis. He found that deficiency normally occurs when the phosphorus content of the feed is less than 0.20% or even 0.26%. High-yielding cows need phosphorus in excess of that available in pasture but since cereal grains contain large amounts, deficiencies are unlikely to occur. If hypophosphataemia is suspected, a rapid response can follow the feeding of dicalcium phosphate (150–200 g/day) or bone meal. It is important to ensure that the ratio of calcium to phosphorus is 1:1.

Selenium and vitamin E

It is difficult to separate the effects of selenium and/or vitamin E deficiency since both have a ubiquitous antioxidant function which protects a wide range of biological systems from oxidative degradation. In addition, they can exert a sparing effect upon each other. Probably because it is now possible to measure the selenium status of cows, by estimating the enzyme glutathione peroxidase in heparinised blood, the influence of selenium on reproductive function has been investigated. Deficiency occurs when soils contain < 0.5 mg/kg, or diets < 0.05 mg/kg selenium. Vitamin E deficiency occurs when animals graze post-mature pasture, receive other diet components that contain < 0.7 mg/kg of the vitamin, or are fed diets that are high in polyunsaturated or rancid fats (McClure, 1994). The active derivative of vitamin E is α-tocopherol.

Diagnosis of selenium deficiency can be made by measuring circulating concentrations of selenium or, better, by measuring selenium stores in the liver. Measurement of levels in feed, pasture or soil is often also indicated. Supplementation is widely practised, especially in areas where soils are known to be marginal or deficient. However, it should be remembered that excessive selenium is toxic, especially where it has been given by injection.

In early studies (Trinder et al., 1969), it was shown that selenium and vitamin E injections reduced the incidence of RFM and, as a consequence, would improve the fertility of herds. However, since this initial study, the results published have been decidedly equivocal. Some studies have confirmed the beneficial effect of supplementation (Julien et al., 1976a, b; Segerson et al., 1980; Harrison et al., 1984), whilst others have failed to identify a positive response (Gwazdauskas et al., 1979; Schingoeth et al., 1981; Hidiroglou et al., 1987). Supplementation with selenium and vitamin E has also been shown to reduce the incidence of metritis and cystic ovaries when administered prepartum (Harrison et al., 1984). However, in this latter study it is worth stressing that even after supplementation with vitamin E and selenium the incidence of postpartum metritis was 57% and that of cystic ovarian disease 19%, both values being very high.

To demonstrate the contradictions in many of the studies, comparisons were made of blood selenium concentrations and cystic ovarian disease (Mohammed et al., 1991). In cows with cystic ovaries, the mean blood selenium concentration was 141 ng/ml compared with 136 ng/ml in normal cows. When a logistic regression analysis was performed, cows with selenium concentrations in blood that were greater than 169 ng/ml had twice the risk of developing cystic ovaries than cows with selenium values less than 108 ng/ml.

Studies involving selenium-deficient Friesian–Holstein heifers have shown improved pregnancy rates after treatment (MacPherson et al., 1987). Taylor et al. (1979) found a lower abortion rate after selenium supplementation, while McClure (1986) reported a higher first-service pregnancy rate after selenium supplementation. Vitamin E deficiency is directly associated with embryonic loss in cows and, through its role in the immune system, may also affect the rate of uterine involution after calving (Lean et al., 1998).

Vitamin A and β-carotene

It is difficult to separate the effects of vitamin A and β-carotene since β-carotene is the plant precursor of vitamin A.

Vitamin A deficiency has been known to delay the onset of puberty in heifer calves and to cause cows to give birth to weak and abnormal calves (Byers et al., 1956). Madsen and Davis (1949) fed cows at different levels of carotene ranging from 30 to 240 mg/kg body weight per day over a number of years. They found that at the lowest level of 30 mg/kg no pregnancies occurred; at the 45 mg/kg level pregnancies occurred but the calves were born with clinical signs of vitamin A deficiency. A response of improved fertility was apparent when cows were fed at a level of 90 mg/kg. Evidence of an effect of vitamin A deficiency on reproduction is given by the study of Kuhlman and Gallup (1942), who reported 1.99 services per pregnancy in 21 cows receiving 86 μg of β-carotene per kg body weight during the 90 days before service. The fertility was improved when β-carotene intakes were increased.

There has also been much interest in the direct influence of β-carotene (not as a precursor of vitamin A) upon reproduction in cattle. This has arisen because of the feeding of maize silage, which is known to have a low β-carotene content of 2–4 mg/kg dry matter (Lotthammer, 1979), in association with poor-quality hay and straw. Diets that are deficient in β-carotene, but are adequate in vitamin A, have been shown to increase the prevalence of extended follicular phases, and cause delayed ovulation, silent oestrus and anovulation with follicular cysts (Lotthammer et al., 1978). Cooke (1978) compared two groups of cows, one fed on maize silage which contained 2.22 μg/ml of β-carotene and the other on grass silage which contained 7.3 μg/ml. The fertility for the two groups showed that the first-service pregnancy rates were 45 and 62%, and the number of services per pregnancy were 2.12 and 1.64, respectively. Reduced pregnancy rates were identified by Lotthammer et al. (1978). Bovine luteal tissue has one of the highest β-carotene contents of any tissue (Friesecke, 1978) and it has been suggested that β-carotene may be involved in ovarian steroid production or corpus luteum formation (Jackson, 1981).

As with many studies involving the influence of specific nutrients on reproduction, conflicting results have been obtained. In Israel, Folman et al. (1979) reported that rations deficient in β-carotene

Table 22.7 Dietary sources, active forms, sites of action and mechanism of action of the major antioxidants (from Lean et al., 1998; reproduced with permission)

Dietary input	*Biologically active antioxidant*	*Site of action*	*Mechanism of action*
Selenium	Glutathione peroxidase	IC, membrane	Reduces peroxides
Copper	Cu/Zn superoxide dismutase	IC	Scavenges O_2^-
	Caerumoplasmin	EC	Binds Cu, oxidises Fe
	Superoxide dismutase	EC	Scavenges O_2^-
Zinc	Cu/Zn superoxide dismutase	IC	Scavenges O_2^-
	Superoxide dismutase	EC	Scavenges O_2^-
	Metallothione	EC	Binds metal ions
Manganese	Mn superoxide dismutase	IC	Scavenges O_2^-
Iron	Catalase	IC	Reduces peroxides
	Transferrin	EC	Binds iron
Cobalt	Vitamin B_{12}		
Vitamin E	α-tocopherol	Membrane	Blocks peroxidation
Vitamin A	Retinol	EC	Maintains cell integrity
β-carotene	β-carotene	membrane	Singlet oxygen
	Retinol	EC	Maintains cell integrity
Glucose	Ascorbate	EC	Radical scavenger
Sulphur-containing amino acids	Glutathione	IC	Replenishes glutathione peroxidase
Protein	Various	EC	Binds metal ions Scavenges OH

IC, intracellular; EC, extracellular

had no adverse influence on reproductive performance in dairy heifers. In a similar study, involving 160 Friesian heifers (Ducker et al., 1984) fed on a diet based primarily on maize silage, although plasma β-carotene concentrations were low in the control group and high in the β-carotene-supplemented group, reproductive performance and growth rates were similar. β-Carotene supplementation of maize silage-fed cows did not alter the concentrations or variations in plasma LH or progesterone (Bindas et al., 1983). The reproductive performance for the supplemented and control groups were similar, i.e. the average intervals from calving to first oestrus were 74 and 64 days, the average calving to conception intervals were 95 and 102 days and the average numbers of services per pregnancy were 1.7 and 1.9, respectively. β-Carotene deficiency was reported to have no effect on the incidence of ovarian cysts or their responsiveness to treatment (Marcek et al., 1985).

The reasons for the different responses are difficult to explain. Perhaps in those studies where reduced reproductive performances occurred there was a concurrent vitamin A deficiency. Alternatively, perhaps β-carotene deficiency occurs at levels well below those normally found in practice, or perhaps the association between β-carotene deficiency and fertility is a reflection of some other unspecified deficiency (Ducker et al., 1984).

Zinc

Zinc deficiency has been shown to have an adverse effect upon reproductive function in the male of many species. Its influence on reproductive function in the cow and heifer is not clear. Uptake of zinc is impaired by copper, calcium, iron, molybdenum and cadmium. Excessive levels of zinc supplementation can lead to perturbation of essential fatty acid metabolism, which affect prostaglandin synthesis. Its potential role as an antioxidant is considered below.

Antioxidant function

A number of the aforegoing micronutrients are now believed to exert actions upon reproductive performance primarily through their role as antioxidants. Many metabolic actions produce superoxides, which, through the Fenton reaction, produce highly destructive free radicals (Fettman, 1991). Normally, the tissue-damaging effects of these free radicals are prevented by the presence of antioxidants. Many of these are essential micronutrients, so when these micronutrients become deficient, free radical tissue damage occurs (see Lean et al., 1998). Free radical damage includes the creation of toxic lipids, reactive proteins, free radical cascades and nucleic acid damage. Transition metals, by virtue of their ability to change oxidation states, are the key component of many antioxidant systems.

McClure (1994) lists selenium, α-tocopherol, β-carotene and copper as the key antioxidants. To this list, Lean et al. (1998) add manganese, zinc, iron, cobalt and vitamin A. Glucose, sulphur-containing amino acids and various proteins probably also exert some antioxidant role. Dietary sources, active forms, sites of action and mechanism of action of the major antioxidants are listed in Table 22.7.

Phyto-oestrogens

When cows ingest large quantities of these substances they become anoestrous, with large ovarian cysts, vulval and cervical enlargement and poor conception rates (Morris, 1976). Such substances are found in subterranean clover, certain strains of red and white clover and lucerne.

OTHER FACTORS AFFECTING REPRODUCTIVE PERFORMANCE

Heat stress

The effects of high temperature upon oestrous cyclicity are discussed above. However, the main effect of thermal stress upon the reproductive performance of cows is upon pregnancy and calving rates, rather than upon cyclicity.

Many studies have shown that conception rates are reduced when ambient temperatures are high (Stott and Williams, 1962; Dunlap and Vincent, 1971; Barker et al., 1994), an effect that can be overcome by cooling cows with, for example, shade (Vermeulen, 1988), water sprays (Omar et al., 1996) or sprays used in combination with forced ventilation (Flamenbaum et al., 1988; Lu et al., 1992).

The effects of heat stress are primarily upon the early embryo. Most studies have shown that fertilisation rates are normal (see Thatcher and Collier, 1986; Wise et al., 1988), but that embryonic death occurs between fertilisation and day 16 (i.e. before luteal maintenance would be stimulated by the maternal recognition of pregnancy). Indeed, embryonic death rates are high during the early cleavage divisions (Roman-Ponce et al., 1981), and the proportion of embryos exhibiting retarded growth on day 8 is greater in stressed than in normal cows (Putney et al., 1986). Moreover, when normal day 8 embryos have been transferred into heat-stressed recipients, pregnancy rates have been equivalent to those of unstressed controls (Putney et al., 1989). In other words, embryonic death occurs primarily between fertilisation and blastulation. However, the effects of heat stress are not confined to the pre-blastulation period, for Biggers et al. (1986) noted suppression of embryonic growth rates and a trend towards reduced numbers of embryos amongst cows that were heat-stressed between days 8 and 16 of pregnancy. These effects are probably mediated through an increase in core body temperature. Thatcher and Collier (1986) also showed that the weight of each component of the placenta was reduced in cows that calved in hot months, compared with those calving in cooler months. Thus, heat stress can not only affect pregnancy rates if it occurs at the time of fertilisation, but can impair pregnancy throughout its course.

Heat stress also affects reproductive endocrine parameters. Thatcher & Collier (1986) showed that heat-stressed cows had increased concentrations of progesterone during the luteal phase, but lower concentrations of oestradiol during the preovulatory period, confirming an earlier report of higher progesterone concentrations in heat-stressed cows (Vaught et al., 1977). Lu et al. (1992) reported that preovulatory oestradiol concentrations were higher in heat-stressed cows that were cooled than in those that were not. It has been suggested that the additional progesterone in heat-stressed cows is derived from the adrenal, whereas the lower oestradiol concentrations are a result of impaired LH secretion (Madan and Johnson, 1973; Lee, 1993).

Marai et al. (1998) found that the effect of heat stress was less in cows that had undergone $PGF_{2\alpha}$-based induction of oestrus than in those that had received progesterone, oestradiol or GnRH. However, when heat-stressed cows were synchronised with $PGF_{2\alpha}$, pregnancy rates were higher in animals that had had the timing of ovulation controlled by GnRH administration than in untreated cows (Sota et al., 1998). Ullah et al. (1996) reported that heat-stressed cows, which had received GnRH at the time of insemination, had higher concentrations of progesterone during the luteal phase than did untreated controls, a difference which they associated with the difference in pregnancy rates between the two groups. However, Schmitt et al. (1996) found that the induction of accessory corpora lutea by GnRH or hCG in heat-stressed cows was not associated with any improvement in pregnancy rates.

Hence, heat stress causes a significant impairment of many parameters of reproductive performance, notably causing anoestrus, lowered pregnancy rates and, possibly, reduced calf birth weight and slower postpartum uterine involution. Simple management procedures mitigate or alleviate the problem. Simply providing shade and adequate access to water helps considerably, while cooling with water and/or forced ventilation is highly effective.

The high-yielding cow: a genetic effect?

The last few years have seen a gradual recognition in both the popular and scientific literature that the fertility of dairy cows has been declining. For example, McGowan et al. (1996), O'Farrell et al. (1997) and Dillon and Buckley (1998) all observed declining fertility in highly productive cows in the UK, Eire and New Zealand, respectively. This decline was initially attributed to the many changes in the husbandry and management of dairy cows that have occurred over the past couple of decades, which might be expected to result in reduced fertility.

However, a more recent debate has examined whether the intensive selection for high milk yield that has taken place (particularly in the Holstein–Friesian breed) has been associated with a parallel, unintentional selection for worsening fertility. The traditional view has been that the heritability of fertility traits is very low, and the magnitude of any genetic correlations between production and fertility traits so marginal, that any genetic effect of selection for high yield upon fertility traits would be negligible. Thus, as an example, Raheja et al. (1989) reported that in Canadian Holsteins, heritability of fertility was low, but that correlations between fertility and production traits were positive and moderate in magnitude. Likewise, Weller (1989) concluded that Israeli Holsteins displayed no adverse relationship between fertility and milk yield. Mantysaari and van Vleck (1989) could find no detrimental effect of selection for productivity amongst Finnish Ayrshires. Similarly, Arendonk et al. (1989) concluded that genetic correlations between fertility and production in Dutch Friesians ranged between –0.08 and 0.33, values at which the effect of declining fertility upon productivity was considered to be marginal.

In 1993, Wheadon, responding to farmers' concerns over potential decreases in conception rates of cattle bred to high breeding value (BV) sires, could find no evidence in the New Zealand national herd of such a trend. However, in 1994, Boichard and Manfredi examined fertility and production data from French Holsteins, finding genetic correlations of –0.6 between first-service pregnancy rate and milk yield and –0.42 and –0.36 between first-service pregnancy rate and milk fat and milk protein yields, respectively. Likewise, Hoekstra et al. (1994) recorded small phenotypic correlations between production and fertility traits (–0.05 to –0.18), but much larger genetic correlations (–0.14 to –0.62). McGowan et al. (1996) found cows' milk yield to be positively related to the number of services per conception (i.e. negatively related to pregnancy rate) and heifers' yield to be related to the calving to first oestrus interval. In 1998, Dematawewa and Berger recorded genetic relationships between fertility and production traits in US Holsteins that were high and negative. Even in the Swiss Simmental, often regarded as a dual purpose rather than a dairy breed, a negative genetic association was found between yield and fertility (Hodel et al., 1995).

O'Farrell (1998) considered the evidence for a genetic link between high yield and declining fertility to be circumstantial. However, he advocated the incorporation of a fertility index into bull selection protocols as a means of counteracting any such trends. For the Irish dairy industry, which has a high dependence upon seasonal patterns of pasture growth, such a step is clearly prudent. For the New Zealand dairy industry, which typifies an industry that is absolutely seasonal, fertility indexing has recently been introduced into sire proving schemes (Burton and Harris, 1999). Previously, Mantysaari (1989) had advocated the use of multitrait selection of bulls (for production and fertility traits), while Averdunk (1994) suggested the use of a fertility index in the selection of bull dams. Very recent studies (Taylor, 2000) have shown evidence of aberrant reproductive function in cows of high, compared with a cohort of average, genetic merit; in the former there was a longer delay before the return of cyclical ovarian activity postpartum, and more with evidence of prolonged luteal phases associated with persistence of the corpus luteum.

Hence, there is an increasingly strong body of evidence for a negative genetic relationship between fertility and production traits. Whilst many still regard this association as circumstantial, breeding organisations servicing dairying systems in which fertility is an important component of productivity are taking steps to ensure that any decline in fertility occurs through management practice, rather than through changes in genetic composition of the national dairy herd. For systems in which fertility does not have a strong economic value, the question remains largely academic. Importantly, given that the debate between genetic versus environmental effects upon fertility remains unresolved, a number of very large-scale experiments are (at the time of writing) being set up in those nations whose dairy industry is obligatively seasonal to provide unequivocal data on the subject.

The 'Repeat Breeder' syndrome

By mathematical chance, if cows have a 60% pregnancy rate, about 6.4% of the animals in a herd will not have conceived after the third mating (2.6% after the fourth mating), whilst with a 50% conception rate, about 12.5% or 6.2% of animals will not have conceived after the third or fourth matings (Table 22.8). It was originally considered that chance was the only factor in determining whether these animals failed to conceive. However, when the cows that had repeatedly failed to conceive were examined in more detail, it was found that they were not the random group of animals that mathematical probability would suggest, but contained a subset of cattle that were actually subfertile. The term 'Repeat Breeder' was coined to describe cows that failed to conceive after three or four services.

Early work on Repeat Breeders was responsible for the identification and subsequent elimination of some of the major venereal pathogens of cattle; yet even when this had been achieved, there remained an irreducible proportion of cows that experienced repeated pregnancy failure. Some Repeat Breeders are simply cows that have gross pathology of the reproductive system, have some functional form of infertility or experience some managemental predisposition to infertility. A

Table 22.8 Numbers of cows conceiving to each service: incidence of Repeat Breeders

A. 60% conception rate per service

Service	*Bred*	*Conceived*	*Failed to conceive*	*Total pregnant*
1	100	60	40	60
2	40	24	16	84
3	16	9.6	6.4	93.6
4	6.4	3.8	2.6	97.4

B. 50% conception rate per service

Service	*Bred*	*Conceived*	*Failed to conceive*	*Total pregnant*
1	100	50	50	50
2	50	25	25	75
3	25	12.5	12.5	87.5
4	12.5	6.3	6.2	93.8

The shaded boxes show the proportions of cows that are classified as Repeat Breeders by being non-pregnant after three or four services number of reviews have enumerated the causes of reproductive failure in such animals (Roberts, 1986; Lafi and Kaneene, 1988; Eddy, 1994; Levine, 1999). Many of these causes have been considered previously and can be diagnosed by careful clinical examination and history-taking. The Repeat Breeder cow that presents the greatest clinical and managemental challenge, however, is the animal that continually returns to service in the absence of any obvious pathological disease.

Ayalon and co-workers (1968) undertook a number of pivotal studies of the Repeat Breeder cow. They found a slightly reduced fertilisation rate in Repeat Breeder compared with normal cows, but after fertilisation the two groups of cows exhibited very different patterns of embryonic survival. Repeat Breeders suffered a significant level of embryonic death (Ayalon et al., 1968; Ayalon, 1972, 1978; Maurer and Echternkamp, 1985) at around the sixth day of pregnancy and further losses at around day 17–19 (Table 22.9). These timings are associated, with firstly, hatching from the zona pellucida and, secondly, with failure of the maternal recognition of pregnancy on day 16. Moreover, embryos derived from normal cows failed to survive in the uteri of Repeat Breeders, whilst embryos derived from Repeat Breeders had normal survival rates in normal cows (Almedia et al., 1984; Ayalon; 1984). Hence, the problem of the Repeat Breeder is primarily in the uterine environment, rather than representing a deficiency of the embryo itself although, by the seventh day of gestation, the in vitro developmental capacity of embryos derived from Repeat Breeders is compromised (Tanabe et al., 1985), as

Table 22.9 Embryonic death in normal and Repeat Breeder cows (compiled from Sreenan and Diskin, 1986 (normal cows) and Ayalon, 1978 (Repeat Breeders))

Day	*Percentage of animals with embryos*	
	Normal	*Repeat Breeder*
2–3	85	71
11–13	74	50
14–16	73	50
17–19	60	43
35–42	67	35

is their morphological development (Gustafsson, 1985).

Thus, provided one has excluded obvious pathological lesions, mismanagement of mating and infectious diseases that impair reproductive performance, two main causes of repeat breeding remain as causes of an impaired uterine environment: luteal deficiency and chronic degeneration of the endometrium.

Damage to the endometrium

Levine (1999) did not place great importance upon chronic uterine infection as a cause of repeat breeding, citing studies by Hartigan et al. (1972), DeKruif (1976), Hartigan (1978) and Roberts (1986) as evidence of a generally low infection rate and moderate bacterial recovery rates. Interestingly, recent studies from the Indian subcontinent do not support this view, since, for example, Ramakrishna (1996) found 46 out of 60 Repeat Breeder cows to have significant bacterial isolates from cervical discharges; Malik et al. (1987) found 370 out of 396 mucus samples from infertile cows to be infected. Moreover, infection does not have to be active at the time of sampling for infection-related uterine damage to have occurred. The effects of infection upon endometrial scarring in the mare are well known (see Chapter 26), but, even in the cow, there is increasingly clear evidence that chronic uterine damage results from infection. Gonzalez (1984) associated infertility with the degree of endometrial damage that was present in uterine biopsies, while DeBois and Manspeaker (1986), in their review of endometrial biopsies in cattle, noted that mild chronic endometritis is one of the most common causes of repeat breeding. Hence, it was their opinion that endometrial biopsy is an essential part of the examination of the valuable cow with unexplained infertility.

Uterine secretions of Repeat Breeder cows have, perhaps unsurprisingly, generally been characterised as differing from those of normal cows (Zavy and Giesert, 1994). Almedia et al. (1984) showed qualitative and quantitative differences in the ionic composition of uterine flushings of normal and Repeat Breeder cows. No clear-cut associations between biopsy lesions and uterine secretion characteristics are evident in the literature and it might be argued that differences in uterine secretory profiles are as likely to reflect differences in luteal activity (or, at least, progesterone status) as the degree of histological change to endometrial structure.

Luteal deficiency

Progesterone is necessary for the maintenance of pregnancy. Until 150–200 days of pregnancy, and perhaps in some cases to term, the main source of the hormone is the corpus luteum; so that if this is not completely formed or it is not functioning adequately then insufficient progesterone is produced and the pregnancy fails. Luteal deficiency has been suspected of causing infertility for many years and, although proof is difficult, treatment of Repeat Breeders is frequently based on this assumption.

The relationship between progesterone concentrations and pregnancy rate has been investigated on many occasions and under many circumstances. The evidence from studies of plasma concentrations has been somewhat equivocal. Erb et al. (1976), Lukaszewska and Hansel (1980) and Hansel (1981) found very early (days 6 to 10) divergences of progesterone concentrations in cows that conceived from those which failed to conceive or which were unmated. In others studies (Parkinson and Lamming, 1990), the differences between pregnant, non-pregnant and unmated cows were not evident until the mid-luteal phase, while yet others (e.g. Shemesh et al., 1968; Sreenan and Diskin, 1983) found no differences until the onset of luteolysis. Given the variability of such results, others have examined concentrations of progesterone in milk. Since progesterone is sequestered in the fat component of milk, progesterone concentrations in milk are regarded as being more representative of the total secretion of the steroid in the inter-milking period, whereas circulating concentrations fluctuate rapidly. Many of the studies of milk progesterone have found that concentrations in cyclic, pregnant and non-pregnant cattle diverge at some point before the onset of luteolysis. Most agree that concentrations are similar before day 6, but the time at which concentrations diverge ranges from day 6 (Bloomfield et al., 1986), day 8 (Lamming et al., 1989), day 11 (Edgerton and Hafs, 1973) day 13 (Bulman and Lamming, 1978), to day 16 (Roche et al., 1985). Despite this variability, and the fact that individual cows can conceive in the face of a long period of low progesterone concentrations (A. O. Darwash, personal communication; T. J. Parkinson, unpublished data; Bulman and Lamming, 1978; Jackson, 1981), a view has emerged that deficiencies of luteal progesterone production around the time of the mid-luteal phase are associated with pregnancy failure.

For the purposes of diagnosing luteal deficiency, it is impossible on rectal palpation to differentiate between a normal and an abnormal corpus luteum; there is a natural variation in luteal size and the position of the corpus luteum within the ovary is variable and thus makes estimation of its size very difficult. Likewise, attempts at diagnosis by taking a single sample of milk or blood for progesterone analysis are also of little value. Hence, given that the relationship between low progesterone and pregnancy failure is one of probabilities rather than of absolute values, attempts have been made to find cost-effective ways to augment circulating progesterone concentrations, in the hope of improving the pregnancy rate in an entire herd, especially amongst the Repeat Breeders.

The main luteotrophic hormone of the cow is LH (Simmons and Hansel, 1964; Donaldson et al., 1965). Thus, if LH activity is enhanced (for example, by injection of hCG or GnRH) after ovulation, a stimulation of the development and function of the corpus luteum may result. Despite the long-standing use of hCG as a 'holding injection' in infertile cows, there is no statistically significant effect in improving pregnancy rates in normal cows (Greve and Lehn-Jensen, 1982; Sreenan and Diskin, 1983), even in meta-analysis of many trials. For Repeat Breeder cows, such hormones have been used in an attempt to hasten the timing of ovulation. Again, results have been equivocal. Results for hCG have often been disappointing (Hansel et al., 1976; Leidl et al., 1979). Those for GnRH have been rather more encouraging (Schels and Mostafawi, 1978; Lee et al., 1981; Nakao et al., 1983; Morgan and Lean, 1993), especially by improving pregnancy rates for services other than the first (Maurice et al., 1982; Stevenson et al., 1984). Hence, the use of GnRH in this way is quite common (Malmo and Beggs, 2000).

An alternative approach has been to give hCG or GnRH 11–13 days after breeding. The rationale for this approach is that accessory corpora lutea might be induced (which would be refractory to the effects of $PGF_{2\alpha}$ on days 18–20), or that the activity of the corpus luteum might be augmented. In either case, the intention is to create conditions that allow an embryo, whose maternal recognition of pregnancy signal (interferon-τ) is inadequate, to survive by preventing luteolysis. Studies with GnRH have produced equivocal results. Macmillan et al. (1986) improved first- and second-service pregnancy (conception) rates by 11.5 and 15.6%, respectively, when cows were treated 11–13 days after insemination. Sheldon and Dobson (1993) improved pregnancy rates from 51% in untreated controls to 60% in cows treated with GnRH on day 11. Conversely, Jubb et al. (1990) were unable to show any significant improvement when GnRH was used on day 12. At the time of writing, the consensus view of the effects of GnRH upon pregnancy rate is that it has little effect in a herd which has good pregnancy rates, but that it can produce a significant increase in herds with poor pregnancy rates.

Progesterone implants have been used to try to augment progesterone concentrations during the period when luteolysis is expected. They are expensive and, in the authors' experience, of little value. In a further meta-analysis of many early trials of the effects of progesterone administration upon pregnancy rate, Diskin and Sreenan (1986) concluded that it was ineffectual. However, recently, interest in progesterone administration as a means of augmenting pregnancy rate has been rekindled, and a method that is increasingly widely practised is the reinsertion of previously used progesterone-releasing intravaginal devices (i.e. PRIDs or CIDRs). This practice may increase pregnancy rates (Macmillan et al., 1986) by augmenting circulating progesterone concentrations, but it has an additional advantage that returns to oestrus in non-pregnant cows are either synchronised or occur at a predictable time (Cavaleri et al., 2000a, b).

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23 Specific infectious diseases causing infertility in cattle

Many of the infectious diseases of cattle adversely affect reproductive performance, either by direct effects upon the reproductive system or via indirect effects upon the general state of health of affected animals. In this chapter, the effects of enzootic infectious diseases upon reproductive performance are considered; the effects of non-specific infections of the reproductive tract, such as those which occur after calving, were considered in Chapter 22.

Infectious diseases can affect the reproductive system in the following main ways:

- Impaired sperm survival or transport in the female tract, leading to reduced fertilisation rate.
- Direct effects upon the embryo. This includes infections that result in early embryonic death, and those that infect the more advanced fetus or its placenta, resulting in abortion, stillbirths or the birth of weak calves.
- Indirect effects upon embryo survival. This includes infections that have adverse effects upon uterine function and those that infect the maternal component of the placenta. Again, these result in embryonic death, fetal death with abortion, mummification or stillbirth.
- Systemic illness causing fetal losses (e.g. pyrexia-induced abortion) or a direct impairment of reproductive cyclicity.

The patterns of enzootic infectious diseases that affect reproduction have changed considerably in most developed countries over the past 40–50 years. The classic venereal diseases, campylobacteriosis and trichomoniasis, have been largely eradicated in dairy cattle, by the use of artificial insemination with semen from disease-free bulls. The control has been less effective in beef cattle, in which natural service remains the predominant method of breeding. Most western countries have successfully eradicated brucellosis, through programmes based upon vaccination, blood testing and slaughter. Conversely, other diseases such as IBR-IPV (infectious bovine rhinotracheitis–infectious pustular vulvovaginitis), bovine viral diarrhoea (BVD) and leptospirosis have assumed much greater importance, because of either a genuine increase in prevalence or the development of better diagnostic methods. Other diseases, whose effects upon reproduction were hitherto unrecognised, are now ascribed significance as reproductive diseases. Examples include ureaplasmosis, *Haemophilus somnus* infections and *Neospora caninum*-induced abortion.

Yet, even though there has been a change in the importance of different specific infectious agents in causing infertility, none should be forgotten when investigating subfertility in a herd. Diseases which have been considered as being eliminated can still recrudesce (as recently happened in the UK with trichomoniasis) and can cause catastrophic effects if they gain entry to a herd with a low immune status to that disease.

Estimates of the prevalence of infectious diseases of reproduction largely depend upon the successful diagnosis of causes of abortion. The data provided from this source provide only an approximate guide to the prevalence of diseases, however, since the percentage of fetopathies from which a specific infectious agent is identified is relatively small. In the results from the Veterinary Investigation Service of the Ministry of Agriculture (UK), positive results were obtained in only 4.3–7.4% of cases. However, these data do show that the prevalence of many infectious causes of abortion has been relatively static in the UK since the publication of the Veterinary Investigation Diagnosis Analysis (VIDA II) in 1977 (see Table 23.1).

Table 23.1 Percentage frequency of isolation of pathogens from bovine fetopathies examined by Ministry of Agriculture Veterinary Investigation Centres (Source VIDA II)

	1977	1987	1988	1990	1991	1992	1993	1994	1995	1996	1997	1998
Bovine viral diarrhoea (BVD)	NR	10.8	8.0	14.5	8.0	8.7	8.6	4.5	5.8	5.4	8.2	7.9
Brucella abortus	52.3	0.3	0.2	0.1	0	0	0.1	0	0	0	0	0
Campylobacter spp.	0.4	0.4	0.8	0.7	1.3	1.3	1.1	1.9	1.5	3.0	2.3	2.8
Actinomyces pyogenes	20.2	5.3	3.7	3.5	4.0	3.8	4.3	3.8	4.6	6.0	4.0	5.2
Leptospira	NR	33.5	46.4	45.6	42.1	43.2	33.0	33.2	25.3	22.4	12.4	12.7
Listeria monocytogenes	0.6	1.2	1.7	1.3	1.2	1.4	2.3	2.0	1.6	2.0	1.8	2.4
Salmonella dublin	9.3	15.4	14.4	9.4	11.8	11.8	15.0	13.7	12.8	7.5	10.2	9.2
Salmonella typhimurium	0.5	0.9	0.4	0.6	0.7	0.5	0.8	1.5	0.8	0.9	0.8	0.6
Other *Salmonella* serotypes	0.8	1.0	0.8	1.2	1.7	1.5	1.7	1.3	1.5	2.2	1.0	0.9
Bacillus licheniformis	NR	NR	NR	NR	8.2	8.2	8.0	8.0	10.2	13.1	7.5	7.4
Coxiella burnetti	0	0.5	0.1	0.3	0.4	0.5	0.3	0.4	0.3	0.1	0.06	0.1
Fungi	8.2	9.7	6.1	6.9	6.1	6.0	10.4	13.7	7.4	8.1	5.5	5.4
Infectious bovine rhinotracheitis – infectious pustular vulvovaginitis (IBR–IPV)	NR	5.4	6.1	4.9	4.3	5.2	3.9	3.9	4.5	5.6	3.1	1.9
Other pathogens	7.6	15.7	11.3	11.0	10.0	7.9	10.3	10.4	12.3	13.5	6.1	10.1
Protozoa	NR	NR	NR	NR	NR	NR	0.3	1.6	11.4	38.1	1.3	0.07
Neospora	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	34.7	33.0
Total identified	1675	1524	2297	2205	1617	1604	1504	1815	1896	1689	1790	1486

NR = not recorded

BACTERIAL AGENTS

Genital campylobacteriosis

Infection due to *Campylobacter fetus* (formerly *Vibrio foetus*) has long been recognised as a cause of abortion in sheep and cattle (McFadyean and Stockman, 1913). It should be noted that the term 'campylobacteriosis' has largely replaced 'vibriosis' in describing the disease caused by *C. fetus*. In dairy cows, the importance of the disease has declined over the past 40 years with the use of artificial insemination, because of bull screening at artificial insemination studs and the use of antibiotics in semen extenders. However, where natural service is used (notably in beef herds) its venereal route of transmission means that campylobacteriosis must always be considered as a potential cause of infertility. It is still a major cause of reproductive disease in many countries. In a 15-year study in Argentina, involving over 11 300 bulls, 22% were found to be immunofluorescent-positive (Villar and Spina, 1982), whilst in 400 cows in three dairy herds in California 47% were seropositive for *C. fetus* (Ahktar et al., 1993).

About 90% of infertility due to *C. fetus* is due to the subspecies *venerealis* (*C. fetus venerealis*); however, the subspecies *fetus* (*C. fetus fetus*, of which there are two serotypes) can cause sporadic abortion, but is not spread venerally and is not normally associated with infertility. Saprophytic organisms such as *C. bulbus* and *C. faecalis* may be present in the alimentary tract of cattle and in the prepuce of the bull. In the latter site, they may complicate diagnosis by direct bacteriological examination and fluorescent antibody tests.

Clinical signs and course of disease

Lawson and MacKinnon (1952) and Boyd (1955) studied bovine genital vibriosis under experimental conditions, and have provided an excellent account of the natural history, symptoms, course and diagnosis of the disease. The bull normally carries the infection for life without any interference with its reproductive behaviour or seminal qualities. The organism is confined to the glans penis, prepuce and distal urethra, but there are no lesions associated with the presence of the organism at any of these sites. Thus, the bull acts simply as a mechanical carrier and transmits the infection at service to the female. Since the organism lives in the crypts of the penile integument, the likelihood of bulls becoming persistently infected increases with age, as the crypts become deeper and more extensive (Jubb et al., 1993).

The sites of infection in the cow are the vagina, cervix, uterus and uterine tubes. The organism causes no lesions of the vagina, but can persist in that site for some time. Within the uterus, it causes a mild endometritis. Dekeyser (1986) describes the endometritis as being diffuse and mucopurulent, characterised by periglandular accumulations of lymphocytes and the collection of exudate in the uterine lumen. The endometritis is of a mild nature and cannot be appreciated by rectal palpation of the uterus. There may be a salpingitis (Roberts, 1986). Inflammation of the cervix may also occur, causing an increased secretion of mucus which may become mixed with uterine exudate to form a mucoflocculent vulval discharge after service. This, however, is not nearly so conspicuous a symptom as in trichomoniasis (see below).

The organisms do not interfere with the process of fertilisation but, following their colonisation of the uterus, a tissue reaction occurs which is inimical to nidation of the embryo, or to its continuing nourishment in the uterus. Therefore, in a majority of susceptible females served by an infected bull, fertilisation occurs but is followed by early embryonic death. In a much smaller proportion of infected cows, later abortion occurs between 4 and 7 months.

When infection is introduced into a susceptible herd, a dramatic decrease in pregnancy rate occurs. Embryonic deaths may occur before the maternal recognition of pregnancy, in which case return to oestrus occurs 3 weeks after service. Embryonic deaths occurring after recognition of pregnancy result in later, irregular return to oestrus, often between 25 and 35 days after service. Hence, the first sign of genital campylobacteriosis to be seen by the stockperson will be a marked increase in the number of females returning to oestrus, some regularly and some irregularly, after service by a newly introduced bull. A small proportion of susceptible cows and heifers conceive to first service by an infected bull and carry their calves to full term. Immunity to the organism slowly develops and, as it does so, cows conceive and remain pregnant. Eventually, after an average of five services, the majority of cows become safely pregnant and carry their calves to term. It is always possible, however, that the occasional cow will abort and, at parturition, a few cows may retain the fetal membranes as a result of the disease. Most cows which have had normal gestations after breeding by an infected bull will be free of infection at the time they are next required to be served.

Thus, after experiencing serious infertility for about 6 months, a herd will gradually become immune and thereafter undergo normal gestation, at the end of which most cows will be free of infection. If infected bulls remain with the herd, reinfection of some cows will occur when they are rebred after normal parturition, whereupon a similar, but much less severe, infertility problem recurs. Eventually, after 2 or 3 years, the fertility of such cows becomes acceptable, with only vague and intermittent infertility occurring (Roberts, 1986). However, amongst newly introduced cows and new heifers, which are not immune to the disease, the disease will be perpetuated. Analysis of fertility records of such a herd will reveal acceptable conception rates and a relatively normal distribution of interservice intervals amongst the established, mixed-age cows of the main herd. Maiden heifers, if they are bred to an infected bull, may show low conception rates and irregular returns to oestrus, but if these animals have been bred by a virgin bull, they may not contract the disease until after their first calving. Purchased animals, likewise, show the effects of the disease during their first season in the herd. An example of the pregnancy rates and interservice intervals of a herd with long-standing campylobacteriosis is shown in Figure 23.1. In a 'flying' herd, the symptoms of vibriosis may be perpetuated indefinitely through the non-immune, bought-in females.

The majority of the abortions due to *C. fetus* occur between the fourth and seventh months of

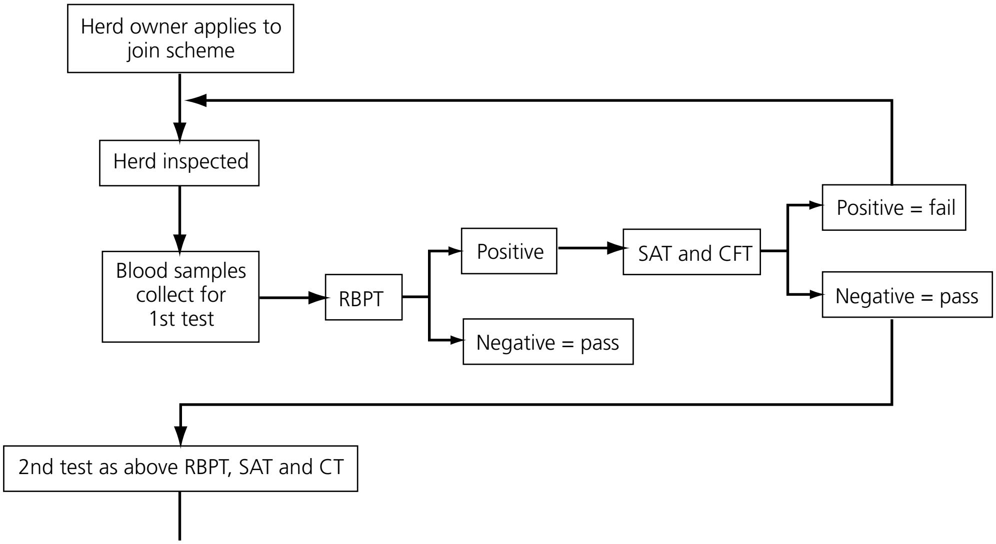


Fig. 23.1 Distribution of interservice intervals in a dairy herd that had enzootic infection with *Campylobacter fetus*. (a) Interservice intervals were normal in maiden heifers that were served by a newly acquired (virgin) bull. (b) In first-calving cows, interservice intervals showed evidence of embryonic death, which were accompanied by poor conception rates. (c) In the older cows interservice intervals and conception rates were relatively normal, although chronic campylobacteriosis remains evident.

gestation. The placenta is often autolysed, indicating that death preceded expulsion by a significant interval. Placental lesions are very similar to, although less severe than, those caused by *Brucella abortus*. Typically, there is necrosis, with yellowish-brown discoloration of the fetal cotyledons and leather-like thickening or oedema of the intercotyledonary allantochorion. Lesions in the fetus are not specific (Jubb et al., 1993).

Diagnosis

Genital campylobacteriosis will be strongly suspected when a majority of cows or heifers are returning regularly or irregularly to service, especially if the infertility coincides with the introduction of a new bull. The possibility that the breeding trouble is due to defective semen of the newly introduced bull should first be eliminated and then specific enquiry for the presence of *C. fetus* should be made.

A variety of diagnostic tests can be used to diagnose *C. fetus* infection. These are:

- identification of the organism in preputial washings
- direct smears, culture and fluorescent antibody tests
- serological tests
- vaginal mucus agglutination.

In bulls suspected of infection, preputial washings or scrapings of the penile or preputial mucosa can be examined (Bartlett, 1948; Dufty and McEntee, 1969; Tedesco et al., 1977). Where samples can be submitted to a diagnostic laboratory on the same day of collection, phosphate-buffered saline will maintain the viability of organisms. Otherwise, a selective enriched transport medium should be used. Antibiotics, such as polymyxin B, inhibit the growth of contaminants, which obviates the need for refrigeration. Even after a delay of 2–5 days, such media can result in good recovery of the organism (Eaglesome and Garcia, 1992).

Preputial samples from suspect bulls and material derived from aborted fetuses can be examined using direct culture or fluorescent antibody techniques. Dufty (1967) advised that a bull can be declared non-infected after four consecutive negative fluorescent antibody tests. At present, it is not possible to differentiate between the two subspecies *venerealis* and *fetus* by this method, although it can distinguish them from other species of *Campylobacter*. Tissues from an aborted fetus (lung, spleen, liver) and abomasal fluid should be removed aseptically and maintained at 4°C until they reach the laboratory. Direct smears of abomasal contents can be examined using phase contrast or dark field microscopy. If the selective enriched transport medium is used, it is normally incubated for 4 days at 37°C before transfer to blood agar plates. In the case of fresh samples, these are streaked on to the plates. Positive cultures are diagnostic, although the fastidiousness of the organism means that negative results should be interpreted with caution. However, Barr and Anderson (1993) considered culture to be of greater value than fluorescent antibody testing.

Serological tests are of little or no value, since genital campylobacteriosis does not engender measurable serum antibody levels.

A vaginal mucus agglutination test was first described by Kendrick (1967) and has been used extensively since. Mucus can be collected by a variety of different methods; however, it is important not to use the copious mucus of oestrus in which the agglutinins will be diluted, but mucus from a cow in dioestrus, which can be difficult to collect in sufficient quantities. A variety of methods have been used; these include a glass or plastic pipette to which is attached a mouthpiece, and a small portable vacuum pump. Probably the simplest and most effective method in cows, as opposed to heifers, is to insert a clean, gloved hand into the vagina and to scoop mucus into the palm of the hand from the ventral fornix. This can be transferred to a wide-mouth collecting bottle. The vaginal mucus agglutination test should be used for herd diagnosis rather than for individual cows. False positives can be obtained if the mucus is contaminated with blood. It is important to ensure that sufficient time has elapsed since animals would have been exposed to infection; thus in investigating a herd it is important to ensure that all non-pregnant cows that were first exposed to service more than 60 days previously should be sampled. One positive reaction is sufficient to establish a herd infection; for this reason, confirmation of an infected bull can be made by allowing test mating of two virgin heifers and performing a mucus agglutination test 60–80 days later. Recently, a method has been developed in which a piece of Whatman filter paper is placed on the lateral wall of the vagina cranial to the urethral opening until it is saturated; secretory immunoglobulin (IgA) is then detected using enzyme-linked immunosorbent assay (ELISA) (Hum et al., 1991).

Treatment and control

Control is based on three epidemiological facts:

- Transmission is venereal.
- Bulls remain permanently infected.
- Infected cows overcome the infection, or become immune, in a period of 3–6 months from service.

Thus, a ‘self-cure’ of the cows will occur if natural service by infected bulls is replaced by artificial insemination. Artificial insemination (AI) is a highly effective means of control, since incoming uninfected animals do not contract the disease and infected animals eventually become immune. Removal of bulls from the herd prevents further venereal transmission of the disease. AI bulls are normally tested on a regular basis (6-monthly in the UK) for the presence of venereal pathogens, and antibiotics are added to semen diluents to ensure that any organisms are destroyed. The drawback to the use of AI is that it is not easily applicable to all types of husbandry – for example, in extensive beef herds; while some pedigree herds require bloodlines that are not available in bulls at the AI centre. A question the attending veterinary surgeon will soon be required to answer is ‘how long is it necessary to persist with artificial insemination?’ It seems certain that in the majority of cows *C. fetus* will not survive a normal gestation, but Frank and Bryner (1953) recovered *Compylobacter spp.* from a few cows as long as 196 days beyond the end of a pregnancy initiated by infected semen. It would seem wise therefore to continue insemination until every exposed cow has completed two normal pregnancies. Natural breeding can then be resumed.

When AI is used to eliminate campylobacteriosis from a herd, it may be considered safe to use a clean bull on the virgin heifers. After the heifers have calved they may again be mated naturally to the clean bull, such that 'clean' and 'infected' herds are maintained during the period of elimination of the organism from the premises. Such a departure from a total AI regimen must be implemented with extreme caution, since segregation of non-infected and infected animals must be absolute. A less satisfactory method is to breed the heifers and any non-exposed cows artificially or to a clean bull – as outlined above – and to continue service by the herd bull on the infected group. In addition to the risk of accidental contamination of the clean bull, there is always the possibility that reinfection of recovered cows may thus occur and that a minor degree of infertility may persist; the much more serious aspect is that such a herd will never be free of infection so long as the infected bull continues to be used.

As *C. fetus* is sensitive to streptomycin (Binns, 1953) this antibiotic has been used to treat the disease in bulls. Dihydrostreptomycin, at a dose rate of 22 mg/kg subcutaneously, together with the local application of the same antibiotic to the penis and prepuce, is effective, although it must be remembered that the bulls will be susceptible to reinfection. An oily suspension of procaine penicillin and streptomycin for intrapreputial infusion was marketed for a long time in the UK for the treatment or prophylaxis of campylobacteriosis in bulls, although this is now no longer available. Dekeyser (1986) reported that a combination of neomycin and erythromycin, in a waxy base, is effective in eliminating *C. fetus* from bulls in which streptomycin has been ineffective. Antibiotics have no beneficial effect in the cow, whether administered locally or parentally.

Vaccination programmes have been successful in controlling the disease in situations where artificial insemination cannot be practised. Using oil adjuvant bacterins with high cell counts of immunogenic strains of *C. fetus venerealis*, good results have been obtained. Vaccination should preferably be carried out 30–90 days before breeding commences and, since the immunity wanes annually, revaccination is recommended for optimum protection as close to the time of service as possible (Hoerlein, 1980). Dekeyser (1986) noted that, although vaccinated females conceive normally, many acquire a vaginal infection if they are served by an infected bull. Vaccination has also been used to cure infected bulls. Bouters (1973) reported that by giving two doses of vaccine at a month's interval, 51 known infected bulls were cured, and this, together with annual vaccination programmes, greatly reduced the incidence of genital vibriosis in areas of Belgium where ambulant stud bulls were used. Some have expressed concern that vaccination may only modify the carrier status (Hoerlein, 1980). However, more recently, it has been reported that protection of the male can be reliably achieved by the use of double doses of vaccine given on two occasions (Cortese, 1999).

Brucellosis (contagious abortion)

Brucellosis in cattle is most commonly caused by *Brucella abortus*. *Brucella melitensis*, which occurs in sheep and goats, can also be transmitted to cattle. *Brucella* causes abortion in the second half of pregnancy, together with metritis and retained fetal membranes (RFM). In bulls, it can cause orchitis, epididymitis, seminal vesiculitis or infection of the ampullae (Nicoletti, 1986).

B. abortus occurs in most countries of the world where cattle are kept in any significant numbers. In 1976, it occurred in 95 out of 153 countries from which information was available (Thimm and Wundt, 1976). Because of the enormous losses that the disease causes to dairy and beef cattle industries, it has been the subject of eradication schemes in many countries.

Epidemiology

Cattle can become infected by ingesting *B. abortus* from contaminated pasture, food or water. Infection may occur by licking an aborted fetus, infected afterbirth or genital exudate from a recently aborted or recently calved cow. It may even occur through the teat by infected milk of another cow, or through the vagina by infected semen. In experimental studies of brucellosis, conjunctival inoculation is usually employed. Infected cows often shed the organism in the milk, thereby endangering public health. Contaminated milk also provides a source of infection for calves, although the main danger of spread to other cattle is at the time of abortion or parturition. The organism colonises the udder and supramammary lymph nodes of non-pregnant animals. In pregnant animals, production of erythritol within the placenta allows rapid multiplication of the bacteria, leading to endometritis, infection of cotyledons and placentitis. The fetus is aborted 48–72 hours after death, by which time a degree of autolysis has occurred. The fetal membranes are very frequently retained. For a day or two before, during and for about a fortnight after abortion the genital discharge of the infected female is highly infected. When the fetal membranes are retained, the uterus may not free itself of infection until about a month after delivery. After the completion of uterine involution, the organisms colonise the udder and supramammary lymph nodes, whence, in the next gestation, infection of the placenta may again occur. Outside the animal body *B. abortus* may live for months in aborted fetuses or fetal membranes, but when exposed to drying and sunshine it is soon killed. Thus, most herd outbreaks have been caused by the introduction of carrier animals.

Occasionally, fetal death occurs and is not followed by abortion, the retained fetus undergoing mummification or maceration. Fetuses from late abortions are often born alive but are frequently weakly and may consequently contract white scour. Calves that derive milk-borne infection throw off infection from the lymph glands of the gastrointestinal tract in 50–80 days. The infantile uterus becomes infected in a very small proportion of animals (Wilesmith, 1978).

Clinical signs

The disease causes serious economic loss, primarily due to abortion in the second half of gestation, although earlier abortions occur at the beginning of an outbreak. In addition, some calves will be born alive but they will be weak and unthrifty. Infected cows usually abort once and seldom more than twice, although in subsequent pregnancies the uterus may be reinfected from the udder even though the cow carries the fetus to term. RFM is more common in cows that abort in later gestation and those that carry to term. Such animals show delayed involution of the uterus, and are prone to secondary bacterial invasion with resultant puerperal metritis.

Some evidence also suggests that brucellosis causes other effects on reproduction beyond those of abortion and puerperal metritis. Plommet (1971) concluded, from a review of many earlier studies, that pregnancy rates, number of services per conception and numbers of Repeat Breeders are poorer in infected than in non-infected cows.

Diagnosis

The organism can be identified in stained smears prepared from suspected contaminated material. Special staining techniques using a modified Koster and Ziehl-Nielson method are quite successful (Brinley Morgan and MacKinnon, 1979). A more specific method of direct identification is a fluorescent antibody technique, which enables differentiation from other infectious diseases such as Q fever (Brinley Morgan and MacKinnon, 1979). *B. abortus* can be cultured from the fetal stomach of an abortus, or from fresh afterbirth, or uterine exudate. Because culture of the organism is time-consuming and expensive, alternative methods of identification have been devised. A colony blot ELISA using monoclonal antibodies provides a rapid, inexpensive and reliable method of identifying *B. abortus* (Eaglesome and Garcia, 1992). Where contamination is probable, the suspected material is inoculated into guinea pigs, in which characteristic lesions occur and from which the organisms can be cultured.

Numerous serological tests have been used to diagnose brucellosis, using a wide range of biological materials such as milk, whey, serum, vaginal mucus and semen. These have then been subjected to agglutination test, complement fixation test, antiglobulin test, fluorescent antibody test and immunodiffusion or electroimmunodiffusion tests (Brinley Morgan and MacKinnon, 1979).

The rose bengal plate test was introduced into the UK in 1970 as the main initial screening test of serum samples in the brucellosis eradication scheme (Brinley Morgan and Richards, 1974). It is recognised that it is oversensitive and may identify non-infected animals as being positive. For this reason, positive samples are re-examined using the serum agglutination test (SAT) or complement fixation test (CFT); rose bengal-negative samples are not normally retested.

A SAT is very widely used, provided that the antigen is standardised against the international standard anti-*Brucella abortus* serum. It has some deficiencies: it detects non-specific antibodies as well as specific antibodies from *Brucella* infection and vaccination; during incubation it is the last of all the possible tests to indicate the presence of infection and after abortion it may be the last to detect diagnostically significant levels; in the chronic stage of the disease the agglutinins wane, thus giving a negative result when other tests would give a positive result (Brinley Morgan and MacKinnon, 1979).

The CFT is a more definitive test than the SAT, especially in differentiating titres arising from infection from vaccination. The CFT identifies infected adults before the SAT, and, as the disease becomes chronic, the titres detected by the SAT tend to fall below diagnostic levels whereas titres detected by the CFT persist at diagnostically significant levels. In calves vaccinated with Strain 19 (S19, see below), titres detected by the CFT become negative in most cases by 6 months after vaccination, whereas an 18-month period is required for the SAT. The milk ring test, which detects *Brucella* antibodies in milk, is very useful in screening the presence of brucellosis in herds by collecting bulk milk samples or in individual animals. Positive results can then be followed up by using other diagnostic tests on individual animals.

The vaginal mucus agglutination test can be used on samples from individual cows; it is not very reliable.

It should be noted that, during an active infection of a herd, results of agglutination tests should be interpreted with some caution. Negative reactions will occur during the incubation period and, furthermore, it is quite common to get a negative reaction at the time of, and for a few days after, a brucellosis abortion. Infected bulls sometimes fail to react to the blood test, and it is considered that if the agglutination test is performed on seminal plasma, rather than blood, a better indication of infection will be obtained. In recent years, purification of specific *B. abortus* antigens has allowed the development of enzyme immunoassays. Using some competitive ELISAs, it is possible to discriminate between vaccinated and non-vaccinated infected animals (Nielsen et al., 1989). Such methods play an important role in the control of the disease (see below).

Control

Brucellosis is not only a cause of abortion in cattle, but it also causes a serious disease, undulant fever, in man. Hence, control of the disease has to be directed at both its animal health and its public health aspects.

From the animal health viewpoint, abortions can be prevented in herds by calfhood vaccination, using the *B. abortus* S19 live antigen. But, since this vaccination programme does not eliminate the infection from cattle, such a method is unsatisfactory from the public health perspective as there is an on-going risk of undulant fever in those who consume the raw milk.

In order to meet both of these requirements, a number of governments have implemented a two-stage programme of brucellosis control. In the first stage, widespread vaccination is encouraged in order to reduce losses due to abortion and its sequelae. Thereafter, a national eradication scheme is undertaken. In some countries, states, areas or even herds where rigorous measures of hygiene can be enforced, eradication has been achieved without recourse to vaccination.

The European Union considers a bovine herd to be officially brucellosis-free if it contains no animals vaccinated against brucellosis (except females vaccinated at least 3 years previously), if all bovine animals have been free from clinical signs for at least 6 months and if all cattle over 12 months old have passed the SAT test at less than 30 i.u. Animals in these herds are subject to twice-yearly blood tests. In those herds where milk is collected into churns, the milk ring test may be used. Replacement cattle must be certified from a brucellosis-free herd and officially tested if over 12 months old. Testing replacement cattle need not be required if infected herds have not exceeded 0.2% for at least 2 years, and certification need not be required if at least 99.8% of herds are officially free, and infected herds are under supervision.

Vaccination. In 1941, S19 vaccine was officially introduced into the USA, since when it has been employed in several other countries. S19 is a smooth variant of a strain of *B. abortus*, of reduced virulence but of high antigenic quality. It was intended for use on calves before the onset of puberty. The ages at which calves have had to be vaccinated have varied between schemes, typically vaccination occurs at some time between 2 and 10 months of age (Roberts, 1986). Vaccination of calves causes a febrile reaction and rapid seroconversion, with titres declining over the next 12 months in 90% of animals.

In self-contained herds, calfhood vaccination is sufficient for life, but where adult cattle are brought in, or in the presence of active infection, cows should be revaccinated after their first calving. When infection is introduced to an unvaccinated herd, all adult female stock (except those with possible blood agglutination titres), as well as calves and cows pregnant up to 4 months, should be vaccinated. The S19 vaccine gives a better immunity when used on cows rather than calves, but in sexually mature cattle, higher and more persistent agglutinating titres are produced. Adult cattle may also display a greater general reaction, with serious interference with subsequent milk yield. Vaccinal titres occurring in adult cows may be confused with natural infection, but they seldom rise above 1:200 and decline with passage of time.

The greatest disadvantage of S19 is that the titres that follow vaccination of adults cause difficulties when an eradication programme is dependent on the interpretation of the SAT. For this reason, vaccines prepared from killed cultures of McEwan's *B. abortus* S45/20 with adjuvant, which cause only insignificant titres, have been recommended for use on cattle of all ages; pregnant cows may also be safely vaccinated. However, the widespread application of S19 vaccination has greatly reduced the losses from contagious abortion, and when its use is restricted to calves (as originally intended) the results are excellent. When the brucellosis eradication scheme was introduced in Britain (Brucellosis Accredited Herds Scheme), the use of S19 vaccines was restricted to calves between 90 and 180 days of age.

It is not usual to vaccinate bull calves, mainly because brucellosis of bulls is uncommon and also because a vaccinal titre might throw suspicion on the bull and would preclude its purchase for artificial insemination or for export. In addition, it has been reported that S19 may produce permanent infection in bulls which is similar to the natural disease, and thus should not be used (Nicoletti, 1986).

Eradication. Eradication can be undertaken by a programme of testing and slaughter of seropositive animals. Radostits et al. (1994) suggested that the incidence of infection has to be reduced to about 4% of the bovine population before a slaughter-based eradication programme is likely to be feasible. In order to undertake such a scheme, statutory powers are usually required to implement a compulsory programme.

The main facets of a brucellosis eradication scheme are:

- Positive identification of cows and their calves.
- Traceable movements of cattle, so that potential carriers and in-contact animals can be found.
- Secure boundaries to individual farms or to eradication areas are also needed, in order that uncontrolled movements of animals are prevented.
- Regular testing of all cows, followed by immediate slaughter of reactors (often accompanied by their calves at foot). For dairy cows, periodic blood tests can be augmented by continuous monitoring of bulk milk. For beef cattle, continuous monitoring is less straightforward, but some information can be provided by collection of blood from animals at slaughter. Compensation payment for slaughtered animals is needed to ensure farmers' full participation in the scheme.
- Isolation and testing of any cows that abort or have premature calvings. In the UK, any animal calving at less than 271 days of gestation has to be sampled for brucellosis.

Although an essential component of the initial stages of eradication, vaccination becomes detrimental to the completion of eradication once the incidence of disease in the population declines to below about 0.2%, due to difficulties in differentiating between vaccinal titres and titres from natural infections (Radostits et al., 1994). Hence, calfhood vaccination is terminated and the national herd maintained in a brucellosis-free state by the biosecurity regimens of its borders.

In practice, the method of control depends upon the prevalence of the disease. Thus, in positive herds with no recent history of abortion, repeated herd blood samples are taken, and if these disclose inactive infection with a small proportion of reacting animals, it is advisable to sell the reactors. Further herd blood samplings are undertaken with a view to obtaining a certificate of freedom from the disease. Such herds become controlled as in the first category. If there are too many reactors for immediate disposal to be an economical proposition, the disease is controlled as far as possible, on the farm; reactors are separated from non-reactors and are strictly isolated when they calve or if they abort. Rigorous cleaning, disinfection and disposal of infective material is practised. The complete isolation of the reactor from 4 days before calving or abortion to 14 days afterwards is the key to successful reduction in incidence of the disease on the farm. Calfhood vaccination should be performed in these infected herds. When the incidence of infection is sufficiently reduced, the reactors may be slaughtered.

Lastly, in heavily infected herds with current abortion, the spread of infection must be controlled in every possible way. It is best to isolate all parturient or aborting animals from 4 days before to 14 days after parturition. Disposal of infected material, thorough cleansing and disinfection after an abortion and segregation of reactors are practised. There will be a shortage of young stock on such a farm, and this can be made good by buying in calves from free herds; these calves and all other young stock are vaccinated. When the disease becomes quiescent – as shown by further blood tests – disposal of reactors may begin.

Cows in controlled herds should be served only by non-reacting bulls, or inseminated with semen from *Brucella*-free bulls.

Scandinavia was amongst the earliest regions in which brucellosis was eradicated, with the different countries reporting a brucellosis-free status between 1952 (Norway) and 1962 (Denmark). Several eastern and central European countries became brucellosis-free during the 1960s. Luxembourg and the Netherlands became brucellosis-free in 1993. Cyprus, Israel, Japan, Jordan, North Korea, Papua New Guinea, the Philippines and the UAE are free of the disease. In Canada, brucellosis was eradicated in 1989. In the USA, although the prevalence of brucellosis has been very greatly reduced, it is yet to be eliminated. Australia and New Zealand became brucellosis-free in 1989 (Anon, 1997).

In the UK, the eradication scheme was initially very successful, such that the country was reported to be brucellosis-free during the early 1980s. However, following recrudescence of infection during the mid-1980s, the country did not finally become officially brucellosis-free until 1993. The scheme that was successfully used to eradicate the disease from Britain is illustrated in Figure 23.2, which is modified from Brinley Morgan and MacKinnon (1979). A voluntary scheme, with incentives to encourage herd owners to become brucellosis-free, was initially introduced, followed later by a compulsory eradication scheme.

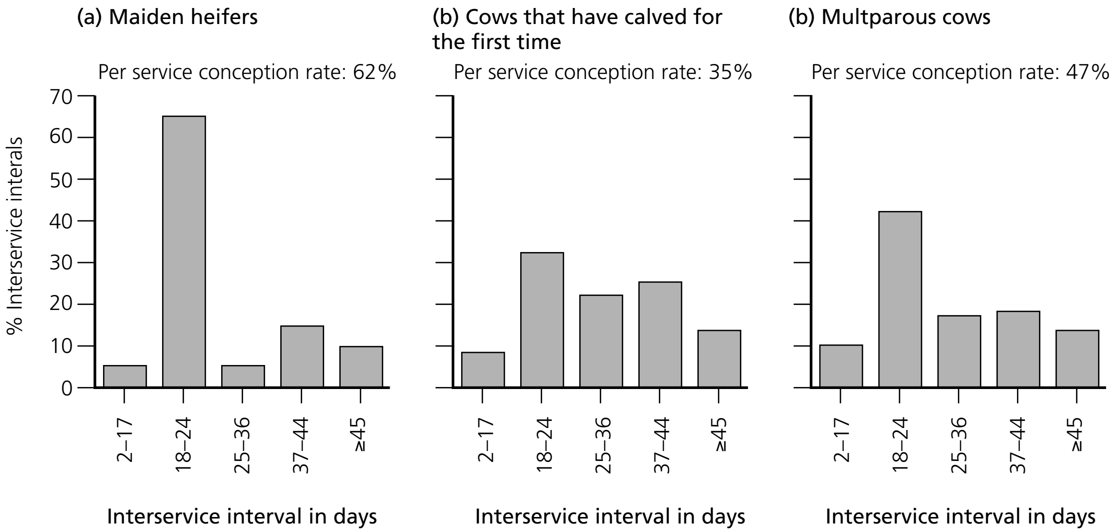
Tuberculosis of the genitalia

Bovine tuberculosis has been eradicated in many countries of the world. However, before eradication schemes were implemented it was an important cause of infertility and thus, where bovine tuberculosis still exists, it should always be considered as a possible cause. Infection may reach the tract either by spread from the peritoneum via the uterine tubes, or by penetration of the serosa, or by bloodstream invasion, in which case the endometrium may be involved in the absence of serous or tubal lesions. Occasionally, primary uterine infections may arise from contaminated instruments or hands during gynaecological or obstetrical interferences.

Williams (1939) classified uterine tuberculosis as being of three clinical types – peritoneal, glandular and epithelial.

Peritoneal

The outstanding feature is extensive adhesions of the uterine horns to themselves, the parietal peritoneum and adjacent organs. The adhesions often contain multiple abscesses, which may attain several centimetres in diameter.



g 23.1

Fig. 23.2 Brucellosis eradication scheme that was undertaken in the UK. RBPT, rose bengal plate test; MRT, milk ring test; SAT, serum agglutination test; CFT, complement fixation test (after Brinley Morgan and MacKinnon, 1979).

Glandular

This type involves chiefly the glandular layer of the mucous membrane and is characterised by marked hypertrophy of a diffuse or nodular nature. Caseous or casepurulent foci of variable size are found throughout.

No clear line of demarcation exists between these types, but one generally predominates. The condition is generally bicornual and, to a degree, symmetrical. The presence of a vulval discharge varies, depending on the degree to which the mucous membrane is involved. In advanced cases there is a profuse mucopurulent discharge, pyogenic infection being added to the tuberculous one. In these cases the uterine tubes are almost invariably involved.

Epithelial

This type generally originates in the bloodstream and the lesions take the form of multiple pinhead sized granulomata. Often there is no appreciable enlargement of the uterus, but a vulval discharge, from which acid-fast organisms can readily be isolated, is the rule. The discharge may be serosanguineous or frankly purulent.

Tuberculosis of the uterus is not an inevitable barrier to reproduction, for quite frequently a calf is born from a grossly infected uterus (the calf itself being affected by the congenital form of the disease), but it is probable in such cases that the uterine infection was acquired or, at least, rapidly developed during pregnancy. The epithelial form is especially liable to develop after parturition.

The uterine tubes are frequently involved in tuberculosis of the genital tract. They become progressively thickened, often attaining a diameter of 1 cm, and may contain local abscesses. There are generally adhesions of the bursa to the ovary. An ovary itself may be the site of tuberculous abscesses. The cervix is rarely affected.

The diagnosis by rectal examination of early cases may be difficult, but particular attention should be paid to the uterine tubes, for the detection of thickened, tortuous tubes is diagnostic. (In this connection care must be taken that the terminations of the uterine horns are not confused with the uterine tubes.) In advanced cases, diffuse or nodular enlargement of the uterus will be readily detected. In infected herds, an animal showing a chronic vulval discharge continuing beyond the puerperium should always be examined for acid-fast organisms, and abortions or premature births should be regarded with suspicion.

Leptospirosis

Leptospirosis is an important zoonotic disease of cattle and other mammals which is caused by pathogenic spirochaetes of the species *Leptospira interrogans* (Eaglesome and Garcia, 1992). Distribution of the organism is world-wide and cattle can be infected by several serovars that have specific effects upon the genital system, causing fetal death, abortion, stillbirth and weakly live calves. The main serovar of *L. interrogans*, whose maintenance host is cattle, is *hardjo* (*hardjobovis* and *hardjoprajitno*; Radostits et al., 1994). Serovar *hardjo* is probably the most common strain that infects cattle world-wide. However, serovars whose maintenance hosts are species other than cattle are also regularly isolated from cattle. Serovar *pomona* is very common in the cattle of many countries, while *ballum, canicola, copenhageni, grippotyphosa, icterohaemorrhagiae* and *tarassovi* are also regularly encountered.

Several surveys have shown how common the disease is in cattle. For example, following a bacteriological examination of 60 cows and heifers selected at random at an abattoir, *L. hardjo* was isolated from 65% of the animals. The spirochaete was isolated from the vagina in 21.7%, the ovary and tubular genital tract in 57% and the urinary system in 62% of the animals. When the results from the microscopic agglutination test (MAT) of sera collected from the same animals were studied, the prevalence of antibodies to the serovar *hardjo* was lower than that from the microbiological study. Overall, 48% (1 in 10) and 27% (1 in 100) had detectable titres to *L. hardjo* (Ellis et al., 1986). In 109 herds surveyed in New South Wales, using the MAT on serum at a dilution of 1:100, only 28% were negative, with a prevalence of 27% positive to *L. pomona*, 16% positive to *L. hardjo*, and 31% positive to both (King, 1991). In New Zealand, 81% of herds have active or previous infection with *hardjo* and 36% show evidence of *pomona* infection (Hellstrom, 1978; Blackmore, 1979). Infection due to *pomona* is also common in Australia and the USA, whilst in parts of Africa, Russia and Israel infection with *grippotyphosa* is the most important incidental leptospiral infection of cattle (Ellis, 1986).

Leptospirosis is also of considerable public health importance, as it causes a zoonotic disease in man. In New Zealand, a very high incidence of human leptospirosis occurred during the 1950s, due to the high prevalence of the infection amongst dairy cows and the high proportion of that nation's labour-force that worked in dairying (Kirschner and McGuire, 1957). The risk of human leptospirosis was considered of such significance that various programmes were introduced to limit the spread of the disease to humans, culminating in a vaccination programme of dairy cows (Oertley, 1999). In excess of 90% of New Zealand dairy cows are now vaccinated against *L. interrogans* serotypes *hardjo* and *pomona*, and, where human leptospirosis does occur amongst farm workers, up to 90% of cases are associated with herds that are unvaccinated (Marshall and Chereshsky, 1996). In other countries, the smaller proportion of the workforce that are employed in dairying means that the proportionate risk is lower, yet the low level of vaccination of herds against the disease in such countries means that workers are at significant risk of exposure to the disease.

Clinical syndromes

Infection can enter via skin abrasions or through the mucous membranes of the eye, mouth or nose. It can also be transmitted in semen after natural service or AI. After infection, a short latent period (5–14 days) is followed by a bacteraemia, which persists for about 4–5 days until the animal mounts a immune response against the leptospires. Thereafter, the organisms localise in tissues that are inaccessible to antibodies, notably the kidney tubules, cotyledons and fetus (Erskine and McNutt, 1956; Higgins et al., 1980). The consequence of colonisation of the kidney is a variable period of excretion of leptospires in the urine, providing a source of environmental contamination and of direct infection both of other cows and of humans. Urinary excretion normally occurs for several weeks (Thiermann, 1982) and it can be for the animal's lifetime (Ellis, 1984). Renal damage can be severe, which is more serious in non-maintenance hosts than in maintenance hosts. Likewise, other pathological changes, such as haemolysis, nephritis and hepatitis, can be serious in non-maintenance hosts. Hence, the clinical signs of leptospirosis depend upon the infecting organism, the route and dose of organisms and the immune status of the cow.

Leptospires can be present in puerperal discharges for up to 8 days (Ellis, 1984), and can persist in the pregnant and non-pregnant uterus for up to 142 and 97 days after infection, respectively.

The role of the bull in the transmission of the disease has been questioned since, according to Ellis et al. (1986), outbreaks of *hardjo* infection have frequently been associated with the introduction of a bull into a herd. The same authors, using material collected from seven stock bulls slaughtered at an abattoir, were able to demonstrate leptospires subgroup *sejroe* in the genital tracts of three bulls, particularly in the vesicular glands, as well as the urinary system. Venereal transmission is thus a possibility.

Clinical syndromes include:

- An acute febrile disease, characterised by temperatures of 40°C or more, together with haemoglobinuria, icterus and anorexia. Leptospiral mastitis may also be present. This syndrome is usually caused by strains such as *pomona, canicola, icterohaemorrhagiae* and *grippotyphosa*. Deaths may occur, especially in calves, and there may be abortions.
- A less acute type of disease where there is no pyrexia; this is most frequently associated with *hardjo*, which was first isolated from cattle in 1960 (Roth and Galton, 1960) and has now been shown to be endemic in the cattle population of the UK (Ellis et al., 1981) and many other countries (Ellis, 1984). The resultant reproductive effect of infection with *L. hardjo* is abortion, stillbirth or the birth of weakly calves. Abortion can occur at all stages of gestation from the fourth month to term; it is most common after 6 months. It can occur in the absence of any clinical signs of disease (Thiermann, 1982), but can also be accompanied by leptospiral mastitis or the 'flabby bag' milk-drop syndrome (Radostits et al., 1994). The level of abortion that results from *hardjo* infection varies between countries. In Australia, *hardjo* is not a major cause of abortion but, in the UK, it results in significant abortion losses, and is an important cause of reproductive failure (Ellis 1984). Examination of 472 aborted fetuses, 20 stillborn calves and 13 weakly calves revealed the presence of *hardjo* in 56, 70 and 85% of the cases, respectively.
- Leptospiral mastitis and milk-drop syndrome. In some herds, abortions have occurred after a 'leptospiral mastitis' or agalactia has been observed during the previous 3 months (Ellis and Michna, 1976). Infection causes a bacteraemia with or without a concurrent pyrexia. There is a precipitous fall in milk yield, especially in cows that are in early lactation. From all four quarters the milk that is obtained is thick and colostrum-like with clots, and is frequently blood-tinged. The udder is soft and flaccid. Agalactia lasts about 2–10 days, after which milk production usually returns close to normal although, in cows near the end of their lactation, milk production may not recover.

Dairy heifers usually become infected at 2–3 years of age, either from older cows or an infected bull; sometimes they become infected when they are introduced into the main herd after calving (Ellis, 1984–85). Most beef heifers become infected as calves because of contact with adult cattle, and for this reason leptospiral abortions are less common in these breeds. The number of animals infected in any one herd will vary from over 50% of cows during the 2-month period of an epidemic in a highly susceptible herd, to sporadic problems amongst first and second calf cows in a resistant herd (Hathaway and Little, 1983). In the UK, abortion occurs all year round but, after correcting for any seasonal variations in calving, it is most prevalent in September and October.

Diagnosis

There are no lesions that are specific for leptospirosis; thus diagnosis of leptospirosis as a cause of abortion is based almost entirely upon demonstrating specific antibodies in fetal sera or by demonstrating leptospires in fetal organs, particularly lungs, kidneys and adrenal glands, by culture or immunofluorescence.

The MAT is used extensively in the diagnosis of leptospirosis, using serum from animals that have aborted or are suspected of being infected. It is of limited value in individual animals, but it can be useful as a herd screening test for both serovars *pomona* and *hardjo*, particularly in herds where the infection is endemic without clinical signs of the disease, and where certain groups might be at risk, i.e. heifers, newly purchased animals and farm staff. The screening of all animals in a herd is expensive; however, it is possible to sample a minimum number in order to obtain reliable information on the disease status (Table 23.2).

Table 23.2 Number of cows to be sampled for the diagnosis of leptospirosis

Total herd size	*Number of cows to be sampled*
20	16
40	21
60	23
90	25
120	26
160	27
300	28
450+	29

The various categories within the herd, i.e. heifers, dry cows, cows in milk, should be sampled proportionately. When a partial or herd test reveals *hardjo* seropositive animals, then if the titres are below 1:400 and are confined to older animals in the herd which have mixed freely in the herd, then the infection can probably be considered to be historical rather than active.

Where more than 20% of the herd are seropositive or if titres are over 1:1600, then an active infection is present and further spread of the disease is possible (Anon, 1992).

Single samples from individual cows are of little value and it is impossible to separate infected from vaccinated animals. However, a high titre in a cow (> 1:1000) at the time of abortion is generally proof of infection; unfortunately, low titres < 1:100 can occur in infected animals (Ellis et al., 1982). Paired samples from individual animals are of no value, since there is usually an interval of 6–12 weeks between infection of the dam and fetal expulsion, by which time the dam's antibody titre is either falling, static or not detectable (Ellis, 1984–85).

Treatment and control

General control measures related to good hygiene, thus minimising the risk of infection with leptospires from other host species, should be implemented. These include the strict segregation of cattle from pigs, rodent control and the draining or fencing off of contaminated water sources. The role of sheep in the epidemiology of serovar *hardjo* is still not clear; however, since they have been shown to excrete the organism in their urine, it seems prudent not to graze them together.

There are two methods of specific treatment and control: the use of a vaccine or parenteral streptomycin/dihydrostreptomycin, or a combination of both. The antibiotic should be used at a dose rate of 25 mg/kg by intramuscular injection with no greater a volume than 20 ml at any one site. Milk should be withdrawn for 7 days and meat for 28 days. Repeated doses may be necessary. Streptomycin is effective in clearing *pomona* from the urine of infected cattle and treatment with antibiotic plus vaccination has been effective in arresting the progress of an abortion storm. Dihydrostreptomycin is less effective in treating *hardjo*, for which other antibiotics may be preferable (Prescott and Nicholson, 1988; Radostits et al., 1994).

In closed herds, vaccination of all members of the herd should be done annually. In open herds, the frequency should be increased to 6-monthly intervals; this is particularly important for heifers between 6 months and 3 years of age (Ellis, 1984). Vaccines are based upon bacterins, which produce relatively low antibody titres, but which confer protection for about 12 months. There is little or no cross-protection between the main serovars that affect cattle, so the use of bivalent vaccines (*hardjo* and *pomona*) or trivalent vaccines (*hardjo*, *pomona* and *copenhageni*) is common (Radostits et al., 1994). In situations where the losses due to leptospirosis are low, vaccination may not be cost-effective. However, the zoonotic risk of the disease is such that, even when losses are not great, public health authorities may (as in New Zealand) exert considerable pressure to ensure that susceptible cattle are vaccinated.

Salmonellosis

Salmonellosis-induced abortion has been reported from many countries. In Britain it has persisted as a continuing, although not a major, problem for some time (see Table 23.1). The main organism involved is *Salmonella dublin* which is responsible for 80% of salmonella abortions (Hinton, 1973). *S. dublin* is not evenly distributed throughout the world. It is common in the UK (notably Dorset, Somerset and south-west Wales) and Europe, South Africa and parts of South America. In the USA, it was confined to California and other regions west of the Rockies until recently, but has spread eastwards through the movement of infected cattle (Bulgin, 1983; Radostits et al., 1994). *S. typhimurium* is endemic in cattle throughout the world, but is not a major cause of reproductive failure. *S. newport* is probably the most common of the ‘exotic’ salmonellae to infect cattle, but a wide variety of other species are isolated during individual outbreaks.

Clinical signs

The disease is contracted following the grazing of pasture possibly contaminated with slurry from animal units, human sewage or infected river water.

The classical signs of salmonellosis in adult cattle include a marked pyrexia (> 40°C), severe diarrhoea and dysentery, which may be associated with abortion. More frequently, salmonella abortions occur in late pregnancy in the absence of any other clinical signs, although malaise, pyrexia and inappetance have also been recorded (Hinton, 1973).

In the UK, salmonella abortions are more prevalent in the period June to December. Hinton (1973) recorded 81% of salmonella abortions occurring during this time of the year. In most outbreaks only one or two animals are affected on each farm, although occasionally five or six cases may be reported at one particular time. However, explosive outbreaks can also occur, in which a large number of cattle both develop enteric signs and abort. In one such outbreak, that was attended by the author, nearly 20% of a herd of 450 cows aborted.

RFM is a common sequel, although there is no adverse effect upon fertility (Hall and Jones, 1977).

Pathogenesis

Following experimental infection of pregnant heifers with *S. dublin*, the organism rapidly spreads to the liver, spleen, lungs and adjacent lymph nodes of the dam; this is associated with pyrexia. Six to eight days later it spreads to the placentomes, causing a second bout of pyrexia. The placentome is damaged, probably by endotoxin, causing necrosis, placental failure, fetal death and abortion (Hall and Jones, 1977).

Diagnosis

A definite diagnosis depends upon the isolation of the organism from fetal tissues and membranes, uterine discharges or vaginal mucus. Serological tests can be used, especially the SAT, although agglutinins fall to low titres fairly soon after the event (Hinton, 1973).

Control

Cows that have aborted only excrete the organism for a very short period of time, unlike the continuous or intermittent excretors that occur following enteric infection. Potential excretors need to be isolated until vaginal discharge ceases; fetuses and fetal membranes together with contaminated bedding should be disposed of safely. Adequate cleansing and disinfection of premises should be performed.

Vaccination has been used to control salmonellosis. *S. dublin* can be controlled by vaccination with the Strain 51 live vaccine (which also gives significant protection against *S. typhimurium*), when its use is combined with a closed-herd policy and effective hygiene measures. Killed vaccines and bacterins have also been used, largely against *S. typhimurium*, but their effectiveness has been a matter of debate (see Radostits et al., 1994).

Listeriosis

Listeria monocytogenes is primarily a pathogen of the central nervous system in sheep and cattle, in which it causes encephalitis. It is consistently, if not frequently, isolated from bovine abortuses, and is also a cause of abortion in sheep and goats (Chapter 25).

Clinical signs

Usually abortions are sporadic, occurring towards the end of gestation. However, there are rare reports of serious outbreaks, or abortion storms, in some herds. In some individuals, there may be pyrexia before, at the time of or after abortions have occurred. The aborted fetus frequently has characteristic multiple yellow or grey necrotic foci in the liver and cotyledons, similar to those described for sheep.

Diagnosis

This is dependent upon the identification of the organism in the abomasum and liver of the fetus, and in the placenta and vaginal discharges by a direct smear or by immunofluorescence. Culture of the organism is not easy, although a series of subcultures following refrigeration has proved to be successful. Serological tests are not used in its diagnosis.

Transmission and pathogenesis

L. monocytogenes is ubiquitous in the environment, being present in the soil, sewage effluent, bedding and foodstuffs; it persists as it is particularly resistant to the effects of drying, sunlight and extreme temperature. There is good evidence that there is an association between listeriosis and the feeding of poor-quality silage of higher-than-normal pH. Cross-infection between sheep and cattle is possible.

The organism gains entry by ingestion or by penetration of mucous membranes of the respiratory system or conjunctiva, as well as the central nervous system. *L. monocytogenes* has a predilection for the placenta, causing a placentitis, and affects the fetus to cause abortion. A latent infection can occur with abortion occurring after a time lag and triggered by stress.

Treatment and control

The possibility of preventing further abortions occurring in a herd might be considered by using oxytetracycline or penicillin; however, this is rarely practicable. If silage is being fed this must be considered to be a potential source of infection and, if possible, withheld from pregnant cows. There is evidence that some individuals become symptomless carriers, excreting the organism in faeces and milk.

Haemophilus somnus

Haemophilus somnus is a fairly common inhabitant of the genital tracts of male and female cattle. The strains of *H. somnus* that infect cattle are different from those which cause disease in sheep (Ward et al., 1995). The organism can be routinely isolated from the mucosal surfaces of the urogenital tract of normal healthy cattle (Eaglesome and Garcia, 1992), in the absence of any macroscopic lesions. In the literature the organism has been isolated from 28% of normal cows (Slee and Stephens, 1985) and 90% of normal bulls (Janzen et al., 1981).

H. somnus infection in cattle causes septicaemia, polyarthritis, pneumonia/pleurisy and thrombotic meningoencephalitis (Radostits et al., 1994). It has been reported to affect reproduction adversely in a number of different ways. It causes abortion, endometritis, vaginitis and cervicitis. It may also be one of the organisms responsible for granular vulvovaginitis (Roberts, 1986). Strains of *H. somnus* that are responsible for reproductive diseases are often considered to be separate from those which cause systemic problems. Even when reproductive and non-reproductive diseases occur concurrently, the strains of *H. somnus* are likely to be different (Szalay et al., 1994), although Miller et al. (1983) found that experimental infection of a strain of *H. somnus* that caused abortion also caused systemic signs in some cattle.

H. somnus is a significant, but relatively uncommon, cause of abortion in cattle. It causes abortion after experimental infection of pregnant cows (Stuart et al., 1990) and has been isolated from a number of field cases. Thornton (1992) found it in 0.4% of diagnosed abortions in New Zealand, and Kiupel and Prehn (1986) reported the organism to have caused 1.7% to 3% of abortions in Germany. Kaneene et al. (1987) and Ruegg et al. (1988) further associated *H. somnus* infection with early embryonic death in cows, while Stephens et al. (1986) found *H. somnus* to be present in a disproportionately high number of cows with metritis or cervicitis. Patterson et al. (1984) reported similar findings.

H. somnus is also regularly isolated in the semen of bulls. Most commonly, the bull is asymptomatic, but the organism can cause testicular degeneration (Barber et al., 1994) or even frank orchitis (Corbel et al., 1986). *H. somnus* also causes bovine epididymitis, producing a large, multiloculated abscess, usually within a single epididymis (Jubb et al., 1993).

Diagnosis can be made following culture of the organism, which can be difficult because of overgrowth by contaminants. Recognition of the organism may not always be straightforward, as it is pleiomorphic. Serological tests are currently unreliable. In aborted fetuses, lesions are scanty and non-specific. Lesions of the placenta occur mainly within the cotyledons, consisting of an acute, non-suppurative placentitis (Jubb et al., 1993).

There are few reports on the treatment of infected cows. Penicillin and streptomycin have been reported to have been used successfully in treating cows where *H. somnus* was frequently isolated from cervico-vaginal mucus, and where fertility was depressed (Eaglesome and Garcia, 1992). Since the organism colonises the genital tract of the bull and can be isolated from semen, this may well be an important source of infection of cows and heifers. Good hygiene and the use of combinations of antibiotics should control infection following artificial insemination.

Other bacterial causes of infertility

Bacillus abortion

It is only in the last decade that abortion due to *Bacillus spp.*, in particular *B. licheniformis*, has been demonstrated. In some parts of the UK, notably northern Scotland and Cumbria, *B. licheniformis* is the most commonly diagnosed cause of abortion in cattle (Counter, 1984–5).

Clinical signs. Sporadic cases occur in late gestation although there are reports of small outbreaks in two consecutive years (Counter, 1984–5). Sometimes live calves can be born with some evidence of placental lesions.

The placentitis due to *B. licheniformis* is similar to that following mycotic infection. The allantochorion is dry, leathery and yellow or yellowish-brown in colour. There is often oedema of the allantochorion, especially around the cotyledons, which appears almost as if there are vesicles present. The cotyledons are haemorrhagic and necrotic.

The fetus may be infected and, if so, there will usually be evidence of a fibrinous pleurisy, pericarditis and peritonitis. There are no systemic signs of disease in the cow (Counter, 1984–5).

Diagnosis. This depends upon the appearance of the placenta and the culture of the *Bacillus* from the fetus (especially the abomasum), placenta and vaginal swab.

Transmission and pathogenesis. *B. licheniformis* is ubiquitous; however, a common source of infection is silage, especially when water, other foodstuffs and bedding are contaminated with silage effluent. Wet, spoilt hay can also be a source.

The method of infection is not known, but it is probably haematogenous following entry via the gastrointestinal tract.

Control. Infected silage or hay should not be fed.

Arcanobacterium pyogenes

Table 23.1 shows that *Arcanobacterium (syn. Actinomyces, Corynebacterium) pyogenes* is frequently isolated from bovine fetopathies, although it would appear to be less prevalent than in the 1970s and early 1980s. The significance of the presence of this organism is difficult to assess since *A. (C.) pyogenes* is a frequent secondary invader following the effect of the primary pathogen. Nevertheless, its presence in a fetopathy is usually significant.

A. pyogenes is believed to reach the uterus by a haematogenous route to produce a suppurative placentitis. Organisms found in the fetal bronchioles probably originate from aspiration of contaminated amniotic fluid. A fetal septicaemia can occur by transplacental passage (Smith, 1990).

Abortion may occur at any stage of gestation, although the organism is most frequently isolated from abortions that occur in the last trimester.

Diagnosis is usually made by the isolation of the organism from the placenta, abomasal contents or fetal tissues. There are no serological tests. Since the abortions are sporadic there are no suitable methods of treatment or control.

Escherichia coli

Sporadic abortions due to *E. coli* have been reported (Rowe and Smithies, 1978; Moorthy, 1985). It is suggested that, following stress, the organism reaches the fetus and placenta via haematogenous spread or ascending the genital tract.

MYCOPLASMA, UREAPLASMA AND ACHOLEPLASMA INFECTIONS

Mycoplasma

There has been much controversy concerning the relationship between mycoplasmas and genital disease in cattle ever since *Mycoplasma bovigenitalium* was demonstrated in the genital tract of infertile cows and the semen of bulls (Edward et al., 1947; Blom and Erno, 1967). Evidence for pathogenicity has mainly been indirect, largely based upon their isolation from diseased rather than healthy tissue, and from limited experimental studies (Eaglesome and Garcia, 1992). The two species which appear to be of greatest importance in cattle are *M. bovigenitalium* and *M. bovis*.

M. bovigenitalium is found in the vaginal mucus of normal and Repeat Breeder cows, which has led to speculation concerning its role as a pathogen (Langford, 1975; Nakamura et al., 1977). It has, however, been found in cows of low fertility in which no other cause of infertility could be found (Kirkbride, 1987). The organism may also cause granular vulvovaginitis (Afshar and Stuart, 1966), although the evidence for its role in natural occurrences of the disease is not unequivocal. Spread of the organism from infected bulls and resultant infertility have also been demonstrated. However, the results have not been unequivocal, and hence it has been suggested that a considerable degree of strain-to-strain variability in pathogenicity exists (Saed and Al-Aubaidi, 1983).

M. bovigenitalium also inhabits many parts of the reproductive tract of the bull. It has been suggested that the prepuce and urethral orifice are the primary locations of the organism (Fish et al., 1985), but it has also been recovered from virtually every part of the male tract. It has been isolated from 15 to 32% of semen samples (see Kirkbride, 1987). It has been implicated as a cause of seminal vesiculitis, as it both is isolated frequently from clinical cases and can infect the vesicular glands after experimental inoculation. When it infects the testes or epididymides, *M. bovigenitalium* may cause detrimental changes to semen quality, especially after cryopreservation.

M. bovis causes mastitis in adult cattle and polyarthritis in calves. It is a successful pathogen of the uterus, causing extensive lesions of the uterus, uterine tubes and even peritonitis. It persists in the uterus and vagina for long periods (1 and 8 months, respectively) after infection (see Kirkbride, 1987). *M. bovis* has been shown to cause abortion in both natural and experimental infections (Stalheim et al., 1974). Since it is seldom found in the reproductive tract of normal cows, isolation of the organism from the placenta or aborted fetus can be considered significant (Kirkbride, 1990b). *M. bovis* is found in bovine semen less often than *M. bovigenitalium* and its pathogenicity for the bull has not been established.

Other *Mycoplasma* species (e.g. *M. bovirhinis, M. arginini, M. alkakescence, M. canadense* and *M. gallisepticum*) have been isolated occasionally from abortuses, but for these, as well as for *M. bovigenitalium*, the evidence for being the initiating cause of abortion is not clear-cut, since mycoplasmas have frequently been isolated from spontaneously aborted fetuses. For example, Langford (1975) cultured them from 8.7% of aborted fetuses but none from normal fetuses. In a study of 245 bovine abortions in Northern Ireland, Ball et al. (1978) recovered mycoplasmas from 23.7% of aborted placental material and none from normal controls, and from 4.4% of aborted fetuses and 1.3% from non-aborted controls. This latter study emphasises the difficulty of interpreting isolations of mycoplasmas from aborted material; *A. laidlawii* was frequently isolated, but it is ubiquitous and generally considered saprophytic. Hence, post-abortion contamination may account for many such isolates. *M. bovis* can, however, be regarded as a causal organism of bovine abortion.

Ureaplasma diversum

Ureaplasma diversum is a common inhabitant of the genital tract of the cow. It persists only briefly in the uterus and uterine tubes, but is most commonly found in the vagina and vestibule. Differences in virulence of strains probably account for the presence of the organisms in normal reproductive tracts.

One of the conditions attributed to *U. diversum* infection is granular vulvovaginitis. Acute infection produces granules around the clitoral region and on the lateral walls of the vagina, which are accompanied by hyperaemia of the vulva and a profuse, mucopurulent vaginal discharge. Large, purulent lesions may also be present, which resemble those of IPV (see below). These may give way to less obviously inflamed, chronic lesions.

U. diversum can also produce endometritis and salpingitis (Kirkbride, 1987). These lesions have been associated with high levels of embryonic death and returns to oestrus, which are accompanied by a mucopurulent vaginal discharge. Abortions may also occur, but *Ureaplasma* may often be isolated as an incidental finding from calves that have been aborted for other reasons. Hence, unless there are histological lesions in the abortus that are characteristic of ureaplasmosis (Murray, 1992) or the presence of a virulent strain is demonstrated, *Ureaplasma* isolations should be interpreted with a degree of caution.

U. diversum can infect the penis and prepuce of the bull and has occasionally been isolated from all parts of the male tract. It is generally regarded as non-pathogenic in the male, although some have attributed low-grade lymphoid granulomas on the penile integument to the presence of the organism.

The main means of transmission of the infection is by the venereal route. Infected semen used in AI seems of particular importance, since its deposition into the uterus allows the development of chronic endometritis, rather than of acute vulvovaginitis. However, infection of virgin females and males has been described and it has been suggested that direct transmission between females, or even transmission by dogs sniffing the vulvas of cows (Doig et al., 1979), may occur. Whether it is transmitted between bulls is uncertain.

Acholeplasma

Three species of *Acholeplasma* have been isolated from cattle: *A. modicum, A. laidlawii* and *A. axanthum* (Kirkbride, 1987). Of these, *A. laidlawii* has been isolated most often, largely from the bull. It is possible that *Acholeplasma* infection of cows may cause pathological changes in the genital tract, but the case is far from proven. It is often isolated from aborted calves, but as described above, may not be the causal organism. It probably causes no pathological lesions of the bull.

Diagnosis

Most bovine mycoplasmas are easily recovered in conventional mycoplasma media, although some may require special supplements or conditions for optimum growth (Eaglesome and Garcia, 1992).

The development of ELISA and other diagnostic tests is likely in the near future.

Treatment and control

Natural service, if used, should be suspended and semen should be collected and cultured for the presence of mycoplasmas. Instead, animals should be inseminated with semen that is known to be free of contaminant organisms. Infected bulls should be rested for 3 months and treated systemically for 5 days with tetracyclines, together with sheath irrigation.

A number of antibiotics have been incorporated in semen for the control of these organisms. A combination of lincomycin, spectinomycin, tylosin and gentamycin added to raw semen, and non-glycerolated whole milk or egg yolk-based extenders has been shown to control *M. bovis, M. bovigenitalium* and *Ureaplasma spp.* (Shin et al., 1988). If artificial insemination is used, the standard Cassou pipette should be protected by a disposable polythene sheath to prevent vulval or vaginal contamination before it is introduced through the cervix. The uterus can be infused with a solution containing 1 g of tetracycline or spectinomycin 1 day after insemination, a treatment that has been shown to improve pregnancy rates.

Stress, associated with intensive management systems, is said to predispose to the disease; thus transfer to pasture of affected animals should be considered. This may reduce spread by direct contagion.

PROTOZOAL AGENTS

Trichomoniasis

The recognition of *Trichomonas (Tritrichomonas) fetus* infection as a cause of infertility was an important advance in our understanding of the role of specific venereal pathogens in cattle (Riedmuller, 1928; Abelein, 1938; Stableforth et al., 1937). Enzootic *T. fetus* infections were brought under control in the dairy herds of many countries by the widespread introduction of AI during the 1950s and 1960s. In passing, it should be noted that the impetus for the development of AI in Europe and North America during the 1940s was as much the need to control venereal pathogens as for the development of selective breeding programmes. However, world-wide, *T. fetus* remains a major cause of reproductive failure. In California, recent surveys have shown that between 5 and 38.5% of beef bulls and 8.7% of dairy cows are infected (Skirrow and BonDurant, 1988; BonDurant et al., 1990). Similar high levels of infection have been reported elsewhere in the USA, Australia (Dennett et al., 1974), South Africa (Eaglesome and Garcia, 1992), Canada (Copeland et al., 1994) and, indeed, most of the major cattle-producing regions of the world. Geographical isolation has permitted the virtual eradication of trichomoniasis in the UK and New Zealand, yet even in these countries, occasional recrudescences of the disease can occur from time to time (Taylor et al., 1994; Oosthuizen, 1999). Hence, whenever natural service is used, trichomoniasis must not be overlooked as a cause of infertility.

Clinical signs

Trichomoniasis is a classic venereal disease that is transmitted to cows from asymptomatic carrier bulls during coitus. The causal organism is a flagellate protozoan (Figure 23.3).

The bull. Bulls become infected by serving an infected cow. The infection rate from cows to bulls is high; Roberts (1986) reported that about 50% of bulls become infected from one service of an infected cow. Bulls can remain infected for life, remaining asymptomatic throughout. Interestingly, however, some bulls have also proved highly resistant to infection; about 20% of bulls failed to become infected after numerous matings with infected cows. It is also evident that younger bulls are less liable to become persistent carriers than are older bulls. The organism lives within the crypts and folds of the penile integument and preputial mucosa. The lack of development of these structures in younger bulls is probably the reason that the organism is less able to establish itself in them (Table 23.3). Control of trichomoniasis through AI can only be achieved if the stud bulls are free of the disease, since trichomoniasis can also be spread from bull to bull via contaminated artificial vaginas and *T. fetus* survives cryopreservation quite well.

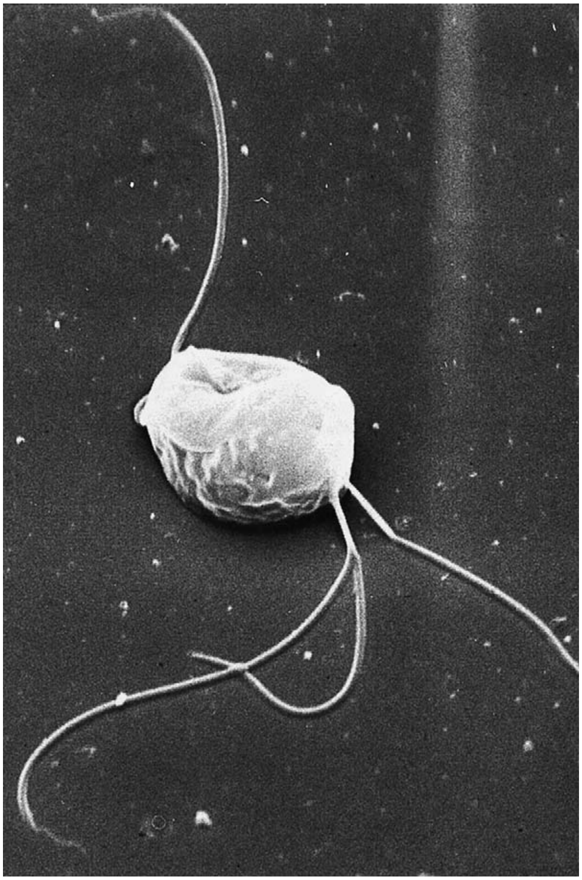


Fig. 23.3 Scanning electron micrograph of *Trichomonas fetus* (×16 500).

Table 23.3 Relationship between age and *Trichomonas fetus* infection in Californian beef bulls (from BonDurant et al., 1990)

Age of bulls (years)	*Number of bulls*	*Number infected*	*Percentage infected*
<2	38	0	0
2	221	1	<0.5
3	137	7	5.1
4	156	5	3.2
5	86	8	9.3
6	55	7	12.7
>6	31	2	6.5
Summary			
≤2	259	1	<0.4
>2	465	29	6.2

The cow. Although the number of trichomonads needed to establish an infection in the cow is large (probably several thousand: Clarke et al., 1974), transmission rates are high. Under conditions of heavy work, the number of trichomonads present in the preputial area of the bull is reduced, so transmission may be less than 100%, but under normal conditions, it is common for virtually every cow that is mated by an infected bull to become infected. In addition to natural service, cows can be infected via insemination with contaminated semen. Rarely, infection can occur following the use of contaminated instruments such as vaginal specula.

In the cow, *T. fetus* colonises the uterus, cervix and vagina, but it survives poorly on the vulva. Within the uterus, the organism produces a catarrhal endometritis and vaginitis, with oedema of vulva, perivaginal tissue and uterine wall. It does not generally invade through the epithelial surface. Affected animals show an intermittent vulval discharge. Manipulation of the uterus often provokes a discharge from the vulva in which motile trichomonads can generally be demonstrated. The disease does not prevent fertilisation, but causes embryonic death at an early stage of gestation. Typically, embryonic death occurs after the maternal recognition of pregnancy (day 16), causing an irregularly extended return to oestrus, although some animals exhibit normal, or even short, returns to oestrus. Many pregnancies fail at between 30 and 50 days of gestation. BonDurrant (1997) suggested that this corresponds with the time of establishment of placentomes, which the parasites disturb by disrupting the physical and endocrine contact between fetus and mother. Embryonic death is not infrequently (up to 10% of cases) accompanied by the development of pyometra, in which the uterus is filled with enormous quantities of trichomonad-filled, thinnish pus. Vaginal discharge of this pus is common.

Many cows experience a series of embryonic deaths before they become pregnant and carry the calf to term. Epizootics of the disease in the 1940s were characterised by an average of 5 returns to oestrus before conception occurred (Bartlett, 1947). The return to fertility is dependent upon the development of immunity to the parasite. However, immunity is slow to develop, for even if the cow is only served once by an infected bull, subsequent services will not result in successful pregnancies until the trichomonads have been eliminated from the uterus. Antibody-mediated (IgG and IgA) immunity develops over several months (Skirrow and BonDurant, 1990), although antigens to some components of the protozoan are present much sooner (after 2–3 weeks: BonDurant et al., 1996). However, infected cows will conceive to both infected and non-infected services and eventually carry to term once immunity has developed. Nevertheless, although cows are free of parasites after a normal gestation (Bartlett, 1947), immunity has been lost by the end of gestation, so that cows again become susceptible to infection from an infected carrier bull.

Some abortions occur between the second and fourth months of gestation, but very few occur after the fourth month. In later-term abortions, trichomonads can be found in the chorion, fetal lung and fetal gut. The fetus is smaller than that appropriate to the period of gestation, due to growth retardation. In such abortion cases, the fetus, which is grey in colour, is generally expelled complete in its membranes. There are no signs of putrefaction and *T. fetus* can readily be demonstrated in fetal fluids. Parasites quickly disappear from the vaginal discharges after abortion (usually within 7 days).

Hence cows and heifers, which have been exposed to infected service, fall into the following clinical groups:

- become pregnant and carry to term without clinical signs of infection developing
- return to multiple services, but show no obvious signs of infection; oestrous cycles may be regular or irregular
- fail to become pregnant and develop an oedematous condition of the endometrium with a mucoflocculent discharge
- become pregnant, but abort at 2–4 months of gestation.
- develop pyometra and become acyclic.

Diagnosis

Diagnostic samples. Although clinical signs and history may be strongly supportive of a diagnosis of trichomoniasis, diagnosis in the female cow is best achieved by demonstrating the presence of trichomonads in uterine pus, vaginal discharges, cervical mucus or abortus material. The best source of material is the fetal membranes or the organs of an aborted fetus (especially the abomasum). Elimination rates of infection are highly variable after an infected mating, so failure to demonstrate the presence of the organism does not necessarily imply its earlier absence. Also, the organism degenerates very rapidly after death, so unless samples are handled properly, the organisms may be absent by the time the samples are examined. Material contaminated with faeces should be discarded, because non-pathogenic trichomonad-like organisms (Taylor et al., 1994) may be present.

In the bull, diagnosis is made by the collection of preputial scrapes or preputial washes. Vigorous scraping of the preputial mucosa, to obtain as much smegma as possible (Eaglesome and Garcia, 1992), is the traditional method of collection. Stoessel and Haberkorn (1978) suggested that 'rough' scraping of the prepuce was needed to diagnose the presence of trichomonads, but Oosthuizen (1999) reported a very high reliability of preputial washings (using about 50 ml of phosphate-buffered saline or lactate Ringer's solution) collected from heavily sedated bulls. The bull should be allowed a period of 5–10 days of sexual rest before sampling so that the number of trichomonads can increase. Alternatively, the presence of the infection in a bull can be demonstrated using a test mating with a virgin heifer (Ball et al., 1983). Cervical mucus should be collected 10–20 days later to demonstrate the presence of *T. fetus* by direct examination or culture.

Demonstration of the organism. Whatever the source of the material which might contain trichomonads, it should be examined as soon as possible after collection. Preputial washings are centrifuged in order to concentrate the organisms.

Various media can be used for culture, including:

- trypticase-yeast extract-maltose (TYM)
- Diamond's medium (TYM + 1% agar); for this method, an incubation period of up to 9 days is required (BonDurant, 1990)
- InPouch system (Biomed Diagnostics Ltd).

Organisms are visualised after culture.

Various methods have been developed in an attempt to increase the efficiency of diagnosis of trichomoniasis, including immunohistochemistry (Rhyan et al., 1995) and polymerase chain reaction (Ho et al., 1994). Agglutinating antibodies are developed locally in the vagina and uterus in response to infection; their identification can be used as a herd test in the same way as described previously for *C. fetus*. For diagnosis in the cow, work is underway on developing a serological assay to a shed antigen of *T. fetus* (Vasquez-Flores et al., 1995).

Between 10 and 20% of infected bulls will not be detected by these means (Schonmann et al., 1994), so a second or third examination is needed to ensure that a bull really is negative.

Treatment and control

Control can be attempted by:

- eliminating bulls and replacing natural service by AI
- 'active' management of groups of cows and use of bulls
- treatment and/or vaccination of cows and bulls.

Artificial insemination. Control through artificial insemination (AI) is based upon the assumption that recovery in the female is spontaneous, and that infection of healthy animals cannot occur if natural service is replaced by AI with semen from non-infected bulls. Of all of the available methods, the elimination of bulls from the herd and AI with uncontaminated semen is by far the most effective and efficient means of control. The method does require that cows should be bred exclusively by AI throughout at least one and, preferably, two seasons. Pregnancy rates to AI are likely to be poor during the initial period of its introduction, since many of the cows may still be infected.

It should be noted that *elimination of bulls* does mean exactly that, all potentially infected bulls (i.e. all bulls) being slaughtered or, if exceptionally valuable, vigorously treated and repeatedly sampled to ensure that they no longer harbour the parasite. Simply putting bulls away into a remote paddock for a couple of years does no good at all. They will still be infected at the end of the period.

Group management. Many different ideas have been suggested as ways of managing trichomonad-infected herds without resorting to the total use of AI.

In principle, when it is established that *T. fetus* infection exists in a herd, the females should be grouped as follows:

- those which are definitively known not to have been exposed to infection. This group will comprise maiden heifers and any recently calved cows that have not been served since the introduction of an infected bull
- all other cows whose disease-free status is not definitively established.

All bulls on the farm should be regarded as being infected, unless individuals' disease-free status is beyond debate.

The 'clean' group (Group 1) is bred to known uninfected bulls. The other group can be bred to any bull until they conceive, after which those bulls (by now infected) are eliminated. After a full-term pregnancy the Group 2 cows should be free of infection. Absolute separation (distance and fences) is a prerequisite for such a scheme to work, although the apparent simplicity of the programme belies the huge practical difficulties of its implementation.

An alternative strategy relies upon the limitation of effects of the disease by only using young bulls for breeding. It is argued that, since 2-year-old bulls are relatively resistant to infection, their use in breeding will result in less spread of the disease than occurs with older bulls. This may be true, but herd fertility remains below normal. Nevertheless, whilst relying upon the resistance of young bulls is unlikely to result in elimination of infection, their use may well help to reduce the level of infection that is present.

Treatment. As a general principle, carrier bulls should be culled since, unlike the infection in the female, it persists indefinitely. However, in a valuable animal whose blood line it is desirous to maintain, treatment may be considered.

Treatment of the bull can be attempted by the use of topical substances infused into the prepuce or applied to the penis. The original method used by Abelein (1938) and Swangard (1939) involved the withdrawal of the penis under epidural anaesthesia, bilateral internal pudendal nerve block or with the aid of a tranquillising drug, followed by thorough manual application to the penis and prepuce of an ointment which contained trypaflavine and a protozoacidal agent. Iodine-based compounds, acriflavine and imidazoles have all been used. Success rates are variable, elimination of infection is not reliable and the application of such substances is anything but straightforward.

Systemic treatment was first attempted by Bartlett (1948), who used sodium iodide at a dosage of 5 g/45 kg body weight in 500 ml water, by intravenous injection on five occasions at 2-day intervals. More recently, treatment with imidazoles has been reported as both feasible and effective. Dimetridazole can be given orally (50 mg/kg per day for 5 days: McLaughlin, 1968), but has unpleasant side-effects of rumen stasis, inappetance and digestive disorders. When given intravenously, different side-effects occur, including respiratory distress, ataxia, short-term recumbency and weakness. In either route of administration it is, however, effective. Metronidazole (i.v., various dosage regimens) is also fairly effective. Ipronidazole can be used, but has to be preceded by the use of broad-spectrum antibiotics to kill non-specific bacteria in the prepuce that break down the imidazole. Resistance to the entire group of imidazoles is easily induced by the use of subtherapeutic doses. Unfortunately, none of these therapeutic substances is licensed for use in cattle in the UK or USA. A new antibiotic, trichostatin, has been found to be effective against *T. fetus* in vitro and in vivo (Otoguro et al., 1988).

Even when treatment of individual animals is effective, it has no impact upon the presence of disease in the herd unless other steps are taken to ensure its eradication.

Treatment of cows is largely unnecessary, as the disease is self-limiting and, indeed, there is no evidence that the treatment of cows or heifers hastens the time to self-cure. Cows with pyometra may be induced into oestrus with prostaglandin $F_{2\alpha}$. Intrauterine administration of Lugol's iodine, antiseptics or acriflavine at regular intervals has been advocated for many years as a means of treating trichomoniasis, but is probably useless (Roberts, 1986). Maybe treatment with imidazoles is occasionally indicated.

Vaccination. Many attempts have been made to develop a vaccine against *T. fetus*. Initial work used killed trichomonads in a mineral oil adjuvant (Clarke et al., 1983), which helped eliminate infection from bulls. However, most development has been based upon fragmented cells or isolated membrane fractions, which stimulate a significant antibody response (Schnackel et al., 1990). These too have helped prevent and/or eliminate infection in cows and bulls (Kvasnicka et al., 1989; Hall et al., 1993; Hudson et al., 1993a, b).

In the USA, a vaccine against *T. fetus* has been available, which reduces the incidence and duration of infection of cows after service by an infected bull (BonDurant, 1997). Efficacy of trichomonas vaccines is estimated to be, at best, 60% (Cortese, 1999). Hence, as the vaccine does not completely protect, it can only be used as an adjunct to other control or prevention methods. Curiously, although the initial studies in Australia suggested that vaccination conferred a high degree of protection upon bulls, more recent American studies have found vaccination has little effect upon either the incidence or the duration of infection in the male. This is in contrast to the response to vaccination against *Campylobacter*, in which protection of the male can be achieved by the use of double doses of vaccine given on two occasions (Cortese, 1999).

Neospora caninum

Neospora caninum was first discovered as a protozoan parasite which causes encephalomyelitis of dogs (Dubey, 1999). Neosporosis is now recognised as a significant cause of bovine abortion in most of the major cattle-producing regions of the world. It has been recorded in the UK, the USA (Dubey and Lindsay, 1996), Canada (Alves et al., 1996), Argentina (Campero et al., 1998), South Africa (Jardine and Last, 1995), Zimbabwe (Wells, 1996), Australia (Obendorf et al., 1995) and many other countries. It is now probably the most important cause of abortion in New Zealand (Thornton et al., 1991). It has been estimated that neosporosis costs the Californian dairy industry US$35 million per year (Berry et al., 2000). Pfeiffer et al. (1998) estimated that the disease cost the New Zealand cattle industries NZ$24 million per year. One of the earliest reports of neosporosis in the UK was that of Otter et al. (1993), who found histological and immunocytochemical evidence of *N. caninum* in the interventricular septal myocardium, brain and placental cotyledon of aborted bovine fetuses. Tenter and Shirley (1999) suggested that *N. caninum* may be responsible for 6000 abortions per annum in the UK.

Infected dams can produce calves which are apparently normal, but are congenitally infected (Thornton et al., 1991), or which are born alive with neurologic limb defects (Barr et al., 1993). Abortions due to neosporosis can be sporadic, but abortion storms, in which up to 30% of calves are lost, are also common. Cows can abort in successive pregnancies (Anderson et al., 1995).

The dog is both the definitive host and an intermediate host for the parasite (McAllister et al., 1998), although occysts have only been found in the faeces of experimentally infected dogs. Tachyzoites are found in neural and vascular cells, together with a number of other tissues of the body. Tachyzoites are also found in bovine placental and neural tissue. Bradyzoites are found in bovine neural (brain, spinal cord and retinal) tissue (Anthony and Williamson, 2000). In some outbreaks, vertical transmission has proved to be the main route by which cattle became infected (Heitala and Thurmond, 1997). However, although the means by which horizontal transmission could take place are poorly understood, epidemiological evidence from abortion storms suggests that a point source of infection was implicated (Anthony and Williamson, 2000). Routes of horizontal infection could include colostrum, fetal membranes and fluids from infected cows or oocyst-contaminated feed. None of these routes of infection has been convincingly proved. However, there is a clear association between the presence of *Neospora* infection in farm dogs and the risk of abortion in dairy cows (Bartels et al., 1999; Wouda et al., 1999). On the other hand, post natal seroconversion is uncommon (Heitala and Thurmond, 1999) and abortion storms could originate from previously infected animals which become synchronously immunosuppressed by, for example, BVD infection (Anthony and Williamson, 2000).

In consequence of this poor understanding of the mode of transmission of *N. caninum*, it has been difficult to devise effective control strategies for the disease. Culling is probably not a viable option under most circumstances, due to the high prevalence of the disease in some herds. Prevention of access by dogs to fetal membranes and abortuses may help reduce horizontal spread, as may prevention of soiling of feed stores by dog faeces. However, there is little or no epidemiological evidence to show whether such methods are effective. Since cows that are apparently immune to the disease (i.e. are seropositive) can still undergo repeat abortions, the prospect for control of neosporosis is not promising, although putative vaccines are under development (Choromanski et al., 2000).

There are several tests which can be used to detect the disease in dairy herds. Immunofluorescent antibody (IFAT) and ELISA tests can detect serological responses against *N. caninum*. However, because of the widespread prevalence of seropositive cows, a positive result does not necessarily indicate infection at the time of testing, only that the cow had been exposed to the disease at some previous time. Abortion diagnosis should therefore be made by a combination of serology, with immunohistochemistry and histopathology of aborted fetuses (Berry et al., 2000).

VIRAL AGENTS

Bovine viral diarrhoea (BVD)

BVD was initially recognised as a cause of diarrhoea during the 1940s. Although it was originally considered to be a simple virus-induced diarrhoea, more recent understanding of the infection has shown that it also causes infertility. Moreover, the importance of fetal infection in the epidemiology of the disease means that it should, perhaps, be considered primarily as a disease of reproduction. BVD was first recorded as a cause of abortion in cattle in the UK in 1980 (see Table 23.1).

The BVD virus is a *Pestivirus*, which is related to the viruses of Border disease of sheep and classical swine fever. There are two main biotypes: a cytopathic and a non-cytopathic strain.

Transmission and pathogenesis

Infection with the non-cytopathic strain in utero between about days 30 and 125 of gestation leads to the birth of a calf that is persistently infected with the virus. Such calves are immunotolerant and, if they are subsequently infected with the cytopathic strain of BVD, they may develop mucosal disease. Persistently infected animals shed the virus throughout life. The incidence of persistently infected calves (carriers) is about 1 per 100–1000 calves born, but such animals are a major source of infection and are important in maintaining the BVD virus in nature (Bolin, 1990a). Persistently infected cows can transmit the disease vertically through transplacental infection to their calves, although the majority of persistently infected calves are born to normal cows that were susceptible to infection during the first 4 months of gestation. Animals that are persistently affected, or have acute infections, shed large amounts of virus in occulonasal discharges, saliva, urine and faeces.

Infection of cows at other stages of pregnancy causes early embyronic death and abortion, with aborted fetuses exhibiting abnormalities of the central nervous and ocular systems. Infection in the last third of pregnancy does not cause immunotolerance, but results in the birth of a calf that is immune to the disease.

Infection of susceptible adult animals that are not immunotolerant produces a transient disease, which is characterised by a period of pyrexia plus a leucopenial viraemia that persists for up to 15 days. In susceptible herds, there will be diarrhoea, with a high morbidity but low mortality rate, occulonasal discharge and mouth ulcers. There is usually a drop in milk yield in dairy cows. The virus has a profound immunosuppressive effect, which can increase the susceptibility of the host to other diseases. Most adult animals, however, seroconvert without showing any overt signs of illness (Barr and Anderson, 1993; Radostits et al., 1994). It is the mild clinical form of the disease that is likely to have the greatest effect upon reproductive function, since the mild pyrexia and modest mucosal lesions generally go undetected.

Bulls have been shown to excrete the virus in their semen following spontaneous, persistent and chronic infection (Barlow et al., 1986; Reyell et al., 1988), and also following experimental infection (Kirkland et al., 1991). In the latter study, the virus was shed after the viraemia had subsided; the vesicular glands and prostate were the main sites of virus replication.

Mucosal disease is usually seen in younger animals (6–24 months old). The disease is characterised by pyrexia, anorexia, watery diarrhoea, nasal discharge, buccal ulceration and lameness. The morbidity rate is generally low, but, amongst affected animals, the mortality rate is high.

Effects upon reproductive performance

The effect of the BVD virus on reproduction depends upon the stage of pregnancy at which the cow becomes infected. Acute infection, with either biotype, can severely affect the embryo or fetus. During the first month of gestation, infection results in the death and resorption of a high proportion of embryos. The only signs of reproductive disease that such affected cows or heifers exhibit is returning to oestrus at normal or extended intervals. Pregnancy rates are therefore reduced in affected animals. For example, Houe et al. (1993) associated the spread of BVD through a group of susceptible cattle with poor pregnancy rates. Likewise, McGowan et al. (1993) demonstrated poorer pregnancy rates in *Bos indicus* cattle that were infected with BVD at the time of insemination than in animals that were immune. BVD is probably directly embryotoxic (although studies of contaminated embryos do not necessarily demonstrate such an effect in vitro) and it can cause ovaritis (Ssentongo et al., 1980) and impairment of follicular function (Grooms et al., 1998).

Low pregnancy rates also result from the insemination of semen that is contaminated with BVD virus, whether by AI or natural service. In a study of the effects of inseminating BVD-contaminated semen, seronegative cows had first-service pregnancy rates of 22.2% compared with 78.6% in those that were seropositive (Virakula et al., 1993). Although Wentink et al. (1989) showed normal pregnancy rates in small groups of heifers after service by a persistently infected bull, Meyling and Jenson (1988) demonstrated that BVD can be transmitted via semen and can give rise to the birth of persistently infected calves. BVD can also be transmitted through virus-contaminated embryos (Avery et al., 1993).

From the second to the fourth month of gestation, infection may be followed by abortion, death with mummification, growth retardation, developmental abnormalities of the central nervous system and alopecia; some infected cows or heifers will carry calves to term, but these may well become persistently infected. It has generally been assumed that infection before 125 days of gestation is necessary for the carrier state to occur in calves (McClurkin et al., 1984), although Roeder et al. (1986) reported an earlier time of 81 days or less.

From the fifth and sixth months of gestation, there can be abortion or the birth of calves with congenital abnormalities of the central nervous system and eyes. Typically, there is a time interval of between several days and 2 months between infection with BVD virus and abortion (Bolin, 1990a).

Irrespective of the biotype, infection of the fetus late in pregnancy will lead to the birth of an immune calf, since the fetus can develop a measurable antibody response to the organism by 5–6 months of gestation (Bolin, 1990b). However, fetal infection can also be followed by the birth of normal premature live, stillborn or weakly calves, as well as those with congenital abnormalities.

Diagnosis

The recent introduction of a persistent infected cow or heifer into a susceptible herd should be viewed with concern (Duffell and Harkness, 1985). There may be a history of the overt disease. However, since in most cases there may only be slight pyrexia, inappetence and respiratory distress which may go undetected, the first signs are likely to be abortions and birth of congenitally deformed calves. The fetuses may be fresh, autolysed or mummified (Bolin, 1990a). Some histological lesions are characteristic of the infection.

The virus can be isolated from the fetus, particularly lymphoid tissue such as the spleen. Immunocytochemical identification of BVD viral proteins in fetal tissue, especially kidney, lung or lymphoid tissue, can sometimes be detected, even though the virus cannot be demonstrated. A substantial rise in neutralising antibodies in herds experiencing abortions and the presence of antibodies in the serum of newborn calves or the thoracic fluids of abortuses is diagnostic of infection. In the case of live calves, serum must be obtained *before* colostrum is ingested.

In countries where vaccination has been used, contaminated modified-live-virus vaccines or poorly attenuated modified-live BVD virus vaccines have been responsible for the introduction of the infectious agent on to a farm (Baker, 1987). In addition, where virus-contaminated fetal calf serum has been used in embryo transfer techniques there is also a possibility of disease transmission.

Control

This can be expensive and may not be cost-effective if it requires extensive culling of persistently infected animals. The basic principles are that farms do not breed from persistently infected cows; that only immune animals are introduced to the breeding herd – this can be achieved by deliberate exposure to persistently infected cattle before breeding; and that any purchased animals introduced into the herd should be screened beforehand. Since there is some suggestion that cross-infection can occur between cattle and sheep and goats (Duffell and Harkness, 1985), the species should be separated.

The absence of antibody titres is generally assumed to indicate the absence of infection. With BVD this is not the case; a seropositive animal would be a safe purchase but a seronegative one requires to be free of virus to assure freedom from risk (Duffell and Harkness, 1985).

Vaccines are used in many countries as a control measure. Killed-virus vaccines can be used in pregnant cows; modified-live-virus vaccines cannot. Concern at the use of the latter has been expressed. Details of vaccination programmes have been described by Ames and Baker (1990).

Infectious bovine rhinotracheitis (IBR) virus

Infectious bovine rhinotracheitis virus (bovine herpesvirus; BHV-1) is present world-wide and causes an acute respiratory disease of cattle with conjunctivitis.

It also causes a disease of the genital organs of the bull and cow, a syndrome that has been recognised for many years, long before the respiratory form of the disease was described or the causal organism identified. The disease of the genital system has been variously called infectious pustular vulvovaginitis (IPV), vesicular venereal disease and coital vesicular exanthema. BHV-1 causes both the respiratory and genital forms of the disease, although the two forms usually occur independently. BHV-1 also causes abortion, more commonly after the respiratory, rather than the genital, form of the disease. BHV-1 infection is also associated with infertility in cows and heifers.

Pathogenesis and transmission

The genital form of the disease (IPV) is readily transmitted venereally, but this is not the only route, since it can occur via contaminated bedding and the mutual licking and sniffing of the vulva and perineum of infected and non-infected animals. Also, it can be transmitted by virus-contaminated semen. Once it has gained entry, it is transported haematogenously in leucocytes.

Some animals can become lifelong latent carriers of the virus, despite the formation of specific antibodies. The infection enters a latent phase in the ganglion cells of the nervous system. Under certain circumstances, such as stress, calving, transportation, vaccination or corticosteroid therapy, the latent infection can be reactivated so that the virus migrates along nerves to the periphery, where it multiplies and is excreted. These animals represent a reservoir of the virus.

Clinical signs

Infectious pustular vulvovaginitis. The onset of vulvovaginitis is sudden and acute. Signs appear 24–48 hours after venereal transmission; heifers tend to be more severely affected than cows. The vulval labia become swollen and tender and, in light-skinned animals, deeply congested. This is quickly followed by the development of numerous red vesicles on the mucosa. These may rapidly rupture or develop into pustules which give rise to haemorrhagic ulcers, 3 mm or so in diameter.

The quantity of vulval discharge is variable, ranging from small quantities of exudate, which adhere to the vulval and tail hairs, to a copious mucopurulent discharge. A speculum is useful to examine the vaginal mucosa but, because of the pain and discomfort, caudal epidural anaesthesia is worthwhile. The lesions are obviously painful since affected animals are restless, with swishing of the tail, frequent urination and straining. There may be transient pyrexia and reduced milk yield, but the systemic effects are variable depending upon the presence of respiratory problems. The acute phase of the disease will subside in about 10–14 days, but a few animals will display a persistent vulval discharge for several weeks.

When females show signs of IPV, the bull must be examined for the presence of lesions, since, unlike the situation with most venereal diseases of cattle, the signs in the bull are dramatic (see Chapter 30).

Infertility. Opinions have varied over the role of BHV-1 as a cause of infertility. Early studies by Parsonson (1964) and Hellig (1965) suggested that it had no effect, whereas Kendrick and McEntee (1967) found that, if semen infected with the virus was used for artificial insemination, there were reduced pregnancy rates, endometritis and shortened interoestrous intervals. Parsonson and Snowdon (1975) also reported that when virus-infected semen is introduced into the uterus, as would occur at artificial insemination, infertility (i.e. poor pregnancy rates) occurs. Experimentally, when infected semen is deposited in the uterus it causes a severe, necrotising endometritis, but lesions remain localised to the site of virus deposition and resolve in 1–2 weeks (Miller and Van Der Maaten, 1984). Hence, Khars (1986) suggested that, since inoculation of IBR virus into the uterus causes endometritis, it was likely to be a cause of infertility. Thus, artificial insemination of contaminated semen is undoubtedly associated with embryonic death; however, the evidence for such an effect of natural service by an infected bull is less clear-cut.

The virus can affect a number of other aspects of reproduction. It can cause a bilateral necrotising oophoritis, to which the corpus luteum appears particularly susceptible, especially during the first few days after ovulation. This damage to the developing corpus luteum may directly affect its function, perhaps resulting in lower than normal progesterone production. In consequence, the survival of the embryo would be compromised. The virus can also directly cause embryonic death, by direct invasion of cells (Bowen et al., 1985; Miller and van der Maaten, 1986). The consequence is embryonic death, with the cow returning to oestrus at a normal interval after insemination (such as was reported by Miller and van der Maaten (1987) after infection of heifers at the time of breeding).

Abortion. IBR virus is an important cause of bovine abortion. Kirkbride (1992) reported that, amongst nearly 9000 abortions that occurred between 1980 and 1990, BHV-1 was responsible for 5.4% of incidents. Murray (1990) found IBR to be the causative agent in 13% of 149 calves that were aborted over 2 years in northwest England. Abortion is a common sequel to infection, with or without previous respiratory tract signs of disease, and also following vaccination with a modified live vaccine (Kelling et al., 1973). The age of gestation at the time of infection appears to be critical, since cows that are $5\frac{1}{2}$ months pregnant, or less, do not abort, whilst those older than this have a 25% probability of aborting (Huck and Lamont, 1979). In beef herds, abortion 'storms' occur, with between 5 and 60% of cows aborting. Such an incident was reported by Tanyi et al. (1993), although in dairy herds abortion is generally sporadic.

Abortions occur from 4 months of gestation to term. Some calves are stillborn, and a few may be born alive, but succumb subsequently. The effects of virus infection may be due to the strain of the virus. Miller et al. (1991) examined the abortifacient properties of each of the three main BHV-1 subtypes (subtypes 1, 2a and 2b) by infecting heifers with the virus at 25–27 weeks after breeding. All heifers developed fever and viraemia within 2–5 days after inoculation. Heifers given subtype-1 aborted between 17 and 85 days later, but those given subtype-2 delivered full-term calves, some of which had BHV-1 neutralising antibodies in precolostral serum. In New Zealand, although IBR is a relatively common disease, the strain that is present does not appear to be able to cause abortion (Durham et al., 1975). Further evidence of strain variation comes from the work of Allan et al. (1975), who caused only mild upper respiratory disease by administration of a genital strain of IPV (IBR) and failed to cause abortions. On the other hand, abortion can occur with little or no accompanying respiratory or occular signs (Anon, 1979), or, because the interval between infection and abortion can be protracted, earlier signs of IBR infection are not always readily associated with later abortions (Barr and Anderson, 1993).

The time interval from infection to abortion varies from a few days to 100 days. In the latter case the fetus is extensively autolysed and may be reported as being too decomposed for diagnostic work-up (Khars, 1986). However, even in such cases, diagnostic lesions are generally present in the fetal liver and adrenal, if a careful search is made.

Epivag. 'Epivag' is a specific bovine venereal disease causing epididymitis and vaginitis in cattle in East, South and Central Africa (Hudson, 1949 and Roberts, 1986). In cows, it causes diffuse infection of the vagina, but not the presence of distinct lesions as occur with IPV. A severe mucopurulent vaginal discharge may be present during the earlier stages of the disease. Most infected cows fail to conceive to service, but most eventually recover. About 15–25% of animals become sterile, due to the presence of lesions of the uterine tubes, such as adhesions, hydrosalpinx, and ovarian and bursal adhesions. Likewise, some cows develop parametritis as a result of Epivag infection (McEntee, 1990), and adhesions may be widespread throughout the pelvis and even extend into the abdomen.

Most bulls have a mild balanoposthitis after infection, although, since this is far less severe than IPV infection, it may not be observed. Subsequently, most bulls develop an induration of the epididymis, particularly of its tail. Orchitis may occasionally occur.

The causal organism has not been definitively characterised. Theodoridis (1978) isolated a series of herpes viruses from cattle with the Epivag syndrome, including some that were related to BHV-1. However, although the vaginitis component of the syndrome could be induced by various of these herpesviruses, the epididymitis could not. Hence, it remains unclear whether the syndrome is caused by BHV-1 and, indeed, whether herpesviruses are the sole causal agent.

Diagnosis

The genital tract lesions of IPV are fairly characteristic of the disease, but must be differentiated from granular vulvovaginitis due to *Ureaplasma spp.* and catarrhal vaginocervicitis.

Some investigators consider that a severely autolysed fetus strongly suggests BHV-1 infection. There is frequently a liquefactive necrosis of the whole of the kidney cortex with peri-renal haemorrhagic oedema. Histologically, there is always focal necrosis of the liver and in many cases there are necrotic lesions in the brain, lungs, spleen, adrenal cortex and lymph nodes. There are characteristic virus inclusion bodies at the periphery of these necrotic lesions in fresh experimental cases but, because of autolysis, they are not always demonstrable in field cases of abortion. The virus has been found in all fetal tissue and is concentrated in the cotyledons.

Nettleton (1986) has recommended that, following abortion, the following samples should be submitted for laboratory examination. Paired serum samples are taken from the dam at the time of abortion and a second set of samples 2–4 weeks later. However, since cows may have been infected up to 4 months before abortion occurs, a significant rise in antibody titres is unlikely to be demonstrated. Serological examination of paired serum samples from at least 10 cows in the herd should reveal seroconversion or a four-fold increase in titres if IBR infection is active in the herd (Kirkbride, 1990a).

For subsequent fluorescent antibody tests, pieces of fetal tissue, particularly kidney and adrenal gland, should be taken together with a piece of placenta. Such tests that demonstrate specific focal fluorescence are diagnostic of the disease. Virus isolation is not particularly reliable but should be used if only placental tissue is available (Kirkbride, 1990a).

Following the presence of genital lesions, vaginal swabs, preputial washings and semen should be placed in virus transport medium. Paired serum samples should be taken from the affected cows.

Treatment and control

Spontaneous recovery of the genital lesions will occur and therefore treatment is not really necessary; however, the administration of emollient creams to the vulva, vagina and penis may be useful. Vulval stenosis and penile/preputial adhesions and phimosis can occur during the healing phase (see Chapter 30).

Infected animals should be isolated and natural service suspended. Vaccination is the most effective way of controlling the disease; a number of live, attenuated vaccines are available, often combined with a bovine parainfluenza virus vaccine. Heifers should be vaccinated after 6 months of age and before their first service; thereafter, annual vaccination is preferable. Pregnant animals should only be vaccinated with a killed vaccine. Both the intranasal and intramuscular routes can be used. Vaccination of bulls is of questionable value since on they will be seropositive blood testing and may be rejected for sale as being infected. Routine examination of semen for the presence of the virus is preferable as a method of control.

Blue tongue

Blue tongue is mainly a disease of sheep and deer, but cattle and wild ruminants are important reservoir hosts for the virus. Blue tongue is found mainly in countries between 40°N and 35°S (Radostits et al., 1994), and is endemic in the western states of the USA. It is not present in Canada, the UK and New Zealand. In Australia, although there is serological evidence of its presence, there is no clinical evidence of disease.

The virus is primarily transmitted by insect bites. *Culicoides* species are the main vectors; in the USA, the main transmitting agent is *C. varripennis* and in Africa, *C. imicola*. A few other species are of importance in other regions and there may be some transmission by ticks, keds and mosquitoes (Radostits et al., 1994). Bulls that are infected by blue tongue virus can transmit the virus in their semen (Bowen and Howard, 1984).

In cattle, clinical disease is rarely caused by blue tongue virus (Radostits et al., 1994), but it does have a number of effects upon bovine reproduction. Infection of susceptible cattle causes a viraemia, during which the virus can cross the placenta. Infection of the post-hatching embryo can result in its death and, if susceptible herds are bred during the season of maximal infection with the virus, seasonal infertility can result. Infection later in pregnancy can lead to abortion or mummification of the fetus. The neuropathogenicity of the virus produces hydrancephaly (Howard, 1986) and abnormal contractures of extremities. Calves may be born alive, which are weak and ataxic, or which are persistent carriers of the infection (Roberts, 1986).

In the aborted fetus, diagnosis of blue tongue can be made by demonstration of central nervous lesions (Barr and Anderson, 1993), or by virus isolation from fetal blood, spleen, lung or brain. Serology can be used to diagnose maternal infection, although the presence of antibody-negative, viraemic animals during an epizootic outbreak can confuse diagnosis (Osburn et al., 1981).

Other viral causes

Catarrhal vaginocavititis

This contagious, mainly venereally transmitted, disease was first described in South Africa (Van Rensburg, 1953); since then it has been reported in many countries. It is caused by an enterovirus from the enteric cytopathic bovine orphan (ECBO) group.

Clinical signs. Affected animals have a profuse, postcoital, non-odorous, yellow, mucoid vulval discharge. The cervix and vagina are inflamed but there are no pustules, such as occur in IPV infection, and no fever. The typical yellow gelatinous exudate frequently accumulates in the vagina, varying in quantity from a few to several hundred millilitres. The disease persists for a few days to a few weeks. Only a few animals show clinical signs of the disease at any one time. As a consequence, pregnancy rates are reduced and there are prolonged, irregular returns to oestrus, presumably due to late embryonic death. In some herds, fetal mummification, abortion and stillbirth have been reported as being a problem.

Bulls may or may not become clinically infected but, in Belgium, Bouters et al. (1964) have provided definite proof of the association of two ECBO serotypes with seminal vesiculitis and infertility lasting up to 90 days. The penis and prepuce do not show the lesions that occur following BHV-1 infection.

Diagnosis. The most reliable method of diagnosis is serological examination of paired blood samples, collected at least 15 days apart, for evidence of rising antibody titres; the first sample should be collected as soon as possible after the disease is suspected.

The virus can be isolated from vaginal mucus, but the recovery rate is frequently low (Huck and Lamont, 1979).

Transmission and pathogenesis. Although the disease is transmitted venereally, it can also be spread by faecal contamination of the vulva, or by animals licking the perineum of infected and non-infected individuals; hence the disease can occur in virgin heifers.

Treatment and control. There is no specific treatment or vaccine. Infected bulls should not be used for service for several months, even after clinical signs of disease have disappeared. Potentially infected animals should be isolated after purchase and, in closed herds, serological examination of potential additions to the herd might be contemplated.

Parainfluenza 3 (PI3) virus abortion

This widely distributed virus has been recovered from aborted fetuses in which it caused a septicaemic disease (Sattar et al., 1968). Experimentally, it can cause fetal death and abortion after intrafetal inoculation, but not after introduction into the maternal respiratory system.

Vaccines to PI3 virus are available commercially, often combined with IBR vaccines. Vaccination can be done during calfhood or in adult cattle to give lifelong protection.

Transmissible genital fibropapillomas

Wart-like tumours commonly occur on the penis of young bulls (see Chapter 30), and occasionally similar growths occur on the vulva, perineum and vestibulovaginal epithelium of heifers. They are caused by a virus of the papovavirus group and are transmitted by contact with infected animals.

These fibropapillomata regress spontaneously in 2–6 months; the speed of regression may be expedited by the use of a wart vaccine (formalised tissue). Except in so far as the larger tumours (which may be removed surgically) might interfere mechanically with coitus, they do not cause infertility in female animals.

MYCOTIC ABORTION

Fungal invasion of the placenta and fetus is a frequent and consistent cause of abortion in cattle (see Table 23.1). Abortions are normally sporadic, although in some herds the incidence may be as high as 5–10%. The frequency of diagnosis is high; in the northeastern states of the USA mycotic abortions accounted for 22% of all infectious abortions and 5.1% of all abortions investigated (Hubbert et al., 1973). Similarly, in South Dakota, USA, a survey over a 5-year period found that 14.6% of all infectious abortions were due to fungi; this was 4.8% of the total number of abortions (Kirkbride et al., 1973).

In the UK mycotic abortion is much more prevalent during the months of December, January, February and March compared with the rest of the year.

The fungi that are most frequently isolated following abortion are *Absidia spp.*, *Rhizopus spp.*, *Mucor spp.* and *Aspergillus spp.* Other fungi such as *Mortiella wolfii* and *Petriellidium boydii* have also been implicated. In the northern hemisphere, *A. fumigatus* (Pepin, 1983) is the most common cause of abortion, while in the southern hemisphere, *M. wolfii* is the most important organism.

Clinical signs

Infection does not always cause abortion, since infected live calves can be born. When abortion occurs, it is usually sporadic in nature, with abortions occurring between 4 and 9 months of gestation, being most prevalent between 7 and 8 months.

The appearance of the lesions on the placenta and the calf are fairly characteristic of mycotic infection. The whole or part of the placenta usually appears discoloured when shed, and is either grey, yellow or reddish-brown; the intercotyledonary areas of the allantochorion are thickened, wrinkled or leathery. Those cotyledons that have attached portions of the corresponding caruncle after the placenta has been shed appear thickened and have a cup-like or coffee bean appearance (Pepin, 1983). Between 25 and 33% of the fetuses are infected (Austwick, 1968; Kendrick, 1975). In a proportion of these, characteristic fetal skin lesions are present which are circumscribed, greyish-white thickened patches similar in appearance to skin ringworm in calves and young cattle.

There are no other clinical signs of disease in the dam associated with abortion due to *A. fumigatus*. Conversely, although abortion due to *M. wolfii* is not accompanied by immediate clinical signs in the dam, a common sequel of abortion is a fatal mycotic pneumonia in the dam after she has aborted.

Diagnosis

The appearance of the placenta is fairly typical in fungal abortion, although some bacteria can produce similar lesions. The fetal skin lesions are almost pathognomonic.

Laboratory confirmation requires submission of placental tissue, preferably the whole organ (Pepin, 1983). Culture from placental tissue is of no value since the placenta is usually contaminated after it has been expelled. Culture from fetal lungs and abomasum is more reliable but contamination can occur.

The reliable and traditional method of diagnosis is the identification of fungal cells in histological sections of the placenta. Since fungal infections are frequently localised, resulting in focal lesions, selection of suitable material is important. Another technique is the potassium hydroxide ‘crush’ mount of non-fixed tissue (Pepin, 1983).

According to Kirkbride (1990c), conclusive diagnosis of mycotic placentitis can be made if:

- the characteristic lesions of placentitis are present in association with the presence of mycotic elements
- the characteristic lesions of fetal dermatomycosis are present in association with the presence of mycotic elements
- there is a fetal bronchopneumonia associated with mycotic elements.

Serological tests are, at present, unreliable and cannot be used for routine diagnosis.

Transmission and pathogenesis

Many of the species of fungi are ubiquitous in the air and environment in which cattle live; however, there is good evidence that mouldy hay and straw and other food such as silage and sugar beet pulp are important sources of infection. Mycotic abortion is most prevalent in the winter months when cattle are housed. This was demonstrated in a survey in southwest Wales involving 531 herds over a 5-year period (Williams et al., 1977). When hay was fed to cows housed in sheds, the percentage of mycotic abortions was 7.14%, compared with that for other systems of management, including the feeding of hay in loose housing, where it was between 1.32 and 0.19%.

There is still speculation about how the organism reaches the uterus and infects the placenta and fetus. It is generally agreed that there is haematogenous spread following entry into the vascular system from the respiratory or alimentary tracts. There is some evidence that fungal-contaminated semen can cause uterine lesions (Kendrick et al., 1975), although this route is unlikely to be important.

The fetus and placenta are much more susceptible to mycotic invasion than maternal tissues; this may be due to growth enhancement of fungi by the products of conception. Once the fungus has colonised the uterus it probably spreads in two ways: after initial infection of a few placentomes it spreads slowly throughout the placenta until sufficient is affected to cause abortion, at the same time the mycelium will invade the fetus and, after initial infection, there is rapid invasion of the placenta with abortion occurring before the fetus is affected.

Control

The feeding of mouldy forage or the use of mouldy bedding should be avoided.

CHLAMYDIAL AGENTS: BOVINE CHLAMYDIAL ABORTION

C. psittaci is a pathogen of both the male and female bovine genital tract.

In the bull it affects the testes, epididymides and other accessory glands. It causes orchitis (Jubb et al., 1993), possibly in association with *Mycoplasma* species. However, it particularly affects the vesicular glands, where it is believed to be involved in the seminal vesculitis syndrome (see Chapter 30). The organism is sometimes excreted in the semen of affected bulls, although it has also been isolated from bulls that were clinically normal (Eaglesome and Garcia, 1992). Chlamydial infection also affects fertility in the cow. If contaminated semen is used then, after fertilisation has occurred, there will be embryonic death either due to a direct effect upon the embryo or, more likely, via its effect upon the endometrium. *C. psittaci* also causes abortion; this has been demonstrated in the USA and southern Europe. Characteristically, abortion occurs at 7–9 months of gestation without any other clinical signs, although experimental infection is followed by a short period of pyrexia and a leucopenia.

The lesions following abortion are fairly characteristic. The intercotyledonary areas of the placenta are more frequently affected, being thickened and leathery in appearance with a reddish-white opaque discoloration; oedema is quite common. In the aborted fetus, the liver is enlarged with a coarsely nodular surface, firm consistency and a mottled reddish-yellow colour (Shewen, 1986). The organism can be cultured from aborted fetuses and discharges following the use of transport media. Giemsa-stained smears for the identification of elementary bodies or inclusions are also useful. Serological tests such as the CFT have been used but are generally too insensitive. It is likely that the ELISA tests, used to detect the infection in sheep, will be developed for use in cattle.

Tetracyclines could be used to treat pregnant cows that have been exposed to infection, but this it is not really practicable because it requires knowing that the secondary chlamydaemia has not occurred, and animals must be treated until normal calving. Pregnant animals should be segregated from potential sources of infection. Vaccines are available for use in sheep but none has yet been developed for use in cattle. Following abortion there should be a natural immunity.

INFECTIOUS DISEASES OF UNCERTAIN AETIOLOGY: EPIZOOTIC BOVINE ABORTION (EBA)

This disease was first identified in the mid-1950s in California. It is characterised by a high abortion rate of 30–40% during the last trimester of gestation in cows and heifers newly introduced to beef herds in particular areas of the states of California, Oregon and Nevada (Barr and Anderson, 1993). The dam shows no clinical signs other than abortion.

Abortions are confined to the habitat of the argasid tick *Ornithodoros coriaceus*. Hence, it seems that this tick is the vector for the disease. The causal organism has, however, not been definitively identified. Early studies suggested that the disease was due to *Chlamydia psittaci*; however, there is considerable debate about the authenticity of the isolation of the organism and its role in the pathogenesis. Spirochaetes have also been implicated (Osebold et al., 1986). It should, however, be noted that enzootic abortion is a separate disease entity from bovine chlamydial abortion (Barr and Anderson, 1993).

Abortions are seasonal, occurring 100 days or more after exposure to ticks. Once abortions have occurred, animals are immune, so the cattle which are at greatest risk are those calving for the first time and animals which have been moved into a tick-infested region. Infection late in pregnancy can give rise to the birth of live, weak calves (Barr and Anderson, 1993). Lesions in aborted fetuses are characteristic and are used in its diagnosis. Abortuses are not autolysed, and lymph nodes, spleen and liver are enlarged, with lymphocytic hyperplasia of most lymphoid organs (Jubb et al., 1993). Control is attempted by ensuring that susceptible animals are exposed to ticks before they become pregnant.

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24 Veterinary control of herd fertility

In the dairy herd, the main source of income is from the sale of milk, although normally, with the exception of the bull calves of the Channel Island breeds, the calf will also provide an additional source of income. In beef suckler herds, the calf is the principal source of income. In both types of farming enterprise, some income will also be generated from the sale of cull cows; however, this is likely to result in a net loss since the cost of a replacement, either purchased or reared on the farm, will be greater. In addition at the present time in the UK, because of the over-30 months of age slaughter scheme that originated as a result of bovine spongiform encaphalitis (BSE) prohibiting meat from such animals entering the human food chain and requiring that it is incinerated, the value of a cull cow is very low. Poor fertility costs the farming enterprise money. For example, a cow with a vulval discharge, which is invariably due to endometritis, costs £161.58; a cow with retained fetal membranes costs £298.29; or the direct cost of veterinary treatment for a cow in which oestrus has not been observed is up to £12.61 (Kossaibati and Esslemont, 1997: based on 1995 prices). In the UK 36.5% of cows are culled for subfertility/infertility (Esslemont and Kottaibati, 1997). The prevalence and cost of infertility are discussed in Chapter 22.

In dealing with fertility and infertility of cattle, the veterinarian has two tasks to perform. Firstly, he or she may be asked to investigate and determine the cause of infertility in individual animals or in the herd; secondly, he or she may be required to assist in the maintenance of optimum fertility so that the livestock enterprise functions as efficiently and profitably as possible. The latter will be dependent on the breeding strategy of the enterprise, which in turn will be influenced significantly by the part of the world where the enterprise exists and the demands placed on the production system.

In those parts of the world that are heavily urbanised, such as much of Europe and North America and elsewhere around the major conurbations, there is a ready market for the supply and sale of liquid milk. Such systems rely on the feeding of large amounts of cereal and other concentrate feeds resulting in high input–high output dairying systems. In other regions of the world, such as New Zealand and parts of Australia, South America and East Asia, the majority of milk produced is used for processing and the manufacture of milk products. Since cereal prices are much higher relatively, and the climate favours rapid pasture growth, there has been the development of pastoral dairying systems.

In this chapter, veterinary involvement in the control of herd fertility will be described and discussed. This will depend upon a number of factors: firstly, the production system used; secondly, the management policy of the livestock unit; thirdly, the expectations of the management of the livestock unit, and whether they consider that veterinary input adds value to the enterprise. In addition to the control of fertility in dairy herds both pastoral-based and high input–high output and combinations of both, the fertility control of beef suckler herds will also be considered.

NORMAL EXPECTATIONS OF FERTILITY

It has been long recognised that, although a cow that appears to have an apparently structurally and functionally normal reproductive system is inseminated or served at the correct time with fertile semen, she may fail to become pregnant. The herd manager should identify this in the first instance when the cow returns to oestrus. The reason for a cow failing to calve to particular insemination is either that there has been failure of fertilisation, or that fertilisation has occurred but the embryo or fetus has died at some stage during gestation.

With the intensive selection of dairy cows for higher and higher milk production, there is clear evidence from many parts of the world that fertility is in decline. As can be seen in Table 24.1, in the USA over a 40-year period from 1955 to 1995 the pregnancy rate to each AI in cows has declined from 60% to 40% as yields have quadrupled, whereas heifer fertility has improved over the same time course. Thus, the decline is related to the demands of lactation rather than some inherent predisposing genetic factor. A similar correlation between milk yield and pregnancy rates is shown in the study of Nebel and McGilliard (1993) also from the USA (Table 24.2). In a study over a 6-year period involving 34 dairy herds in Ireland, the use of logistic regression analysis showed that there was a consistent and significant ($P < 0.01$) change in calving rates over time, amounting to an estimated decline of 0.54% per annum (Table 24.3). Similarly in the UK, a study comparing the fertility of commercial dairy herds between 1975 and 1982 and 1995 and 1998 showed that the calving rate to first service for all cows declined from 55.6% to 39.7%. When those that were untreated for reproductive disorders were compared, the comparable values had declined from 65.4% to 42.9% (Royal, 1999). During this same time, the average annual milk yield in the UK has increased from 4270 to 5515 kg, which has been associated with the introduction of high genetic merit North American Holsteins. Significantly, in 1975 Holsteins comprised 0% of the cows in dairy herds, compared with 80% in 1995.

Table 24.1 Fertility of dairy cows in the USA over a 40-year period as measured by pregnancy rates to AI (Wiltbank, 1998)

	Pregnancy rate per insemination		*Milk yield per lactation (kg)*
Year	*Lactating cows*	*Heifers*	
1955	60%	66%	2300
1975	50%	65%	5000
1995	40%	70%	9100

Table 24.2 Relationship between milk yield and fertility in American Holstein cows (Nebel and McGilliard, 1993)

Milk yield per lactation (kg)	*Number of herds*	*First AI pregnancy rate (%)*
6364–6818	452	52
7727–8181	678	44
8638–9090	479	43
9545–10000	202	40
> 10 454	53	38

Table 24.3 Trends in the calving rate to first service in Irish dairy herds on the Dairy Management Information System (Dairy MIS) from 1991 to 1996 (from O'Farrell and Crilly, 1999)

Year	*Percentage calving rate*	*Number of cows*
1991	53.0	2305
1992	51.3	2998
1993	51.6	3284
1994	48.9	3301
1995	49.7	3299
1996	48.8	3164

FERTILISATION FAILURE AND EMBRYONIC LOSS

Since Corner's (1923) discovery of the phenomenon of death of conceptuses in sows, it has now been established that there is an incidence of 20–50% embryonic and fetal death in apparently normal healthy animals of all domestic species, including cattle. Extensive studies have shown that there are a number of factors that may cause embryonic death, but the aetiology of a large part of the problem remains unexplained. The existence of this unexplained moiety in rather constant degree in all species led Hanly (1961) to suggest that it was due to a more universally active factor than any of those so far investigated. Bishop (1964) proposed that, because embryonic loss appeared to be a general feature of mammalian reproduction, it probably conferred some biological advantage that might allow the elimination of undesirable genetic material at a low biological cost. If this were so, then a considerable part of embryonic death should be regarded as a normal occurrence and thus unavoidable. This concept of inevitable conceptual loss implies a limit to the chance of a successful outcome to each mating or insemination, which will not be significantly affected by previous success or failure.

This concept of the inevitability of embryonic loss, thus limiting the successful outcome of each service or insemination, has been generally accepted. Chromosome abnormalities are known to be one of the major causes of fetal death in humans (Simpson, 1980). Their involvement in embryonic death in cattle was shown some years ago by McFeely and Rajakoski (1968), who found tetraploid cells in one of eight bovine blastocysts at 12–16 days of age. When they occur, it is likely that there will be early loss of the embryo with return to service; in polytocous species there will be a reduced litter size. Chromosome abnormalities are either inherited or arise de novo during gametogenesis, fertilisation and early cleavage of the embryo (Hamerton, 1971) (see Chapter 4). During gametogenesis, abnormal meiosis can produce gametes with unbalanced chromosome composition, such as duplication and deletion of segments of chromosome, whole chromosomes or the failure of the reduction division. Although abnormal, these gametes are capable of participation in fertilisation so that the embryo has chromosome abnormalities. Chromosome abnormalities can occur because of polyspermic fertilisation, failure to extrude one or both polar bodies, fertilisation of the oocyte and the polar body at the first cleavage division or because of failure at meiosis. Whilst it has been clearly demonstrated that superovulated oocytes quite frequently have cytogenetic abnormalities (up to one-third) due to polyspermic fertilisation and/or mitotic activity of the polar body (King, 1985), those derived from a single ovulation do not. Work on virgin and 'Repeat Breeder' heifers indentified two animals, out of a total of 42 from the latter group, which had 1/29 gene translocations, but the remainder had normal karyotypes (Gustafsson et al., 1985). Gayerie de Abreu et al. (1984) reported that 9% of cow embryos had abnormal karyotypes compared with 6% in heifers.

Single genes that affect embryological development have not been identified in domestic animals, although they are known to cause fetal death and congenital abnormalities in humans.

There is little evidence that inherent genetic abnormalities are the main cause in cattle, since the work in humans, from which the theory has been extrapolated, has been done on aborted human fetuses, not embryos (Land et al., 1983). Furthermore, there is now good evidence that it is possible to select mice genetically for a high rate of embryo survival (Bradford, 1969), and that mammalian gametogenesis and syngamy do not necessarily lead to a high incidence of mortal damage (Land et al., 1983). Perhaps the genetic selection of domestic species for high embryonic survival rates, rather than other genetic traits such as milk yield and quality in dairy cows or food conversion, might be a profitable way to increase the overall fertility rate.

There is increasing evidence that the major reason for embryonic loss is spontaneous asynchrony between dam and embryo, which would appear to be largely mediated by endogenous ovarian steroids as was first identified by Wilmut et al. (1985). Adequate concentrations of progesterone have been shown to be important in the normal temporal development of the embryo by regulating the provision of nutrients and growth factors in the uterus in early pregnancy (Starbuck et al., 1999).

How can the incidence of embryonic loss be determined? If fertilisation occurs, the developing conceptus prevents the return to oestrus by inhibiting the production or release of endogenous luteolysin (see Chapter 3). If the embryo dies before 13 days of age (the time of the maternal recognition of pregnancy; see Chapter 3), then the cow will return to oestrus at the normal interoestrus interval. If the embryo dies after this age, then the interoestrus interval will be extended beyond the generally accepted figure of 18–24 days. Therefore, it is impossible to differentiate, by observing the occurrence of a return to oestrus, between fertilisation failure and embryonic death before 13 days of age. This is particularly important, since it has been postulated that most embryos die before 15 days of age (Boyd et al., 1969; Ayalon, 1972). For many years, the only method available for the study of embryonic death was slaughter at known time intervals after service or insemination, followed by flushing of uterine tubes and horns. In such studies, using first-service heifers, Bearden et al. (1956) reported a fertilisation failure of only 3.4% and an embryonic loss up to 35 days of 10.5%; in Repeat Breeder heifers, Tanabe and Almquist (1953) reported a fertilisation failure of 40.8% and an embryonic loss of 28.7%. In normal fertile cows Ayalon (1978) and Boyd et al. (1969) found fertilisation failure rates of 17 and 15% and embryonic loss rates up to 35 days of 14 and 15%, respectively. In Repeat Breeder cows similar figures for these two categories were 39.7% and 39.2% (Tanabe and Casida, 1949) and 29% and 36% (Ayalon, 1978), respectively. In a large survey of 4286 randomly selected cows the greatest incidence of embryonic loss (14.9%) occurred between 30 and 60 days; at 60–90 days it was 5.5% and at 90–120 days it was 2.8% (Barrett et al., 1948). In a study using milk progesterone determinations, it was found that the incidence of fertilisation failure, together with conceptual loss up to 20 days after artificial insemination, was almost equal to fetal loss between 20 and 80 days (Pope and Hodgeson-Jones, 1975).

The availability of a reliable assay to measure early pregnancy factor (EPF)/early conception factor (ECF) (see Chapter 3) in the peripheral circulation within days of fertilisation will be a useful research and diagnostic tool to study the relative importance of fertilisation failure and early embryonic death in the near future.

There is good evidence that the critical period for embryonic demise is on day 7 after fertilisation when the morula develops into the blastocyst (Ayalon, 1973), and that embryonic loss at this time is greater in Repeat Breeder cows (Ayalon, 1978). In a review using composite data for heifers from nine publications, Sreenan and Diskin (1986) calculated the mean fertilisation rate to be 88%; for cows from four sources the mean fertilisation rate was 90%. The same authors calculated the mean embryonic death rate using data from nine sources involving 468 heifers and cows; the percentage pregnant 2–5 days after artificial insemination was 85%, between 11 and 13 days it was 73%, and for 25–42 days, it was 67%.

The development of embryo transfer techniques for the non-surgical flushing of embryos (see Chapter 35) has enabled a large number of studies to be performed (Sreenan and Diskin, 1983; Roche et al., 1985). Using these methods, it is possible to flush cows and heifers repeatedly at varying time intervals after insemination to recover the embryos. These can be examined critically microscopically, thus allowing differentiation between unfertilised oocytes, normal embryos and abnormal and dead embryos. Furthermore, doubts about embryo viability can also be confirmed by in vitro culture.

There are two main causes of embryonic death, viz. genetic and environmental factors (Boyd, 1965). These have been reviewed in detail by Ayalon (1978), who subdivided them further into genetic factors (both intrinsic and extrinsic), general and local environmental factors (nutrition of the cow, age of the dam, ambient temperature, genital tract infection), and hormonal asynchrony and imbalance.

Thus, even in apparently reproductively normal cows, there are biological constraints on the number of oocytes that become fertilised, and the number of embryos and fetuses that survive resulting in the birth of a normal live calf at term. Thus, there are other reasons why the reproductive performance of an individual cow, and collectively the herd, are suboptimal and can be improved. It should be one of the roles of the veterinarian to ensure that an individual cow's reproductive performance, and that of the herd of which it is a member, are maintained at their required optimum level.

INVESTIGATION OF THE INDIVIDUAL SUBFERTILE COW

Before discussing the investigation of the individual subfertile cow, it is important to define the meaning of the term. This has already been discussed in Chapter 22, but it is worthwhile repeating it here. A fertile cow is one that produces a calf at a regular preferred interval, which will be determined by the management policy for the herd. It must be stressed that a cow must calve at a reasonable time interval to ensure that milk yield does not decline to an unacceptable and uneconomic level. Other factors will have an influence on the required frequency of calving. These include milk yield, variations in milk prices and the requirements to calve at a specific season of the year (this is particularly important in pastoral dairying; see below). A cow that does not satisfy the management requirement for the herd is deemed subfertile, and one that is incapable of ever producing a calf is sterile.

History

Before performing a clinical examination it is important to obtain a detailed and accurate history, particularly a breeding history, of the cow. The following should be obtained:

- age
- parity (there are certain conditions that can be excluded in nulliparous, as opposed to parous, individuals)
- date of last calving, together with information on the occurrence of dystocia, retained fetal membranes or puerperal infection
- dates of observed oestrus since calving when insemination has not occurred (sometimes referred to as oestrus-not-served)
- presence of any abnormal vulval discharge
- dates of services or inseminations, preferably with the identity of the bull
- if uncontrolled natural service is used, then the date when the bull was first allowed access to the cows
- previous fertility records, particularly calving–conception intervals and services per conception
- details of feeding, management and milk yield; in suckler cows the number of calves suckled
- details of health, i.e. signs of milk fever, mastitis, ketosis, lameness
- details of fertility of other cows or heifers in the group or herd.

Clinical examination

A good general clinical examination should be undertaken with assessment of body condition score and possibly live weight. The genital system should then be examined in detail; where it is available, transrectal ultrasonography should be used.

- Inspect the vulva, perineum and vestibule for evidence of current or healed lesions and discharges.
- Examine the base of the tail for signs of rub marks, and back and flanks for hoof marks, which might indicate that the individual has been ridden by other cows.
- Explore the vagina by hand or speculum to examine the mucosa and to inspect the mucus.
- Palpate the cervix per rectum to determine its size and position in relation to the pelvic brim, and the uterine horns to determine if involution is complete (see Chapter 7). Assess the texture of the uterus, the degree of tone, the mobility of the horns and the absence of adhesions. Image the same structures using transrectal ultrasonography. *The absence of any signs of pregnancy should be confirmed*.
- Palpate the uterine tubes for evidence of induration or increased size.
- Palpate the ovarian bursa for evidence of adhesions.
- Palpate the ovaries to note their position, mobility and size and to identify the presence of any structures. Confirm the nature of the structures using ultrasonography.

Diagnostic tests

Single blood or milk progesterone assays are useful to identify the presence of luteal tissue if concentrations are high (4–6 ng/ml in plasma or 12–18 ng/ml in milk); sequential assays over several days are better. Specific serological tests – for example, the mucus agglutination or fluorescent antibody tests for *Campylobacter fetus*, or the investigation of a wide range of infectious agents by taking single or paired blood samples (see Chapter 23) – can be diagnostic for many diseases. Swabbing for subsequent bacterial culture and endometrial biopsy are of limited value. The PSP (phenolsulphonphthalein) test for tubal patency can also be used to demonstrate occluded uterine tubes (see Chapter 22).

Summary of the signs of infertility: the diagnosis, cause and treatment

The following summary describes a procedure for investigating an infertile animal on the basis of the clinical history, signs and examination, with an indication of a possible diagnosis of the cause and its treatment. These are covered in detail in Chapters 22 and 23.

No observed oestrus

Rectal palpation or diagnostic ultrasonography should establish the presence or absence of pregnancy; if the individual is pregnant it should be recorded. However, if there is any doubt, or if it might be pregnancy at a stage that is too early to be detected by the method used, then a re-examination at a later date is required. If there is no pregnancy, then examination of the ovaries is the next step.

Absence of ovaries. This is uncommon. It is due to ovarian agenesis or freemartinism and hence will be seen only in a nulliparous animal. There is no treatment, and thus the animal should be culled.

Small inactive ovaries. If the ovaries are small, narrow and functionless in a heifer, then this is due to delayed puberty or ovarian hypoplasia. There is no treatment; if delayed puberty is suspected, normal cyclic activity should eventually occur.

If the ovaries are flattened, smooth, small and inactive and the horns are flaccid, then this is true anoestrus; confirmation may require a repeat examination or a milk progesterone determination 10 days later. This may be due to high yield, suckling, negative energy balance, intercurrent disease, severe postpartum weight loss or trace-element deficiency.

Assess body condition, and calculate nutrient intake. Correct any deficiencies if present. Insert a progesterone-releasing device (PRID) or a controlled internal drug release device (CIDR) for 12 days; oestrus should occur several days after withdrawal. Alternatively, gouadotrophin-releasing hormone (GnRH) analogues can be used with oestrus occurring in 1–3 weeks. In beef cattle whose milk is not used for human consumption, a norgestamet (Crestar) implant and injection, together with 400–750 IU of equine chorionic gonadotrophin (eCG) or 1 mg oestradiol benzoate at the time of implant removal, can be used (see Chapter 22).

Presence of one or rarely more corpora lutea. There are a number of explanations:

- Pregnancy; if in doubt re-examine later and check records.
- Non-detected oestrus; improve detection with increased frequency of observation, heat mount detectors or tail paint, or induce luteolysis with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or an analogue, followed by artificial insemination at observed oestrus or at a fixed time.
- Suboestrus or 'silent heat'; this is most likely at first ovulation after calving. Treat with $PGF_{2\alpha}$ or an analogue as above.
- Persistent corpus luteum; thoroughly palpate the uterus, using retraction forceps if necessary, to confirm the absence of pregnancy. It may be due to pyometra, chronic endometritis, mummified fetus or, rarely, a non-specific cause. Treat with $PGF_{2\alpha}$ or an analogue.

Small active ovaries. The identification of follicular activity, perhaps together with a regressing corpus luteum or evidence of recent ovulation associated with good uterine tone, indicates that the animal is coming into oestrus, is in oestrus or has been in oestrus (differentiation between a developing and a regressing CL can be difficult ultrasonographically). Careful inspection of the vulva at the time of palpation may reveal clear mucus, and if there is a small amount of fresh bright red blood then the animal has recently been in oestrus (metoestral bleeding). Re-examination in 10 days should reveal the presence of a CL if the cow is undergoing cyclical activity.

Ovarian cysts (luteal or follicular). The presence of one or both enlarged ovaries, containing one or more fluid-filled, thin- or thick-walled structures more than 2.5 cm in diameter, can be confirmed using ultrasonography (see Chapter 22), and should confirm the diagnosis. A repeat examination several days later will confirm their persistence, and a milk or blood progesterone determination will show the presence of luteal tissue. Treat with $PGF_{2\alpha}$ or an analogue if luteal or, in the case of follicular cysts, with GnRH, human chorlonic gonadotrophin (hCG) or progesterone preparations such as a PRID.

Prolonged interoestrus interval

The ovaries and genital tract should be examined per rectum. If the ovaries are normal, subfertility may be due to:

- Non-detected oestrus; if the interval between successive heats is approximately twice the interoestrus interval, i.e. 36–48 days, then this indicates that one oestrus has not been observed or recorded. Irregular intervals that are not the product of the normal interval are likely to be due to incorrect identification of oestrus (see Chapter 22). If large numbers of animals are reported then this suggests that the oestrus detection rate is poor. If a susceptible corpus luteum is present, $PGF_{2\alpha}$ can be used to cause luteolysis and oestrus in 2–5 days' time. Methods of improving oestrus detection should be implemented (see Chapter 22).
- Embryonic or fetal death; the interval between successive heats is unlikely to be an approximate multiple of 21, and thus will be some other interval such as 35 or 46 days. In an individual cow it is probably of no significance, but if a number of animals are involved, especially if natural service is used, specific pathogens should be eliminated (see Chapter 23) and other causes sought.

Regular return to oestrus (Repeat Breeder or cyclic non-breeder)

The ovaries and genital tract should be examined per rectum to determine the presence of gross abnormalities, such as severe adhesions or uterine infection. This condition can occur only if there is a failure of fertilisation or embryonic death before day 12 of the oestrous cycle (before or at the time of the maternal recognition of pregnancy). There are a number of possible causes:

- Infertile bull; if a number of cows and heifers are involved he should be examined as described in Chapter 30. If artificial insemination is done by trained inseminators from an approved centre, then poor AI technique can probably be excluded. It must be remembered that there is considerable variation in the fertility of bulls standing at artificial insemination studs, although they should be above a minimal level. Where possible, semen from a bull with a high fertility should be selected. Where DIY AI is performed by the owner or herd manager, then it is important to ensure that the person is adequately trained and that the procedure is being done correctly. In some animals, the cervix can be very difficult to traverse, even by experienced inseminators.
- Incorrect timing of service or artificial insemination; this is unlikely to occur repeatedly, unless the time of ovulation is asynchronous. If a significant number of animals are involved, advice on the correct time may be worthwhile or else fixed-time artificial insemination after the administration of $PGF_{2\alpha}$ or progestogens (see Chapter 1) should be instituted.
- Nutritional deficiency or excess; check diet.
- Occluded uterine tubes; palpate carefully and use the PSP test to confirm.
- Anatomical defects; palpate carefully. If the animal is nulliparous, look for segmental aplasia; if it is a parous animal, check for ovarobursal or uterine adhesions.
- Endometritis; if there are clinical signs, diagnosis is simple but subclinical disease can be diagnosed only by biopsy. If endometritis is suspected, treat with appropriate intrauterine antibiotics, or $PGF_{2\alpha}$ to shorten the luteal phase preceding insemination. If there is evidence of a persistent discharge, the possibility of urine pooling in the anterior vagina should be investigated.
- Delayed ovulation; diagnosis is difficult. Treat with GnRH or hCG at the time of insemination or repeat insemination on the subsequent day.
- Anovulation; diagnosis depends on ovarian palpation or transrectal ultrasonography 7–10 days after oestrus to demonstrate failure of ovulation by absence of a corpus luteum. Treat with GnRH or hCG at the time of insemination.
- Luteal deficiency; there is evidence that this is quite common although it is difficult to prove. Once other causes have been eliminated, then a luteotrophic agent, such as hCG, might be worthwhile at 2–3 days after subsequent inseminations to improve corpus luteum formation, or at midcycle to stimulate accessory corpus luteum formation.

Alternatively, a GnRH analogue can be administered at day 12 or 13 after insemination, and intravaginal progesterone from about 4 days after insemination.

Short interoestrus interval

This condition is usually identified by other signs of nymphomania and palpation or imaging of ovaries. The cause may be:

- Enlarged ovaries; if either one or, more likely, both contain one or more thin-walled, fluid-filled structures this should confirm the diagnosis of follicular cysts. Treat with GnRH, hCG or a PRID.
- Artificial insemination at the wrong time due to incorrect oestrus detection. This is often preceded or followed by an extended interval so that the sum of the two intervals is 36–48 days. If large numbers of cows have the same history, oestrus detection should be improved (see Chapter 22).

Abortion

This is defined as the production of one or more calves between 152 and 270 days of gestation; they either are born dead or survive for less than 24 hours.

The cow should be isolated, the fetus and fetal membranes should be retained and the case treated as a suspected *Brucella* abortion under the brucellosis scheme. In the UK, this requires any abortion occurring less than 271 days after insemination to be reported to the Ministry of Agriculture, and clotted blood, milk and a vaginal swab submitted for laboratory examination. The physical appearance of the fetus and fetal membranes should be noted, the fetus aged approximately and this confirmed by the service or insemination date if available. One endeavours to eliminate infection as a cause when one is unable to demonstrate organisms in the fetus, fetal membranes, and vaginal and uterine discharges and/or by the demonstration of specific antibodies in body fluids. Where possible the whole fetus should be submitted to the laboratory for cultural examination.

Possible infectious causes of abortion are:

1. *Brucella abortus*; occurs at 6–9 months of gestation.
2. *Leptospira* spp.; occurs at 6–9 months of gestation.
3. *Listeria monocytogenes*; sporadic outbreaks occur at 6–9 months of gestation.
4. *Campylobacter fetus (venerealis)*; occurs at 5–7 months of gestation.
5. *Tritrichomonas fetus*; occurs before 5 months of gestation.
6. *Salmonella* spp., especially *S. dublin*; is usually sporadic with no specific time, although usually about 7 months of gestation.
7. *Arcanobacterium (Actinomyces, Corynebacterium) pyogenes*; is usually sporadic and occurs at any stage.
8. *Myobacterium tuberculosis*; occurs at any stage.
9. Mycotic agents, *Aspergillus* spp., *Absidia* spp., *Mucoralis* group, *Mortiella* spp.; occurs from 4 months to term.
10. *Bacillus licheniformis*; gives rise to sporadic late abortions.
11. *Neospora caninum*; gives rise to late abortions, and is an increasingly diagnosed cause of fetopathy.
12. Infectious bovine rhinotracheitis–infectious pustular vulvovaginitis (IBR–IPV) virus; occurs at 4–7 months of gestation.
13. Bovine viral diarrhoea (BVD) virus; occurs at any stage.

The approach to investigating the cause of abortion will depend upon the frequency. If sporadic, then a full laboratory investigation is probably unnecessary because many abortions are not associated with infection. However, if it exceeds 3–5% of the herd – and it is important to consider stillbirths and premature calvings (excluding twins) in this calculation – then a thorough investigation should be implemented. The approach recommended by Pritchard (1993) should be followed:

Sporadic abortions

1. Perform a statutory brucellosis investigation.
2. Determine if all abortions have been reported and that it is a true sporadic case. If so, proceed to (3); if not, or if there is any doubt,

then follow the procedure for an outbreak investigation (see below).
3. Clinical examination of the cow.
4. Examine the placenta for evidence of obvious lesions, particularly fungi or *Bacillus licheniformis* (see Chapter 23).
5. Submit serum for *Leptospira* serovar *hardjo* serology unless it is a vaccinated herd.
6. Request culture of a vaginal swab for *Salmonella dublin*.
7. Obtain a detailed history of changes in husbandry, movement of livestock, purchase of animals, hiring of bulls, signs of ill-health and age of aborting cows.

Abortion outbreak

1. Repeat (1), (2), (3), (4) and (7) above.
2. Ideally, submit one or more fresh whole fetuses and placentas – *or* several complete fresh cotyledons.
3. Fetal stomach contents (2 ml) should be aseptically collected using a vacutainer or syringe and needle.
4. Collect fluid from thorax or abdomen (2 ml) using the methods described in (3).
5. Submit about 5 g of fresh lung, liver, thymus and salivary gland. All tissues and other samples should be refrigerated and packed with ice, but not frozen.
6. Take air-dried, acetone-fixed impression smears from fresh cotyledons, lung, liver and kidney.
7. Submit formal-saline-fixed cotyledon, fetal liver, heart and lung.
8. Take two 7 ml vacutainers of clotted blood from all cows that have recently aborted.
9. Repeat samples from the same cows as in (8) 2–3 weeks later for possible rising antibody titres in the serum.

If an infectious cause is not identified using routine diagnostic tests it may be necessary to extend the investigation in an attempt to confirm the presence of a less common infectious agent. However, abortions can be caused by many other factors: congenital defects due to genetic factors or teratogens; trauma; allergies; dietary excesses such as high protein pastures (Norton and Campbell, 1990), or deficiencies such as iodine; poisonous plants such as brassicas, hemlock and, in the USA, pine needles (*Pinus ponderosa*); chemicals such as nitrates and chlorinated naphthalene; and hormones such as prostaglandins. Diagnosis is generally based on circumstantial evidence and, in some cases, the presence of pathognomonic lesions.

It should be noted that cause of many abortions is not ascertained, despite meticulous investigation (see Table 23.1).

EVALUATION OF DAIRY HERD FERTILITY

Regular, accurate evaluation of the fertility status of the dairy herd is an essential part of a control programme. In an ‘all-year-round calving’ herd it should be done at least twice a year; in a seasonally calving herd it should be done at times appropriate to the desired calving pattern. Obviously, such evaluations are an important prerequisite when investigating herd subfertility (Eddy, 1980).

In order to evaluate the fertility status of a herd it is necessary first of all to quantify certain reproductive values, and in order to do this it is necessary to have access to records of reproductive events. This presents few problems if details are recorded as described later (pp. 524–529); however, on many farms the information is incomplete and is dispersed in many places such as on milk record sheets, artificial insemination receipts and records or the farm diary. Obviously, the accuracy and value of such calculations will depend upon the quantity and quality of the information provided, and it will be necessary to modify one’s assessment accordingly, depending upon clinical judgement, the history of the herd and the primary complaints of the herd manager or owner.

The minimum information required is identity of cow; last calving date; first and subsequent service or insemination dates; confirmation of pregnancy.

The following measurements of fertility can be made (the terms and definitions used are those stated in *Dairy Herd Fertility: Reproductive Terms and Definitions* (Ministry of Agriculture, Fisheries and Food, Booklet 2476)).

Non-return rate to first insemination

This is the percentage of cows or heifers, in a particular group over a specified period of time, which have not been presented for a repeat insemination within a specific period of time. The periods are usually 30–60 days or 49 days. This is used, particularly in artificial insemination centres, to monitor the fertility of bulls and the performance of inseminators. Figures of 80% are frequently obtained at 30–60 days, which is often more than 20% better than the true calving rate to first insemination. The discrepancy is due to failure to identify, record and report if the cow returns to oestrus; culling the cow after she has returned to oestrus; subsequently using natural service; or prenatal death. It is therefore an imperfect measure of fertility but can be useful if no pregnancy diagnosis is performed.

Calving interval and calving index (CI)

The calving interval is the interval (in days) between successive calvings; for an individual cow the calving index is the mean calving interval of all the cows in a herd at a specific point in time, calculated retrospectively from their most recent calving date. These two measurements have been used traditionally as a measure of fertility, since they indicate how closely the individual cow or herd approximates to the accepted optimum of 365 days.

The disadvantages of these measurements are that they are historical, in that they are calculated retrospectively; furthermore, the calving index can give an overoptimistic assessment of fertility when many of the cows that fail to become pregnant are culled.

More contemporary measurements are the *predicted calving interval or index*, where the estimated date of the next calving is calculated by counting 280 days (mean gestation length) from the assumed date of conception (last recorded service date). This assumes that pregnancy will be maintained; both values should be 365 days.

Calving to conception interval (CCI)

The calving interval (or index, CI) is the sum of two components, the interval from the last calving date to the date of conception (*a*) and the length of gestation (*b*). Thus:

$$\text{CI} = a + b$$

Therefore:

$$\text{CI} = 85 \text{ days} + 280 \text{ days} = 365 \text{ days}$$

The calving to conception interval (CCI) is calculated by counting the number of days from calving to the service that resulted in pregnancy (effective service); this is usually the last recorded service date. The CCI is a useful measurement of fertility but requires a positive diagnosis of pregnancy to be made. It is influenced by two factors: how soon after calving the cows are re-bred and how readily they become pregnant when they have been served. The CCI can be expressed thus:

$$\text{Mean CCI} = c + d$$

where c is the mean calving to first service interval and d is the mean first service to conception interval. Therefore:

$$\text{Mean CCI} = 65 \text{ days} + 20 \text{ days} = 85 \text{ days}$$

The mean CCI is a useful measure of fertility, provided that the interval from calving to first service is stated, since this probably will have the greatest influence upon its length.

Days open

This is defined as the interval, in days, from calving to the subsequent effective service date of those cows that conceive, and from calving to culling or death for those cows that did not conceive. Numerically, it will always be greater than the mean CCI unless all cows that are served conceive, in which case it would be the same. Days open is a popular measurement of fertility in North America.

Calving to first service interval

In the case of a herd that calves all the year round, a mean value of 65 days should result in a mean CCI of 85 days (see above). The factors that influence the calving to first service interval are:

- Breeding policy of the farm. Although cows will return to oestrus after calving as early as 2–3 weeks, they should not be served before 45 days, and in the case of first calvers, high-yielding cows and those that have had dystocia and problems during the puerperium (see Chapter 7) slightly longer should elapse. Thus, in a seasonal calving herd, those animals that calve early in the season will have their first service delayed and, for those that calve late, it may be necessary to advance the date of first service, thereby tightening the calving pattern.

- Delayed return of cyclical activity after calving, i.e. acyclicity or true anoestrus (see Chapter 22).
- Failure to detect oestrus in those cows that have resumed normal cyclical activity.

Factors (2) and (3) can be improved by ensuring that cows have returned to cyclical activity postpartum. This can be done by regular and routine examination per rectum of those cows that have failed to be seen in oestrus by 42 days postpartum and by the use of milk progesterone assays. Detection of oestrus depends upon the herd manager knowing the true signs of oestrus, having a regular routine, recording the events and using oestrus detection aids (see Chapter 22).

Overall pregnancy rate

This (originally called the overall conception rate) is the number of services given to a defined group of cows or heifers, over a specified period of time, which result in a diagnosed pregnancy not less than 42 days after service; the figure is expressed as a percentage of the total number of all services and should include culled cows. The method of pregnancy diagnosis should be specified. The *first service pregnancy rate* is usually calculated separately and obviously refers to first services only. Thus in a 12-month period, if 100 cows receive 180 services, of which 90 resulted in a confirmed pregnancy, the overall pregnancy rate would be 50%.

The pregnancy rate is influenced by:

- the correct timing of artificial insemination (see Chapter 22), which will be dependent particularly on the accuracy of oestrus detection
- correct artificial insemination technique, and handling and storage of semen, especially if 'DIY AI' is used
- good fertility of the bull if natural service is used, and the absence of venereal disease
- adequate nutritional status of cows and heifers at the time of service and afterwards (see Chapter 22)
- complete uterine involution and absence of uterine infection (see Chapters 22 and 23); this is especially relevant to first-service conception rates.

The pregnancy rate to first service and overall pregnancy rate are very useful measures of fertility; the latter is used to calculate the *reproductive efficiency* of the herd (see below). The rates for the first service are usually slightly higher than those for all services, because the latter group will include those cows that may be sterile and receive many services before they are culled. Mean values of 60 and 58%, respectively, are obtainable, although in many parts of the world the figures are much lower (Table 24.4).

In order to identify the influence of management changes, particularly nutrition, it is worthwhile calculating these two parameters on a monthly basis (provided that there are a minimum of 10 services per month), or expressing them as Cu-Sums (see below).

Table 24.4 Herd target and interference levels

Index	*Target level*	*Interference level*
Mean calving to first service interval (days)	65	70
Mean calving to conception (pregnancy) interval (days)	85	95
Mean interval from first service to conception (pregnancy) (days)	20	25
First service submission rate (%)	80	70
Overall pregnancy rate (%)	58	50
First service pregnancy rate (%)	60	50
Reproductive efficiency (%)	46	35
Cows served that conceive (%)	95	90

The above values are those required to achieve a 365-day calving index for the herd; in high-yielding animals such values are not achievable, and the target and interference values must be adjusted accordingly

Oestrus detection

Improving the detection of oestrus has a much greater influence upon reducing the calving to conception interval than improving the pregnancy rates; the latter can only be improved up to a certain level (Esslemont and Ellis, 1974; Esslemont and Eddy, 1977). It is important that *all* observed heats are recorded during the voluntary waiting period (VWP) which will be before the earliest date for service (ideally this should be 45 days, although the required calving pattern for the herd will influence this figure). This enables herd managers to anticipate the time of a subsequent oestrus, and thus should improve the detection rate. It also enables the early detection of acyclical cows.

It is possible to estimate the oestrus detection rate, but it is important to stress that it is an estimate and not an accurate measurement. A number of different methods are used and they all have some measure of error (Esslemont et al., 1985). One method is to determine the number of supposed missed oestrous periods. Thus an interval of 36–48 days (2 × 18–24) suggests that one oestrus has been missed, and an interval of 54–72 days (3 × 18–24) suggests that two have been missed, although this latter range is fairly wide and can lead to errors. The percentage oestrus detection rate (ODR) is calculated thus:

$$\text{ODR} = \frac{\text{No. of interservice intervals recorded}}{\text{No. of interservice intervals recorded + No. of missed oestrous periods}} \times 100$$

This overestimated the heat detection by about 5% (Esslemont et al., 1985).

Another method is to calculate the mean interservice interval for the herd, so that the ODR is calculated thus:

$$\text{ODR} = \frac{21}{\text{Mean interservice interval}} \times 100$$

A large number of short interservice intervals due to inaccurate oestrus detection (see below) can overestimate the oestrus detection rate.

One simple method of assessing the oestrus detection rate at routine sessions of pregnancy diagnosis will be the number of cows that are assumed by the herd manager to be pregnant and thus submitted for examination, but are found to be non-pregnant. Non-pregnant cows should have returned to oestrus since service or artificial insemination, and hence should have been seen in oestrus.

In many apparently well-managed dairy herds where the calving to first service interval is on target, there is a failure to detect returns to oestrus in non-pregnant cows. This will result in a large number of interoestrus intervals that are two or three times the normal interval. Milk progesterone assays can be helpful (see pp. 538–539).

Poor oestrus detection may be due to:

- poor accommodation inhibiting cows from exhibiting overt signs of oestrus
- poor lighting or identification of animals
- failure to record signs of approaching oestrus and signs of true oestrus
- inadequate regimen for observing cows for signs of oestrus (see Chapter 22), perhaps due to the herd manager being overworked.

  Methods of improving and aiding the detection of oestrus are described in Chapter 22.

Distribution of interoestrus or interservice intervals

Analysis of the distribution of interoestrus, or more usually interservice, intervals will provide useful information about a number of aspects of the reproductive status and management of the herd. These intervals are subdivided into the following groups: (a) 2–17 days, excluding those intervals of 1 day associated with double fixed-time artificial insemination (see Chapter 3); (b) 18–24 days, the normal interoestrus interval; (c) 25–35 days; (d) 36–48 days, twice the normal interoestrus interval; and (e) more than 48 days. In a well-managed herd, with accurate detection of oestrus and presentation for service, at least 45% of intervals should be within the 18–24 day range, thus 12% for (a), 53% for (b), 15% for (c), 10% for (d) and 10% for (e) (Anon, 1984). If the percentage for the 36–48-day interval is high and the figures for the 18–24-day interval are low, then this is indicative of poor oestrus detection.

A large number of intervals in groups (a) and (c) suggests inaccurate identification of oestrus, whilst a large number of intervals in groups (c), (d) and (e) could be associated with a late embryonic or early fetal death problem (see pp. 512–514). As with all fertility measurements they should be evaluated together with other parameters.

Using the percentage distribution of the interoestrus and interservice intervals, a single figure referred to as the *oestrus detection efficiency* (ODE) is sometimes calculated as follows:

$$\text{ODE} = \frac{b + d}{a + b + c + 2\,(d + e)} \times 100$$

A good ODE would be 50% or more.

First-service submission rate

Measurements of oestrus detection rates are not very accurate, and for this reason the first-service submission rate can be calculated; this is a measure of how quickly cows are served after they have become eligible for service (after the end of the voluntary waiting period). It is defined as the number of cows or heifers served within a 21- or 24-day period expressed as a percentage of the number of cows or heifers that are at, or beyond, the earliest date at the start of the 21- or 24-day period.

Thus once a cow has reached the earliest time after calving that she is ready for service, i.e. above 45 days in all-the-year-round calving herds, then she should be served or inseminated within the next 21 or 24 days. However, pregnancy rates will probably not reach their optimum for at least 90 days postpartum (De Kruif, 1975; Williamson et al., 1980; Esslemont et al., 1985). Furthermore, cows that have suffered dystocia or an abnormal puerperium should not be served before 60 days postpartum and should be examined routinely before service. It has been shown that there is a good correlation between the physical state of the uterus, as determined by transrectal examination, and the quantity, colour and smell of mucopurulent discharge and the regeneration of the endometrium (Studer and Morrow, 1978).

Heifers, and cows yielding more than 40 litres per day, should not be served before 50 days postpartum.

The submission rate is influenced by the time interval to the resumption of normal cyclical activity after calving, the detection of oestrus in those cows that have resumed normal cyclical activity, and their presentation for service or artificial insemination. A good submission rate is 80%. In seasonally calving herds (see below), it will tend to be higher in those cows that calve earlier than in the later calvers. This is because, with the former, the presence of more non-pregnant cows will ensure greater interaction when they are in oestrus, which should improve its detection (Anon, 1984). The calculation of a rolling average submission rate can be difficult unless it is part of a computer program. A relatively simple method of obtaining a fairly accurate measurement is to list all cows that are ready for service (at or beyond the earliest service date of 45 days, or whatever has been decided upon, since calving) at the start of each 21- or 24-day service period. At the end of this period identify all those that have been served. The percentage submission rate is calculated thus:

$$\frac{\text{No. of cows served that are listed}}{\text{No. of cows that are listed}} \times 100$$

Another method is to list all cows chronologically in order of the calving date. Add 21 days to the earliest date on or after which they are ready for service, i.e. 45 + 21 (24) = 66 (69) days. Thus every cow should be served before the target date of 66 or 69 days postpartum. The submission rate is calculated thus:

$$\frac{\text{No. of cows served on or before the target date}}{\text{No. of cows that should have been served on or before the target date}} \times 100$$

In a tight seasonally calved herd, the earliest service date will be selected in relation to when the cows are required to calve down the following year. Thus, cows that calve early in the season will have a longer time interval before they need to be served compared with those that calve late in the season. The choice of 21 days is based on the assumption that this is the mean interoestrus interval. However, 24 days can be used as it is the normal maximum interval. It is irrelevant which is selected as long as its use is consistent.

Reproductive efficiency

Attempts have been made to calculate a single index that provides an overall measurement of fertility and takes into account many different parameters. One such measurement is the reproductive efficiency (RE) of the herd (Anon, 1984). It is calculated thus:

$$\text{RE} = \frac{\text{Submission rate} \times \text{Overall pregnancy rate}}{100}$$

Thus if the submission rate is high, i.e. 80%, and the overall pregnancy rate is good, i.e. 55%, then the RE is 44. In a herd with a more modest submission rate of 70% and an overall pregnancy rate of 50%, the RE is 35.

The advantage of this measurement is that an artificially high submission rate, obtained by an overzealous herd manager presenting cows for artificial insemination when they are not in oestrus, will be compensated by a reduced pregnancy rate. Conversely, an overcautious herd manager may have a reduced submission rate but although the pregnancy rate may rise to 65%, producing a reasonable RE value, it is not possible to increase this further.

Fertility factor

Another composite measurement can be obtained by calculating the fertility factor (FF) (Esslemont et al., 1985). This is obtained following the calculation of the overall pregnancy rate (OPR) and the estimation of the oestrus detection rate (ODR). It is calculated thus:

$$\text{FF} = \frac{\text{ODR} \times \text{OPR}}{100}$$

Thus if the ODR is 60% and the OPR rate is 50%, then the FF is:

$$\frac{50 \times 60}{100} = 30$$

Another way of calculating this factor is to estimate how many cows in the herd become pregnant during a 21-day period after being detected in oestrus and inseminated; using the figures above it would be 30%. As Esslemont et al. (1985) comments: ‘Most farmers’ estimates would be higher.’

Culling rate

One method of achieving a CI of around 365 days is by culling those cows that are slow to get in calf. This is rarely cost-effective because it will be necessary to replace the culled cow with a heifer. The purchase price or the cost of rearing such a replacement is much greater than the price obtained for the cull. Overall culling figures for infertility should not exceed 5%; thus 95% of the cows that calve and are served should become pregnant again.

Fertility index

Another single index that can be calculated and takes into consideration the pregnancy rate to first service, services per conception, calving to conception interval and culling rate is the fertility index (De Kruif, 1975; Esslemont and Eddy, 1977; Esslemont et al., 1985).

THE COST OF INFERTILITY IN DAIRY HERDS

Poor fertility reduces the profitability of a dairy enterprise. Various figures have been quoted for the financial loss and these are discussed at the beginning of Chapter 22. However, some recent figures are listed in Tables 24.5 and 24.6. It is important to remember that actual values will vary from year to year depending on the economics of the dairy industry.

RECORDING SYSTEMS

Irrespective of the recording system used there are certain basic requirements. Perhaps the most important is the ability to identify easily and accurately every cow from virtually any point whether she is standing or recumbent. This enables all people working on the farm to identify cows in oestrus, thus assisting the herd manager. Each cow should have a permanent freeze brand on the rump that must be kept clean and clipped, together with a collar or large ear tag with a number.

Table 24.5 The FERTEX score for a dairy herd (Kossaibati and Esslemont, 1997)

A. Standard indices and the penalty or bonus incurred for divergence

	Standard values	*Divergence from standard values: penalty or bonus*
Calving index (days)	360	£3.00/day
FTC culling rate (%)	5.3	£770/cull
Services/conception	1.8	£20/service

B. Worked example for a herd. A figure of approximately £88 per cow is obtained

	Actual value	*Target*	*Excess*	*Cost of unit*	*Total cost*	*Cost/100 cows*
Calving index (days)	380	368	12	£3	£36	£3600
FTC culling rate (%)	11	5.3	5.7	£770	£4389	£4389
Services/conception	2.2	1.8	0.4	£20	£8	£800

Total cost/100 cows = £8789

FTC = failure to conceive
These values are for 1995 prices in the UK; they will vary depending on the changes in the costs and the sale price of milk

Table 24.6 Costs for some common diseases, based on DAISY (Kossaibati and Esslemont, 1997)

Disorder or disease	*Mean incidence (%)*	*Direct cost (£)*	*Indirect cost (£)*	*Total/case or cow (£)*
Retained fetal membranes	5.7	83.25	215.07	298.32
Vulval discharge	19.2	70.81	90.77	161.58
Oestrus not observed	12.61	12.61	0	12.61

It will be necessary to record, at least, the following: calving date; all service or artificial insemination dates; results of pregnancy diagnosis. In addition, the following are needed: dates of oestruses during the voluntary waiting period; the identity of the sires used; and parturient and periparturient problems and diseases.

There are many and varied recording systems ranging from simple manual ones involving the use of notebooks and diaries to sophisticated on-farm computers with a keyboard and visual display unit adjacent to the milking parlours and cattle housing. Most systems fall between these two extremes.

The investigation of infertility problems and the maintenance of good fertility require the keeping of accurate records of the reproductive history of each and every cow in the herd. The absence of accurate and accessible records makes the task of the veterinarian difficult, if not impossible. Some information is often available in an apparently unpromising situation: for example, artificial insemination receipts and milk recording sheets, especially if the herd is involved in milk recording schemes.

The value of any recording scheme is dependent upon the weakest link in the chain, which is usually the accuracy of the on-farm raw data. For this reason, the recording system must be designed to accommodate the weakest link. It is preferable to operate a simple but accurate system maintained enthusiastically, than a complex one with numerous errors and omissions.

Manual systems. A simple and reliable system is as follows:

- The herd manager keeps a simple pocket book in which all relevant information is immediately recorded, e.g. signs of oestrus or leucorrhoea, with the date and identity of the cow. This is then transferred to a cow record sheet and a herd record sheet.

- The cow record sheet can be an index card or a page in a book. The record should be permanent and kept in close proximity to the place where the veterinarian examines cows so that it is readily available during visits. Records should be kept clean and up to date. Details of veterinary examinations should be recorded (Figure 24.1).
- The herd record sheet should be kept in the dairy or milking parlour. The information can be recorded with cow identity listed numerically, in the order of the date of first service; however, the preferred system is in chronological order of calving. Every observed oestrus should be recorded (even those when a cow is not served), as should the target date for first service, date of each service, result of pregnancy diagnosis examination, expected calving date and any other information about the reproductive system or general health (Figure 24.2).
- As an alternative to record sheets, various pictorial display charts are available, either circular or rectangular. These have the advantage that by using colour codings for various reproductive states, e.g. freshly calved but not observed in oestrus, served but not confirmed pregnant, confirmed pregnant, they give a good visual display of the reproductive status of a herd. They have the big disadvantage of not providing a permanent record and not being tamper-proof.

Computerised systems. There are a large number of different systems, which can be divided into three main categories: those where the computer hardware is farm-based; those where the computer hardware is veterinary practice-based; those where the computer hardware is based at a bureau remote from either practice or farm, and to which the raw data are sent. Many of these systems also include the provision for recording production and other herd health data.

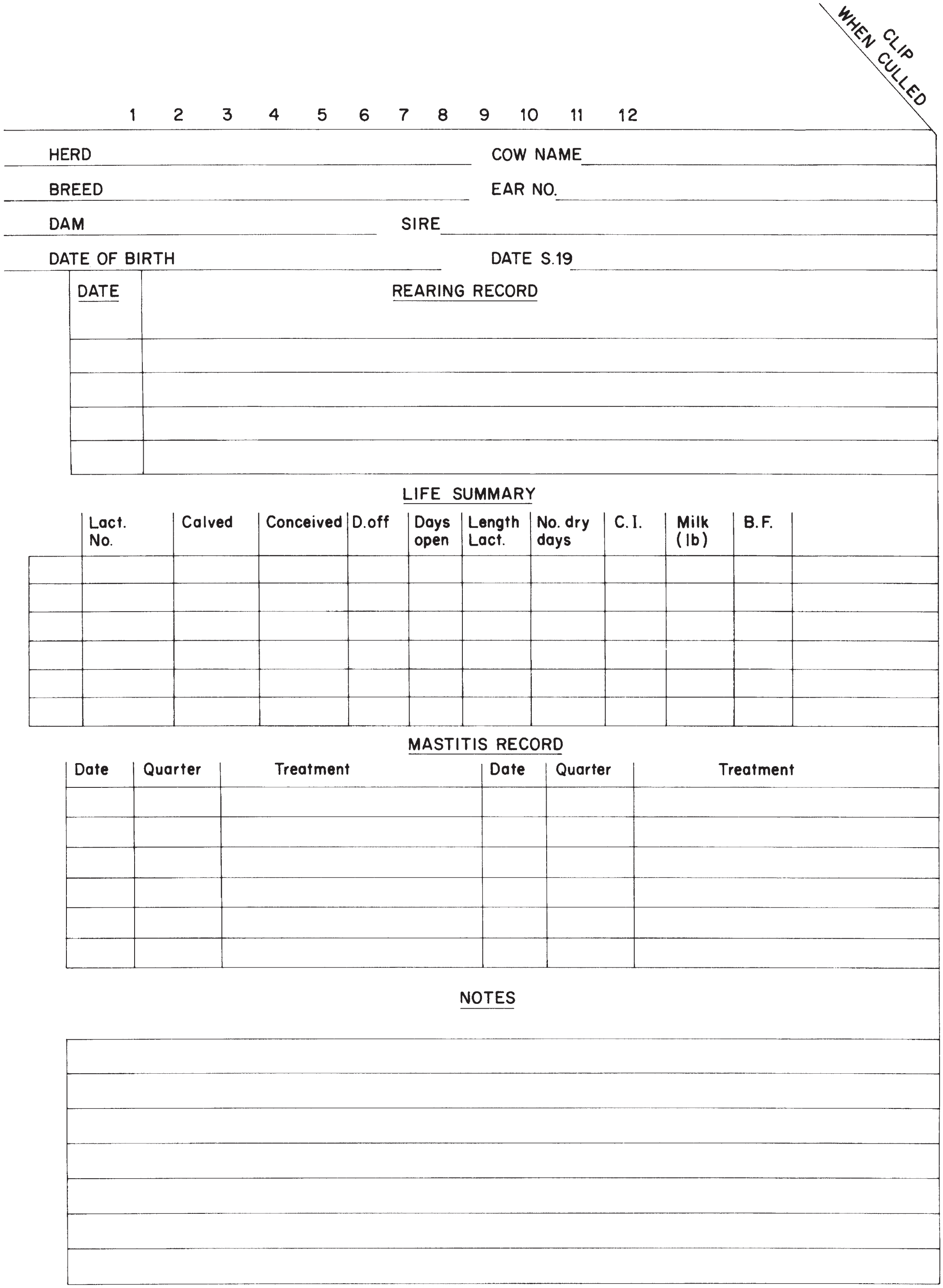
In small herds a manual system is perfectly adequate. However, in large herds of 100 or more cows there are many advantages to computerised systems, particularly the ability to produce action lists for herd manager and veterinarian of cows to be examined, and the ability to produce an analysis of the fertility status of a herd, frequently with graphic display. If a bureau service is used the turnround of information is sometimes too slow.

Visual presentation of data

Simple methods involving the use of herd record sheets (see Figure 24.2) or rotary boards, especially if they have some form of colour symbols, are good ways of presenting data so that they can assist the herd manager in managing the herd. Computerised systems often produce graphic printouts: for example, histograms of frequency of interoestrus /service intervals (Figure 24.3(a)), or pregnancy rates for different days of the week (Figure 24.3(b)) or for different bulls (Figure 24.3(c)).

One useful method of monitoring the contemporary fertility of a herd is to record the pregnancy rates to all services, or first services, as a cumulative sum or Cu-Sum (Gould, 1974) recorded in chronological order. Although several computer programs will produce a printout of Cu-Sums for overall pregnancy rate (Figures 24.4(a) and 24.4(b)) it is quite straightforward to produce one manually; all that is required is a sheet of squared graph paper, preferably marked in 0.1 inch squares.

Half-way down the vertical axis ‘ink in’ or cross the first small square; this represents the first service for the year or season. Move along one column and repeat the same procedure for the next small square; this represents the second service of the season or year. If this resulted in conception, as determined by pregnancy diagnosis, then the square in the line above is marked. If the cow does not conceive then the square in the line below is marked. This procedure is repeated for all the services with each vertical small column representing a cow (Eddy, 1980). If more than one cow is served on the same day then several squares will be marked. The Cu-Sum graph can be completed only after the presence or absence of conception has been confirmed by pregnancy diagnosis. Such a graph is shown in Figure 24.5; a rising graph represents a period when conception rates are greater than 50%, a falling graph a period when conception rates are less than 50%. The dates of the services should be placed on the horizontal axis and any changes in feeding, environment, management



CLIP WHEN CULLED

1 2 3 4 5 6 7 8 9 10 11 12

HERD ____________ COW NAME ____________

BREED ____________ EAR NO. ____________

DAM ____________ SIRE ____________

DATE OF BIRTH ____________ DATE S.19 ____________

DATE	REARING RECORD

LIFE SUMMARY

	Lact. No.	Calved	Conceived	D.off	Days open	Length Lact.	No. dry days	C.I.	Milk (lb)	B.F.	

MASTITIS RECORD

Date	Quarter	Treatment	Date	Quarter	Treatment

NOTES

Fig. 24.1 An example of a simple individual cow record card suitable for permanent recording.

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
Name	Lactation No.	Calving date	Bulling date (not served)	Bull reqd	Target date to serve	Interval to 1st A.I. or N.S.	1st service	Int	2nd service	Int	3rd service	Int	4th service	Int	5th service	Int	6th service	No. serves	Calving/conception days	Next calving due	Dry off date	Remarks
A 94	3	1/9	5/10	PJ	26/10	55	27/10	21	16/11									2	76	25/8		PREGNANT
A 105	2	3/9	14/10	H	28/10	61	3/11											1	61	11/8		PREGNANT
A 54	5	4/9		H	29/10	73	16/11	22	7/12	23	30/12							3	118	8/10		NON-OBSERVED OESTRUS PRID PREGNANT
X 514	8	5/9		–	–													0				CULL. CHRONIC LAMENESS
A 176	1	7/9		PJ	1/11	60	30/11											1	84	8/9		RFM. METRITIS PREGNANT
A 32	6	7/9	19/11	PJ	1/11	70	10/12	40	9/1	22	31/1							3	146	9/11		PREGNANT
X 499	9	10/9		PJ	4/11	55	4/11											1	55	12/8		PREGNANT
A 60	4	10/9	19/10	H	4/11	59	8/11											1	59	16/8		PREGNANT
A 81	3	11/9	23/10	PJ	5/11	63	14/11	21	5/12									2	84	13/9		MASTITIS. PREGNANT
A 98	2	12/9	17/10	ZT	6/11	56	7/11											1	56	15/8		PREGNANT
A 88	3	17/9		ZT	11/11	65	21/11	9	30/11	33	2/1							2	107	11/10		PREGNANT
					Total												Total					
					Mean												Mean					

Fig. 24.2 *For captions, see opposite*

Fig. 24.2 Herd record sheet for manual recording of fertility data. An explanation of the details and important data recorded is given below.

Column	*Comments*
A	Accurate identification is essential, preferably using a permanent identity from a freeze brand or ear tag.
B	Recording of the lactation number is useful but not essential. It enables differences in fertility that might be related to age to be identified, particularly the sensitive first lactation group that have yet to reach maturity.
C	Recording the calving dates is essential; they should be listed chronologically.
D	Recording these dates is not essential but it assists in the early identification of acyclical cows and helps in the anticipation of the first oestrus after the earliest service date.
E	This enables a comparison of the fertility of bulls used in the herd.
F	This date should be entered on the record sheet at the time of calving; the interval should not be less than 45 days.
G	This can be calculated once the date of the first service is known and thus enables the calving to first service interval to be known.
H	It is essential to record this figure accurately.
I to R	It is essential to record the dates of second and subsequent services so that the interservice intervals can be calculated, enabling an assessment of the efficiency and accuracy of oestrus detection to be made.
S	The number of services can be recorded, and this enables the number of services/conception to be calculated.
T	The calving to conception interval is calculated by counting the time interval (in days) from the calving date (column C) and the last recorded service date (columns H, J, L, N, P, R) after the cow has been confirmed in calf.
U	This is based on the assumption that the cow will remain pregnant to term and is calculated by assuming a fixed gestation length (i.e. 280 days) for a particular breed.
V	This is the target date for lactation to end and hence the cow will be 'dried off'. Normally this should be 60 days before the expected calving date.
W	This column enables brief comments to be recorded on facts that might have an influence on reproductive events.

or service procedure recorded as well. This will then give a good visual record of factors which might influence conception rates.

Cu-Sums can be used to represent other fertility parameters. Figure 24.6 is a computer printout for the first service submission rate.

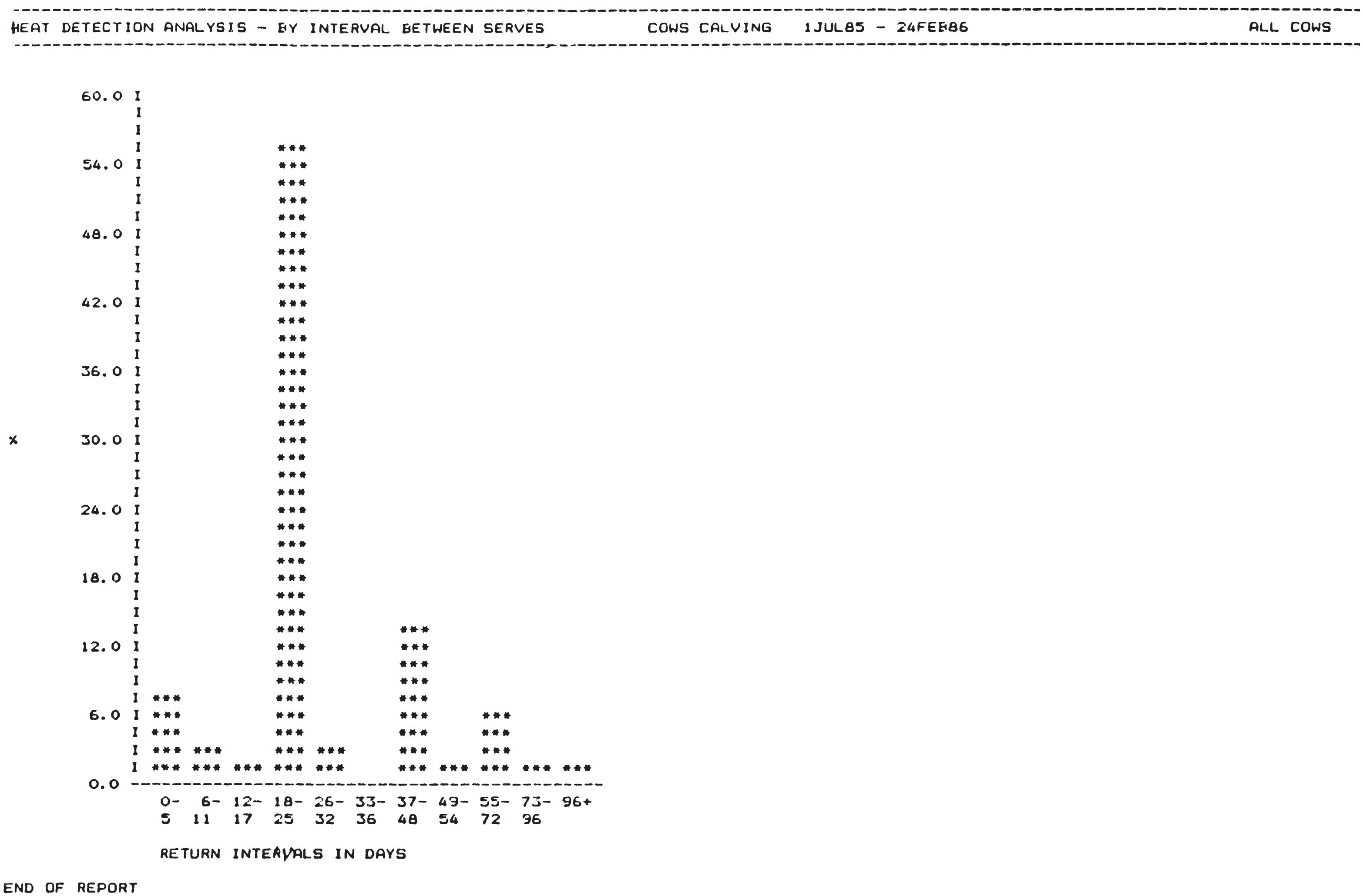
MANAGING FERTILITY AND ROUTINE VISITS IN DAIRY HERDS

Managing fertility so that it is maintained at an optimum level requires the active collaboration of herd manager, farm owner and veterinarian; all three must have a positive commitment to ensure that the system functions effectively.

It is important that fertility targets are agreed upon by all three; these may need some modification, particularly in the early stages of implementing a scheme and in relation to the overall policy and expectations of the farm. As well as agreeing on targets for fertility, it is also worthwhile establishing interference values so that when these are reached they will stimulate a response to initiate remedial action.

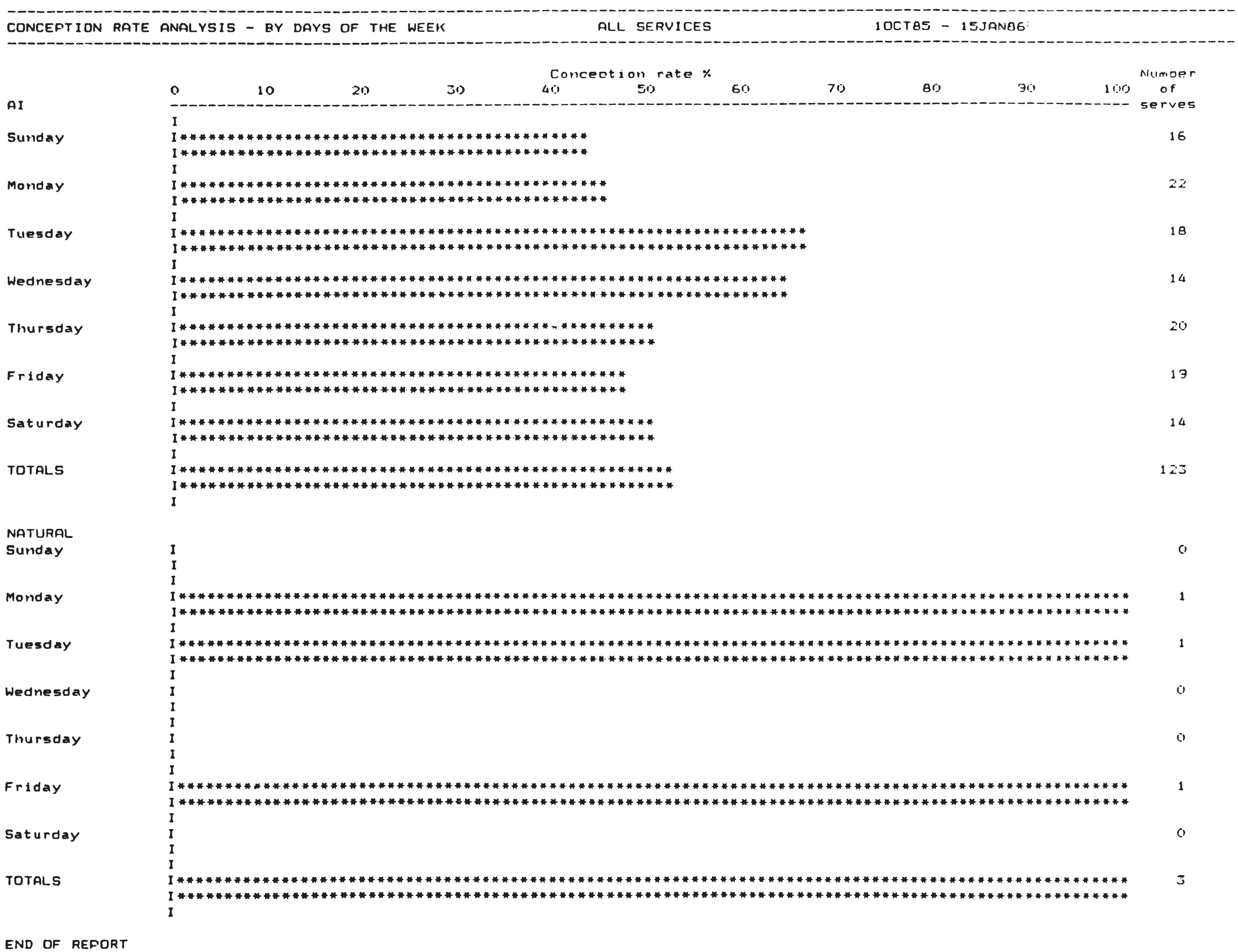
In order to implement a scheme that controls fertility, and thus meets the agreed targets, it will be necessary to visit the herd for regular, routine visits so that certain cows can be examined. The visit frequency will depend upon the number of cows in the herd, the annual calving pattern and the number of cows that can be effectively handled by the herd manager and veterinarian at one visit (probably not more than 40–50). Thus, for a small herd of less than 60 cows, once every 3 weeks should suffice; for herds of 60–150 cows, once every 2 weeks; and for herds of more than 150 cows a weekly visit will probably be necessary. The intensity of the calving pattern will modify the frequency of visits.

One advantage of computerised systems is that they automatically identify the individual animals requiring examination by producing action lists (Figure 24.7). This can also be done using simple manual systems, although it may take a little time to identify the cows. It is important to stress the need for close liaison between the veterinary surgeon and herd manager so as to ensure that the correct animals are presented for examination at the correct time. For this reason,



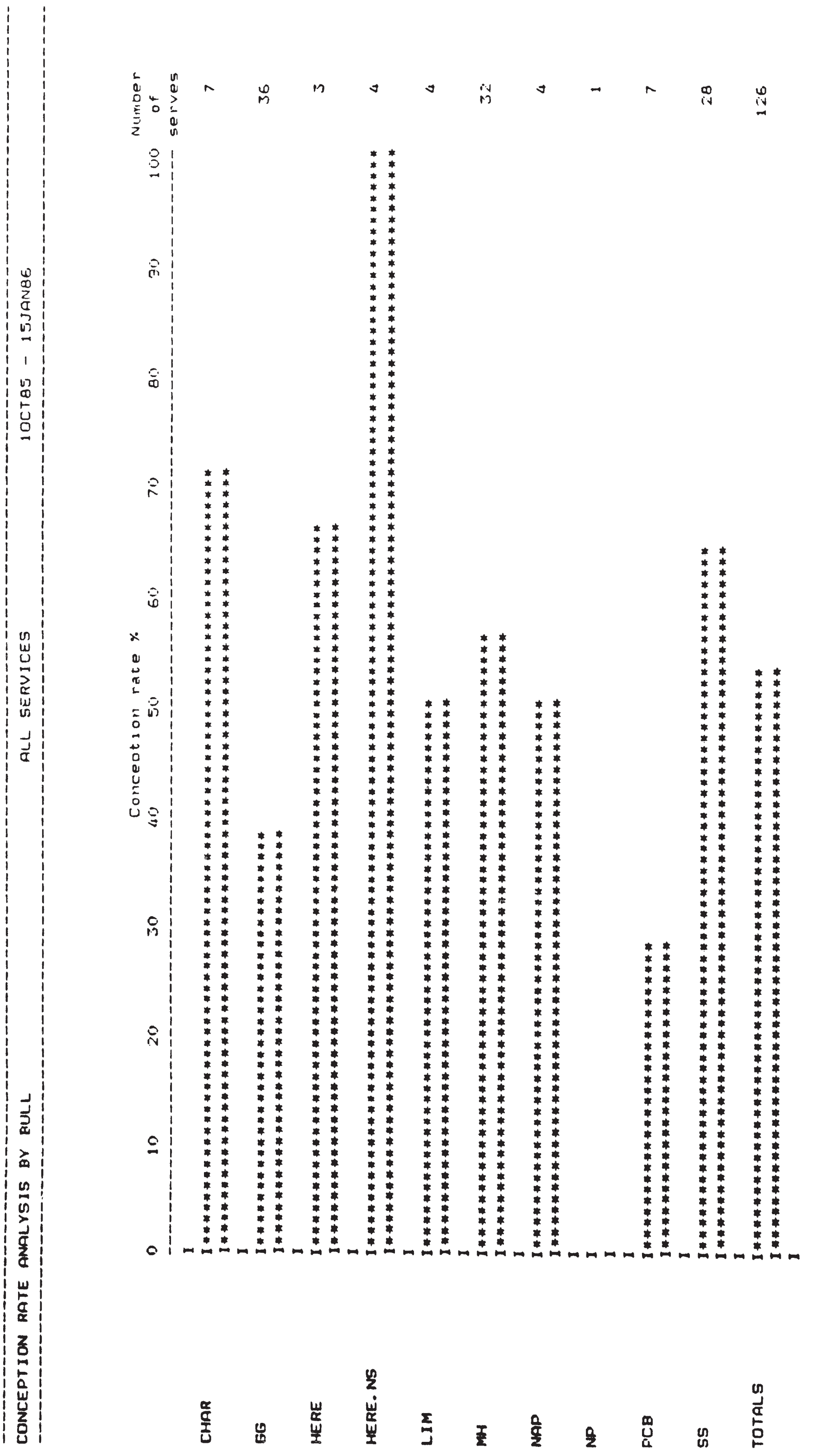
(a)

Fig. 24.3 (a) An example of a computer-produced histogram illustrating the distribution of interservice intervals. Note that in this program the daily intervals are quite short (DAISY). (b) An example of a computer-produced histogram illustrating pregnancy (conception) rates by the days of the week when cows were inseminated (DAISY). (c) An example of a computer-produced histogram illustrating pregnancy (conception) rates to individual bulls whose identities are listed by abbreviations or initials (DAISY).



(b)

Fig. 24.3 *continued.*



(c)

Fig. 24.3 *continued.*

```
HERD :   2 -                                                                                                                 DAISY      P3
                                                                                                                             DATE 30MAR95
                                                                                                                             PAGE        1

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CONCEPTION RATE ANALYSIS  - Q-SUM                                          ALL SERVICES            1JAN91 - 31DEC91
-----------------------------------------------------------------------------------------------------------------------------------

Key :  %=to be CUlled  @=Not to be Served  $=left herd  w=P+ unknown serve  x2=double insemination

Date  Cow    Lac Srv Bull  Res  Int Ca-S Type.......................................................   Comments            Grp  Cow
                                             CR tending to be under 50%*CR tending to be over 50%
 3JAN     6   3   1 LOD    +V     0    36                              .*                            0     6
 9JAN    37$  2   2 LOD    -O    49    93                              *                             1    37
13JAN    55   4   1 LOD    +V     0    79                              .*                            0    55
18JAN    97   5   1 LOD    -      0    49                              *                             0    97
24FEB    37$  2   3 LLY    -O    46   139                             *.           SE FOR PROF NOAKES    1    37
27MAR    28   2   2 LLY    +V    98   139                              *                             0    28
27MAR    79   6   1 LLY    -      0    91                             *.                             0    79
29MAR    31   4   1 LLY    +V     0    58                              *                             0    31
30MAR    69   4   1 LLY    +V     0    70                              .*                            0    69
30MAR   102   1   1 LLY    +V     0    89                              . *                           0   102
30MAR     4$  3   1 LLY    -O     0   112                              .*                            1     4
30MAR    44   5   1 LLY    -      0    73                              *                             0    44
31MAR    68   8   1 LLY    +V     0    54                              .*                            0    68
 7APR    96   5   1 LLY    +V     0    82                              . *                           0    96
 7APR   104   1   1 LLY    +V     0    98                              .  *                          0   104
 7APR    39   3   2 LLY    +V   118   192                              .   *                         0    39
 7APR    97   5   2 LLY    -     79   128                              .  *                          0    97
 8APR    53   9   1 LLY    +V     0    96                              .   *                         0    53
 9APR    87$  4   1 LLY    -V     0   127                              .  *                          0    87
11APR    24   5   1 LLY    +V     0   107                              .   *                         0    24
11APR    47   3   1 LLY    +V     0    54                              .    *                        0    47
14APR    89$  4   1 LLY    -V     0    95                              .   *                         0    89
14APR    51$  2   2 LLY    -V   106   186                              .  *                          0    51
14APR    98$  5   1 LLY    ?      0   152                              . *                           0    98
15APR    79   6   2 UN     +V    19   110                              .  *                          0    79
18APR    95   5   1 LOD    +V     0    66                              .   *                         0    95
 5MAY    51$  2   3 LLY    -V    21   207                              .  *                          0    51
 9MAY    89$  4   2 LOD    -V    25   120                              . *                           0    89
12MAY    44   5   2 LOD    +V    43   116                              .  *                          0    44
12MAY    43$  4   1 LOD    ?      0   131                              . *                           0    43
19MAY    80   6   1 LOD    +V     0    73                              .  *                          0    80
20MAY   101   1   1 LOD    -      0   117                              . *                           0   101
21MAY    38$  4   1 LOD    -V     0   100                              .*                            0    38
21MAY     4$  3   2 LOD    -O    52   164                              *                             1     4
21MAY    37$  2   4 LOD    -O    86   225                             *.                             1    37
26MAY    97   5   3 LOD    -     49   177                           * .                              0    97
26MAY    51$  2   4 LOD    -V    21   228                          *  .                              0    51
 1JUN    89$  4   3 LOD    -V    23   143                         *   .                              0    89
 5JUN   103   1   1 LOD    -      0   135                        *    .                              0   103
```

(a)

Fig. 24.4 (a) An example of a computer-produced ‘Cu-Sum’ for pregnancy (conception) rates to all services (DAISY). (b) An example of a computer-produced ‘Cu-Sum’ for pregnancy (conception) rates to all services; note the use of upper and lower case symbols in the construction of the graph to distinguish between AI and natural service and their respective success rates (Dairy CHAMP) (courtesy of Mr M. Dobbs)

CONCEPTION EFFICIENCY

DairyCHAMP P1B

Date	Animal Id	Bull Id	Lact	Service	#------------------------------O------------------------------
15 OCT 99	1170	TIERENY	2	1	a
16 OCT 99	1158	TIERENY	3	1	A
19 OCT 99	513	ELMA	1	2	a
21 OCT 99	521	ELMO	1	2	A
22 OCT 99	1097	ASTONMARTIN	4	2	n
25 OCT 99	505	VENTURE	5	9	a
29 OCT 99	1175	ASTONMARTIN	2	3	n
29 OCT 99	1131	ASTONMARTIN	3	6	N
4 NOV 99	510	ASTONMARTIN	1	2	n
4 NOV 99	1168	YELLOW	1	1	a
4 NOV 99	1113	ASTONMARTIN	4	2	N
4 NOV 99	1039	YELLOW	7	2	a
5 NOV 99	516	VTEC	1	2	a
5 NOV 99	1195	VTEC	1	2	a
5 NOV 99	1157	ASTONMARTIN	2	1	n
5 NOV 99	1145	ASTONMARTIN	2	4	N
5 NOV 99	1108	ASTONMARTIN	4	1	N
5 NOV 99	1012	ASTONMARTIN	7	4	N
7 NOV 99	1124	YELLOW	3	6	a
7 NOV 99	1016	YELLOW	7	1	A
7 NOV 99	1002	YELLOW	7	4	a
8 NOV 99	1138	TIERNEY	2	6	a
9 NOV 99	1076	YTEC	5	5	a
17 NOV 99	519	TIERNEY	1	1	a
17 NOV 99	518	TIERNEY	1	2	a
17 NOV 99	1191	ASTONMARTIN	1	3	n
18 NOV 99	1160	VTEC	3	3	a
18 NOV 99	1126	VTEC	2	2	a
19 NOV 99	1192	ASTONMARTIN	1	3	N
19 NOV 99	1175	ASTONMARTIN	2	4	N
19 NOV 99	1119	TIERNEY	5	1	a
20 NOV 99	515	ASTONMARTIN	1	2	N
20 NOV 99	1190	TIERNEY	1	1	a
20 NOV 99	1138	TIERNEY	2	7	a
21 NOV 99	1166	CAROUSEL	2	2	a
21 NOV 99	1142	ASTONMARTIN	3	1	n
22 NOV 99	511	VTEC	1	2	A
23 NOV 99	1076	ASTONMARTIN	5	6	n
29 NOV 99	1124	ASTONMARTIN	3	7	N
4 DEC 99	1002	ASTONMARTIN	7	5	n
5 DEC 99	1138	ASTONMARTIN	2	8	n
5 DEC 99	1135	ASTONMARTIN	2	7	n
7 DEC 99	518	ASTONMARTIN	1	3	n
8 DEC 99	1133	THOMAS	3	1	O
10 DEC 99	1170	VENTURE	2	2	O
10 DEC 99	1111	THOMAS	4	8	N
11 DEC 99	1191	THOMAS	1	4	O
11 DEC 99	1127	THOMAS	4	3	n
11 DEC 99	1003	THOMAS	7	2	O
12 DEC 99	1139	VTEC	3	2	O

Key : A – Artificial success a – Artificial failure
N – Natural success n – Natural failure
O – Can not be determined

(b)

Fig. 24.4 *continued*

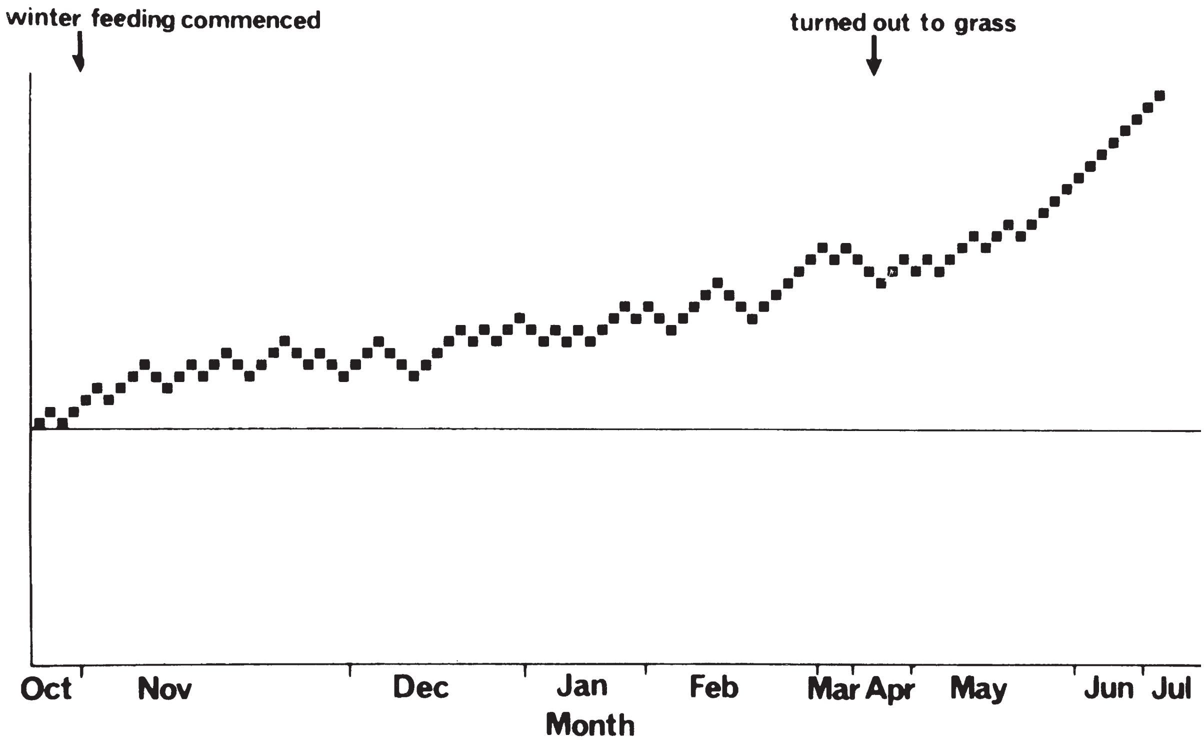
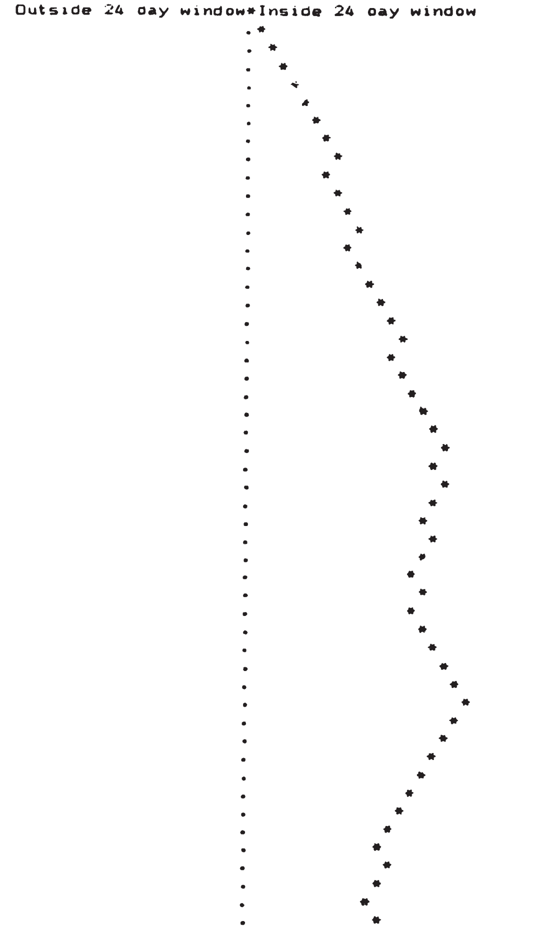


Fig. 24.5 An example of a manually produced 'Cu-Sum' of pregnancy (conception) rates to all services throughout a breeding year; the overall pregnancy rate was 65%.

FIRST SERVICE SUBMISSION RATE - Q-SUM　　　COWS CALVING　1JUL85-24FEB86

ALL COWS

Start of breeding season　1NOV85
Days to 1st allowed serve　45



Calve	Cow	Lac	Date	Bull	Res	Ca-S	Type	Comments	Grp	Cow
1AUG85	738	6	16NOV85	SS	Y	107	M		3	738
2AUG85	65	3	5NOV85	MH	Y	95	O		3	65
3AUG85	21	2	6NOV85	PCB	Y	95	M		3	21
5AUG85	325	2	5NOV85	MH	Y	92	M		3	325
6AUG85	628	6	16NOV85	SS	Y	102	V		3	628
8AUG85	521	3	15NOV85	SS	Y	99	M		3	521
12AUG85	316	7	23NOV85	MH	Y	103	O		3	316
13AUG85	511	3	13NOV85	MH	Y	92	M		3	511
15AUG85	3	2	2DEC85	SS	N	109	M		3	3
19AUG85	35	2	8NOV85	MH	Y	81	M		3	35
19AUG85	493	3	5NOV85	MH	Y	78	M		3	493
20AUG85	22	2	22NOV85	SS	Y	94	M		3	22
20AUG85	41	2	9DEC85	MH	N	111	O		3	41
21AUG85	835	4	21NOV85	SS	Y	92	KA		3	835
22AUG85	63	2	15NOV85	MH	Y	85	M		3	63
22AUG85	351	8	3NOV85	NAP	Y	73	M		3	351
22AUG85	702	6	22NOV85	HERE.NS	Y	92	M		3	702
23AUG85	60	2	16NOV85	SS	Y	85	O		3	60
23AUG85	68	2	5DEC85	SS	N	104	V		3	68
25AUG85	30	2	6NOV85	MH	Y	73	M		6	30
26AUG85	34	2	17NOV85	MH	Y	83	M		3	34
26AUG85	304	1	16NOV85	GG	Y	82	V		6	304
26AUG85	614	6	11NOV85	MH	Y	77	O		3	614
27AUG85	43	2	7NOV85	PCB	Y	72	O		3	43
28AUG85	108	2	7JAN86	HERE	N	132	M		3	108
29AUG85	134	7	2NOV85	NAP	Y	65	M		3	134
29AUG85	48	4	28NOV85	MH	N	91	M		3	48
30AUG85	109	4	1DEC85	SS	N	93	M		3	109
31AUG85	39	3	17NOV85	SS	Y	78	M		3	39
1SEP85	64	3	13DEC85	MH	N	103	M		3	64
1SEP85	305	1	28NOV85	GG	N	88	M		7	305
2SEP85	329	1	6NOV85	GG	Y	65	M		6	329
4SEP85	326	1	29NOV85	GG	N	86	M		7	326
5SEP85	66	3	11NOV85	MH	Y	67	O		3	66
5SEP85	315	1	1NOV85	GG	Y	57	M		6	315
5SEP85	385	8	8NOV85	MH	Y	64	M		3	385
6SEP85	327	1	14NOV85	GG	Y	69	M		6	327
6SEP85	554	7	7NOV85	MH	Y	62	M		3	554
7SEP85	76	3	24FEB86		N	0		NONE	5	76
7SEP85	307	1	26NOV85	LIM	N	80	M		6	307
7SEP85	321	1	2DEC85	GG	N	86	M		7	321
8SEP85	318	1	19DEC85	GG	N	102	O		7	318
8SEP85	333	1	2DEC85	GG	N	85	M		7	333
8SEP85	340	1	14DEC85	GG	N	97	TP		7	340
9SEP85	312	1	27NOV85	GG	N	79	KA		6	312
9SEP85	662	8	1DEC85	CHAR	N	83	M		3	662
11SEP85	319	1	1NOV85	GG	Y	51	M		6	319
11SEP85	54	3	26DEC85	MH	N	106	M		3	54
13SEP85	341	1	23JAN86	HERE.NS	N	132	O		7	341
14SEP85	81	3	17NOV85	SS	Y	64	M		6	81

Fig. 24.6 An example of a computer-produced 'Cu-Sum' for first service submission rates (DAISY).

HERD : 2 -

DAISY A7
DATE 30MAR95
PAGE 1

COWS FOR VET TO SEE : WORKLIST

Number of checks = 16

Date of last visit = 28MAR95
Date of next visit = 30MAR95
Calving-1st serve interval = 63 days
Service-P.D. interval = 42 days
Calving-1st heat interval = 42 days
PD negative,no action for 21 days

Sorted by Reason
then Cow name

*=more than one reason

COW	GRP	REASON TO BE SEEN	Days Calved at visit	----CRUCIAL EVENT----- Type	Date	Days to visit	FURTHER DETAILS	Latest 2 Heats/Serves Days to visit
74*	0	For revisit(PD?)	262	PD?	3MAR95	27		:SE140
74*	0	For vet P.D.	262	SERV	10NOV94	140		:SE140
366	0	For vet P.D.	274	SERV	9DEC94	111		:SE111
315	0	P.D. neg, no action for 21 days	169	PD-	23FEB95	35		:SE107
316	0	P.D. neg, no action for 21 days	172	PD-	23FEB95	35		:SE117
406	0	P.D. neg, no action for 21 days	266	PD-	3MAR95	27	T PRID	:SE 91
55	0	No serve 63 days since calving	185	ONO	15DEC94	105	F ACY T PRID	
56	0	No serve 63 days since calving	79	VLD	28FEB95	30	F FC	
68	0	No serve 63 days since calving	73	ONO	28MAR95	2	F CLLO T PG	
154	0	No serve 63 days since calving	70	VLD	28FEB95	30	T PG	
204	0	No serve 63 days since calving	77	CALV	12JAN95	77		
312	0	No serve 63 days since calving	80	ONO	28MAR95	2		
486	0	No serve 63 days since calving	90	ONO	10FEB95	48	F INV	
513	0	No serve 63 days since calving	92	CALV	28DEC94	92		
T 64	0	No serve 63 days since calving	87	VLD	28FEB95	30		
T 85	0	No serve 63 days since calving	87	CALV	2JAN95	87		

END OF REPORT

Fig. 24.7 An example of a computerised work or action list identifying cows that require veterinary examination at the next routine fertility visit (DAISY).

duplicate copies of action lists should be sent to the herd manager several days before a proposed routine visit. Those requiring examination will be:

- cows that suffered dystocia, retained fetal membranes or metritis, who will require a preservice examination (some veterinarians routinely examine all cows irrespective of their previous history)
- cows with an unnatural vulval discharge
- cows that have aborted
- cows that have shown signs of nymphomania
- cows that have not been seen in oestrus by 42 days after calving
- cows that have not been served or inseminated by 63 days after calving
- cows that have returned to oestrus after service or artificial insemination three times or more (Repeat Breeders)
- cows that have been served or artificially inseminated, and have not been seen to have returned to oestrus after 24 days (have missed one heat) if transrectal ultrasonography is used, or after 42 days (two missed heats) if transrectal palpation is used
- cows that have been diagnosed pregnant but have been observed to be in oestrus.

Rearing a dairy heifer as a replacement for a culled cow is expensive, and until she calves for the first time she has contributed nothing to the income from the herd. It is important not to ignore the replacement heifers in the overall strategy for managing the fertility of the herd. In the case of Friesian-Holstein heifers, there are several important stages when it is appropriate that the herd manager and/or the veterinarian should examine each individual animal. The scheme for the reproductive management is as follows:

- At 10–12 months of age ensure that they are adequately grown and in an appropriate condition.
- Heifers should be served at 14–15 months of age so that ideally they calve slightly before the cows in a seasonally calving herd. This enables them to have a longer calving–conception interval and calve for the second time at the same time as the rest of the herd. They should be approximately 325 kg live weight and growing at 0.7 kg/day. It is advisable to 'flush' them by increasing the feed intake from before the service period until diagnosed pregnant.
- A bull with a low probability of causing dystocia due to fetomaternal disproportion should be used whether by artificial insemination or natural service.
- Pregnancy diagnosis should be made by rectal palpation at 5–6 weeks. Adequate feeding should be maintained.

They should be at a condition score of $2\frac{1}{2}$–3 and about 480–500 kg live weight at the time of calving.

The use of milk (or plasma) progesterone assays in cow fertility management

In Chapter 3 (see pp. 90–92), the milk or plasma progesterone assay is described as a method of diagnosing pregnancy in cows 24 days after service. However, the same assay can in other ways assist both veterinarian and herd manager in managing the fertility of the herd. The assays are expensive and require some degree of laboratory skill and thus they should be used judiciously rather than as a non-selective procedure on all cows at all times. Possible applications have been described by Drew (1986) and are as follows:

Identification or confirmation of postpartum anoestrus before the target service date. At a single rectal palpation of a cow that has not been observed in oestrus since calving it may not be possible to make a definite diagnosis of anoestrus (acyclicity) (see p. 416). A high progesterone concentration in the milk 10 days before (or after) the palpation of ovaries without a corpus luteum is indicative of a non-observed oestrus. A low (or zero) milk progesterone concentration at the same time interval before (or after) palpation when no corpus luteum was identified is indicative of anoestrus. Furthermore, two consecutive low (or zero) milk progesterone concentrations in samples collected 7–10 days apart confirm that the cow is anoestrous.

Ensuring that a cow is close to or in oestrus on the day of insemination. Milk progesterone concentrations should be low on the day of insemination. Thus, this test enables the accuracy of oestrus detection to be checked. If it is done before the cow is due to be inseminated it can prevent the wastage of a dose of semen. It can be used to investigate a herd where poor overall pregnancy rates are obtained and prevent the insemination of cows that are already pregnant.

A single low progesterone sample does not necessarily show that the cow is at the optimum time for insemination but rather that the cow is not in dioestrus. A more accurate assessment of optimum timing (see p. 431) can be achieved if milk samples are collected and assayed every day from day 17 after the last recorded oestrus. Normally the samples on days 17 and 18 will have high progesterone values, day 19 intermediate values, and days 20, 21 and 22 low values. The timing of the high:low values will depend upon the normal cycle length (see p. 19). If oestrus has not been observed, then the cow should be inseminated on the third consecutive day of low progesterone concentrations (Table 24.7); using such a scheme acceptable pregnancy rates have been obtained.

Anticipation of the return to oestrus in the absence of pregnancy. If the milk progesterone concentration is low on day 19 after service or insemination, then the cow can be assumed to be non-pregnant, and her return to oestrus can be anticipated. This can improve the oestrus detection rate after service.

Despite the expense of performing more frequent milk progesterone assays, it has been shown that it can be cost-effective (Eddy and Clarke, 1987). In a study involving four dairy herds, milk samples were collected at either 18, 20, 22 and 24 days or 19, 21 and 23 days after service. The calving–conception intervals in two herds were reduced from 115 to 84 days and from 85 to 74 days with a potential cost benefit of 7.4:1 and 3.4:1, respectively.

Confirmation of ovarian structures identified at rectal palpation. Confirmation of the presence of a corpus luteum or luteal cyst (see Chapter 22) can be made by the presence of concurrently high milk progesterone concentrations.

Assessment of cows' response to therapy. The assessment of the response to therapy is frequently entirely empirical. The assay of progesterone concentrations in milk at the time of treatment and at varying time intervals afterwards can be used to assess the luteolytic response after prostaglandin treatment or the luteotrophic response after GnRH or hCG therapy.

The regular collection of large numbers of milk samples and their assay is another task that, if imposed upon an already overworked herd manager by an overenthusiastic veterinarian, can result in loss of enthusiasm for this and other chores. For this reason selectivity of sampling should be a major aim so that the demands for large numbers of samples should be reduced.

Table 24.7 Timing of insemination in relation to milk progesterone concentration

	Day of previous insemination					
	17	*18*	*19*	*20*	*21*	*22*
Milk progesterone concentration	High	High	Low	Low	Low ↑ AI	Low

PASTORAL DAIRYING

Milk can be obtained most efficiently from pasture when the feed demand curve of the cows coincides with the growth curve of the grass (Holmes et al., 1984). Grass growth is maximal during the spring, declines during the summer and undergoes a brief resurgence during the autumn, before declining to a basal level during the winter. Thus, by calving cows at the start of the grass growth phase (i.e. in the early spring), peak milk yield can be achieved at the time of maximal pasture growth, thereby maximising the efficiency of pasture use (Figure 24.8). Cows are dried off in the late autumn, when grass growth becomes too low to continue to support lactation. Excess pasture can be conserved during the spring, to augment the availability of feed during the period of low growth that occurs in the dry summer period, or it can be retained as a supplement for use during the winter period, when grass availability can limit stocking rates (Holmes et al.,

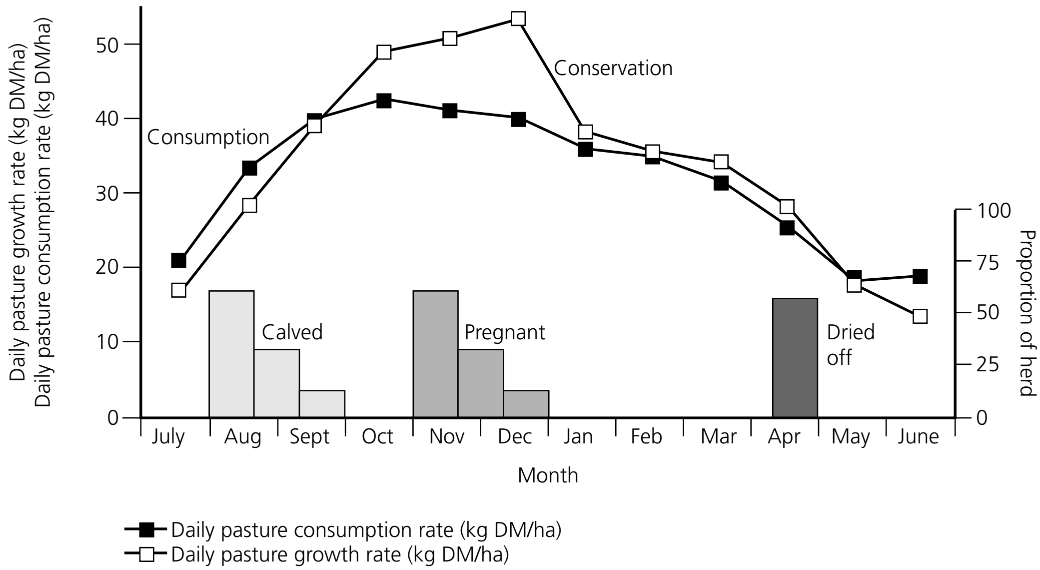


Fig. 24.8 Pasture growth rate and the rate of consumption of pasture in a seasonal, spring-calving, southern hemisphere dairy herd, in relation to the major management events of the annual calendar (from data supplied by C. W. Holmes, reproduced with permission).

1984). Apart from such use of conserved grass, the only other feed inputs to the system may be the use of fodder crops including brassicae (especially turnips; Clark et al., 1996) during the summer dry period, or maize silage. The use of cereal or other concentrates is rare and is usually confined to situations where pasture management has broken down (i.e. either as consequence of adverse climatic conditions or an excess of demand over production).

Many studies of pastoral dairying have shown that the economic performance of the herd is best when the growth of grass is maximised; the harvest of grass is maximised; and the reproductive performance of the herd is optimal (Thomas et al., 1985; Clark and Penno, 1996; Grosshans et al., 1996; Holmes, 1996). Indeed, the accumulated experience of managing pastoral dairying systems is that the greatest efficiency can be achieved when the calving season is as closely synchronised as possible with the onset of grass growth, and is as compact as possible. Moreover, since drying off occurs by calendar date, rather than with respect to days of lactation, achieving a calving pattern that is both early and compact ensures that the mean lactation is as long as possible. These strategies ensure that the harvest of grass is maximised, whilst the unit cost of milk production is minimised. A compact calving season can only be achieved if the mating season is well managed and cows conceive over a narrow window of time. Hence, the fundamental aim of reproductive management of pastoral dairy cows is to ensure that as many cows as possible conceive over as short a period as possible, with a calving interval of no more than 365 days (Holmes et al., 1984).

Overview of reproductive management of a seasonally calving pastoral dairy herd

The main features of the annual reproductive calendar (Holmes et al., 1984; Macmillan, 1998) are illustrated in Table 24.8. For a spring-calving herd, the cows will calve over a relatively short period during late winter/early spring. The subsequent breeding season starts between $2\frac{1}{2}$ and 3 months after the start of calving. This is a calendar date, rather than being derived from calculation of

Table 24.8 Annual calendar of main managemental and reproductive events in a spring-calving, pastoral dairy herd (derived from Holmes et al., 1984)

Early spring
- Cows calving
- Pregnant cows on restricted grazing, often supplemented with hay, silage or maize silage
- Calved cows on unrestricted grazing
- Yearling heifers showing oestrous activity

Late spring
- All cows should have calved within 8–10 weeks. Late-calving cows may be induced
- Tail-paint cows 3–4 weeks before start of mating. Observed for oestrus
- Oestrous cyclicity commences within 50 days of calving. Anoestrous cows treated before the start of the mating period
- Cows with dystocia, retained fetal membranes, metritis or hypocalcaemia for veterinary examination before the start of the mating period
- Planned start of mating about 3 months after the start of calving. All cows should be bred at least once to AI in the first 4–6 weeks of the mating period

Summer
- All cows and 15-month-old heifers should be mated by midsummer
- Pregnancy test cows and 18-month-old heifers

Autumn
- Dry off once pasture becomes scarce and/or milk production declines to uneconomic levels
- Adjust feeding to ensure cows calve at correct condition score. Separate thin animals and feed more generously
- Control DCAD during late dry period

the postpartum intervals of individual cows, with the consequence that individual animals are first bred at a range of postpartum intervals. Artificial insemination, usually to bulls of dairy breeds, takes place for 4–6 weeks, after which sweeper bulls are run with the herd. The bulls are removed after a further 6–8 weeks and, after the appropriate interval, the herd is pregnancy-tested. Any cows that are non-pregnant at that time will be culled when the herd is dried off. Drying off occurs in the late autumn, ideally as late as possible, although the exact time is dictated by the availability of pasture and/or supplementary feeds during the late summer/early autumn. It is common practice to dry off the entire herd on a single day, although batches of low-yielding animals may be dried off in advance of the main herd if it is necessary to conserve autumn grass. Animals may also be dried off early in order to improve the body condition of young cows or cows that are too thin. Non-pregnant cows may be retained in the herd until the last drying-off date, if their production is good, but it is quite common practice to remove them from the herd as soon as summer/autumn grass growth starts to limit the herd's milk production.

In considering the veterinary control of fertility in these herds, it is important to remember that, from the perspective of the herd manager, the individual cow is of relatively low value. Hence, the unit of production is normally considered to be the herd rather than the cow. A few exceptions to this generalisation exist, especially where there has been a significant investment in the breeding of cows of high genetic merit, or where breeding policy has been towards maximising the milk production per cow, rather than using high stocking rates to maximise production per unit area of land (i.e. with many cows producing relatively low individual yields).

Nutrition and reproduction in pastoral dairy cattle

In pastoral dairying systems, there is a crucial synergy between the production system and reproductive performance, such that, unless the feeding strategy is right, reproductive performance will suffer. Likewise, unless reproduction is managed efficiently, it will not be possible to achieve efficient utilisation of pasture and a worthwhile economic return. In this context, and in common with several other dairying systems, it is increasingly well realised that achieving maximum lifetime productivity from animals within the pastoral system can only be achieved when their lifetime nutritional management is optimal.

Hence, a significant component of the veterinary control of herd fertility involves managing nutritional strategies for the lifetime of the cow (Thomson et al., 1991; Thomet and Thomet-Thoutberger, 1999). The rearing of both calves and maiden heifers is a crucial component of this. It is well known that, across a wide spectrum of dairying management systems, achieving satisfactory live weights at the time of first mating (i.e. > 60% of mature liveweight; Penno, 1997) is imperative. For pastoral dairy cattle, this target is of particular importance, since the grass-based ration is unlikely to allow for compensatory growth after the onset of lactation (Holmes et al., 1984). Hence, ensuring that adequate calfhood and yearling growth rates are achieved is a significant step in maximising overall lifetime performance.

Most obviously, feeding has to be managed correctly during the early part of lactation. As in other systems, this depends to a large extent upon management during the dry period, especially with reference to the animals' body condition (condition score) at the time of calving (Grainger and McGowan, 1982; Holmes et al., 1984). Calving at a condition score of 4.75 to 5.0 (0–8 scale: equating approximately to 2.75 to 3 on a 0–5 scale) provides enough fat to allow body reserves to supplement feed available through pasture since, unlike the situation with cereal-based systems, it is rarely possible to meet the entire nutrient demands of the cow in early lactation from grass alone. Nevertheless, ensuring an adequate supply of grass that meets the demands of lactation as closely as possible is a predictably important component of minimising the duration of postpartum anoestrus (Figure 24.9; McGowan, 1981). However, there is an additional complication in the nutritional management of early lactation in the pastoral system: namely, the effects of winter feeding strategies upon the availability of pasture to calving cows and the growth of pasture in the post-calving grazing period. In most other systems, where feeding in early lactation relies upon concentrates that are supplemented with some conserved forage, feed availability is largely independent of previous grazing management. With pastoral cows, decisions that were made about levels of feed intake during the dry period not only affect subsequent performance, but also greatly constrain subsequent nutritional options (Clark et al., 1994).

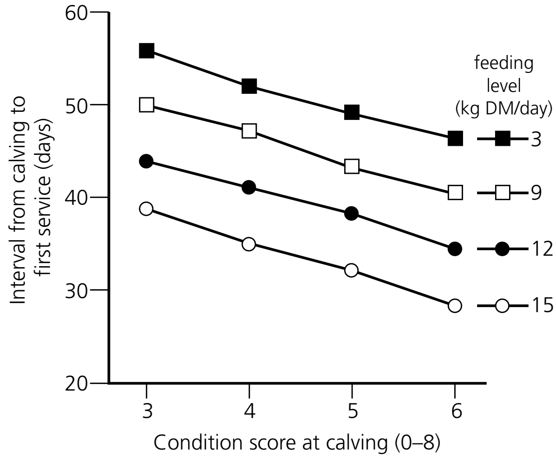


Fig. 24.9 Effects of body condition score at calving and postpartum level of feeding upon the interval between calving and first oestrus. (Adapted from McGowan, 1981)

Feeding after the peak of lactation has less direct effect upon reproductive performance, since all of the cows should be pregnant by 3 months after calving. It does, however, affect the condition score at the time of drying off and, consequentially, the interrelationship between condition score at calving and the pasture cover at calving. Feeding during the dry period also significantly affects the incidence of hypocalcaemia, which, as both a clinical and subclinical entity, has significant effects upon subsequent reproductive performance (McKay, 1994). Since the discovery that the dietary cation–anion imbalance during the late dry period is a key determinant of calcium homeostasis (Wang et al., 1991), a great deal of effort has been put into controlling this aspect of diet. It is not normally possible to achieve a zero cation–anion balance in a pastoral system, since the range of nutritional options that are available to control DCAD are very limited. Nevertheless, supplementation with anionic salts, especially those of magnesium, substantially mitigates the ionic imbalances (Wilson et al., 1998).

Hence, veterinary control of reproduction is heavily involved with decisions regarding nutritional strategies during the various stages of lactation. The main control points are:

- Feeding must be planned throughout the winter, to ensure adequate condition scores and pasture cover at calving and the control of DCAD in the transition period (McKay, 1998).
- Since managing the availability of feed during the post-calving period is vital, significant veterinary input can be made to assessing the nutritional status of animals during this phase.
- Since many pastures are deficient in a wide variety of micronutrients that can limit reproductive performance through limitation of feed intake or through direct effects upon reproduction per se, veterinary control of these aspects of management is also important (Grace, 1983; Holmes et al., 1984).
- Ensuring that good growth rates are achieved during the rearing period, especially up to the time of first mating, requires veterinary input to the management of the pre-weaning calf (largely with respect to enteric disease) and to the rearing of post-weaning yearling stock (with respect to energy intake, parasite control and micronutrient deficiency).

Calving

It is most common for calving to take place at pasture, ideally over more than about 6 weeks. Dystocia is relatively uncommon, although little information has been published on its incidence.

Problems of the calving period

The incidence of retained fetal membranes tends to be low, except where uncontrolled hypocalcaemia occurs, or where there are uncorrected micronutrient deficiencies. Similarly, the incidence of post-calving septic perimetritis or clinical cases of metritis occurring later in lactation is also quite low. To a large measure, this is the consequence of calving at pasture, rather than in indoor calving accommodation where high levels of organisms capable of colonising the uterus can build up in soiled bedding. It is also the consequence of the low incidence of dystocia and hypocalcaemia. Veterinary treatment of retained fetal membranes is often minimal unless the cow is clinically ill, and most cows succeed in resolving the subsequent uterine infection by the start of the breeding season.

Small numbers of cows suffer uterine prolapse, vaginal tears and similar emergencies. However, hindlimb paralysis is a relatively common sequel of the delivery of oversized fetuses by traction. When they occur in the middle of a busy calving season, it is common practice to make decisions about the probability of the survival of a paralysed cow sooner rather than later. Fewer of these animals seem to develop mastitis and metritis that occurs with indoor calving, but keeping affected animals warm, fed and watered can be difficult when they are at pasture.

Calving pattern and its effects upon reproduction

When calving takes place over a short period of time, it is relatively easy to sustain that pattern of calving. Where it takes place over an extended period of time, it is quite difficult to restore it to a compact pattern. The reasons for this difficulty are several, for an extended calving pattern not only affects the time at which cows can be expected to rebreed successfully, but also affects the patterns of reproductive performance and production in replacement heifers.

The calving pattern affects the age at which heifers are first bred. It is usual to breed all of the replacement heifers simultaneously, so that they all calve at (or slightly before) the time when the main herd starts to calve (Macmillan, 1998). If they are born from a compact calving pattern, they will all be of a similar age when they are bred. If, however, they come from an extended calving pattern, they will either be at mixed ages when first bred, or the younger ones will have to be bred asynchronously (Figure 24.10). Hence, in the former situation, some heifers will calve at a relatively younger age than their peers, probably also at a lighter body weight, so will be relatively disadvantaged in competing for food. In the latter situation, the heifer will have a shorter interval between her first parturition and the start of the

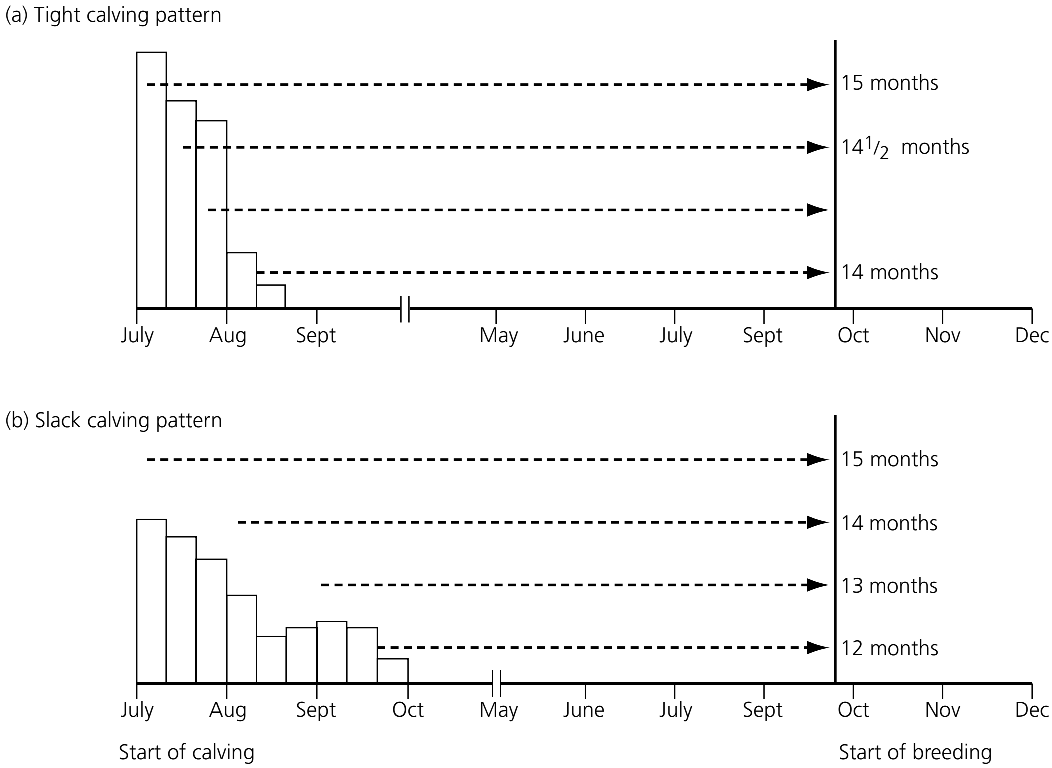


Fig. 24.10 Effect of calving spread on ages of heifers at first joining for a seasonal, spring-calving southern hemisphere herd. (a) When the calving pattern is tight, there is not more than about a month between the ages of the oldest and youngest heifers. (b) The greater spread of ages in heifers derived from a slack calving pattern significantly jeopardises the performance of the younger animals.

breeding season and, hence, a reduced probability of conceiving.

Similar constraints pertain when an extended calving pattern exists in the main herd. For cows that calve early in the breeding season, there is plenty of time for the uterus to undergo involution and eliminate infection, and for the cow to return to positive energy balance by the time of the start of mating. For cows that calve later in the season, there is a proportionally shorter interval between calving and the start of mating, so the uterus will be relatively less well involuted (see Chapter 7) and more likely to be contaminated, and the cow herself is less likely to be out of the phase of negative energy balance. Since the cows that are in negative energy balance are far more likely to be acyclical than cows that are in positive balance, the incidence of anoestrus is also higher amongst the former. Hence, they are less likely to conceive early in the breeding season and, indeed, if they do conceive, it is more probable that they will do so to sweeper beef bulls than by AI to dairy bulls. In consequence, these animals can either become locked into a cycle of late calving-late conception, which is remarkably difficult to break, or they fail to conceive and are culled as non-pregnant animals.

Induction of calving

A further means of synchronising the date of calving with the onset of grass growth is to induce premature calving in cows at the appropriate stage of the spring (MacDiarmid, 1983 and Chapter 3).

However, the use of premature induction of calving for the management of calving patterns is increasingly falling into disfavour (Macmillan, 1995), with the realisation that the duration of the calving season can be better managed by attention to nutrition in the post-calving period, the timely treatment of anoestrous cows during the early part of the breeding season and removing sweeper bulls from the herd earlier in the year. A prevalent view of the use of induction of calving is that induction of early parturition should primarily be used as an emergency measure on late-calving cows and that only relatively young cows with several years of lactation ahead of them and/or healthy cows in good condition should be induced (Moller and MacDiarmid, 1981).

Cows that are induced tend to have an increased incidence of retained fetal membranes (Welch and Kaltenbach, 1977; Malmo, 1993), although this seems to be far lower in pastoral cattle than is reported for housed animals in Europe and North America.

The breeding season

The breeding season typically starts about 3 months after the calving season, usually on a predetermined calendar date. Cows are bred for 4–6 weeks by artificial insemination, thereafter by running sweeper bulls with the herd for a further 4–8 weeks. Thus, the breeding season is rarely more than 14 weeks long. The aim of this breeding programme is to ensure that as many cows as possible are submitted for AI during the early part of the breeding season; the target is to present 90% of cows in the herd for first service within 3 weeks of the start of the breeding season (Macmillan and Watson, 1973; Xu and Burton, 1996; Hayes, 1998).

There are a number of factors that are intrinsic to a seasonal calving pattern that facilitate achieving such high submission rates. The most important of these are related to the seasonal pattern itself (Brightling et al., 1990; Hayes, 1998). When there are large numbers of cows coming into oestrus simultaneously, a large group of sexually active animals forms, which, by its very size, means that there is substantial sexual activity between cows. Hence, the observation of cows that are standing to be mounted is facilitated, since there are many cows both to mount and to be mounted. Secondly, there is considerable anecdotal evidence to suggest that most herd managers can achieve very high oestrus detection rates provided the breeding season is not long. Thus, many herd managers can successfully detect up to 90% of heats when the breeding season is no longer than 6 weeks, even though far fewer can achieve such high rates over a longer period of time.

Most farmers use auxiliary aids to oestrus detection. The most widely used is tail paint (Macmillan and Curnow, 1977; Smith and Macmillan, 1980). This is rubbed off or disturbed when another cow rides an oestrous animal, thereby providing an additional source of evidence that the cow has been ridden. Typically, 70% of cows that have been in oestrus will have most of the tail paint removed, while a further 20% of animals will have a significant proportion removed. The final 10% have little paint removed, thereby requiring observation by the herd manager of other signs of oestrus (Macmillan, 1998). During the concentrated breeding season, quite a lot of reliance is placed on secondary signs of oestrus as a means of confirming provisional diagnoses of oestrus; surprisingly good reliance can be placed upon restlessness, changed order at milking and reduced milk yield. The use of vasectomised bulls, or bulls with penile deviation, to aid oestrus detection is relatively uncommon in New Zealand, but is more widely practised in Australia. Occasionally, other aids to detection are used, although most have not proved to be cost-effective.

The chances of cows being seen in oestrus during the first 3 weeks of the mating period depend upon the interval since calving (Figure 24.11; Hayes, 1998). Thus cows that are calved significantly less than 40 days at the start of the mating period have a significantly lower chance of displaying oestrus than animals that are longer-calved (Rhodes et al., 1998). This is obviously of considerable significance in terms of the chances of an individual animal being presented for service. However, since conception rates depend upon the time after calving (Figure 24.12; Brightling et al., 1990; Hayes, 1998) and the number of oestrous cycles that the animal has had in the period between calving and first insemination (Figure 24.13; Macmillan and Clayton, 1980), the chances of a cow conceiving to AI are highly related to time after calving.

It is increasingly common for farmers to undertake some form of oestrus detection before the

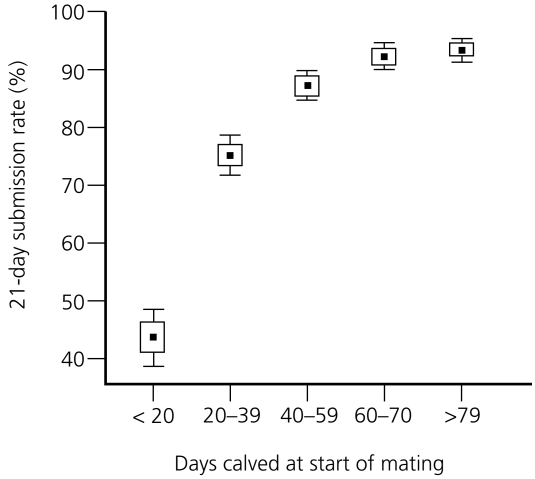


Fig. 24.11 The effect of days calved at the start of mating on the 3-week submission rate. The standard error of the mean is indicated by the box and the 95% confidence interval by the whiskers (from Hayes, 1998; reproduced with permission).

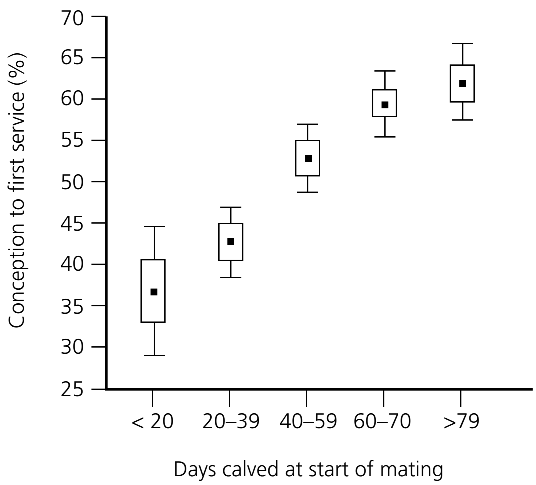


Fig. 24.12 Effect of days calved at the start of mating upon the conception rate to first service. Data were derived from herds using whole herd pregnancy testing. The standard error of the mean is indicated by the box and the 95% confidence interval by the whiskers (from Hayes, 1998; reproduced with permission).

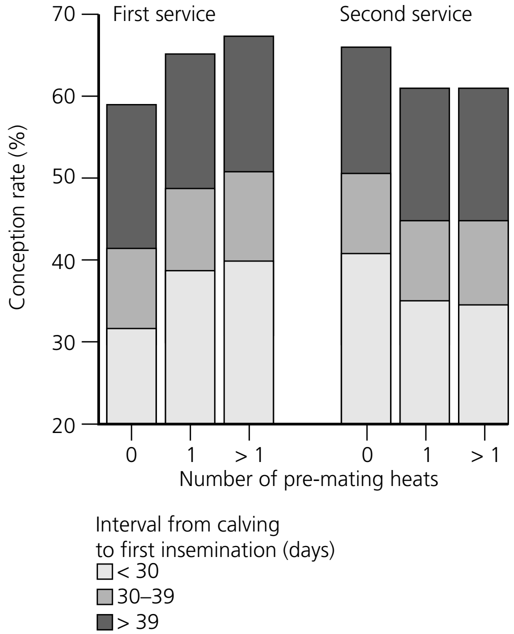


Fig. 24.13 Average pregnancy rates to first or second inseminations after varied intervals from calving to first insemination, in relation to the occurrence of pre-mating heats (derived from Macmillan and Clayton, 1980).

start of the breeding season. This is done for two reasons: firstly, in order that anoestrous cows can be detected and treated before the start of the mating period and, secondly, to allow farm staff to refamiliarise themselves with oestrus detection. Tail paint is applied to cows some 4 weeks before the start of mating. Any cows that fail to exhibit oestrus by the end of 3 weeks are presumed to be anoestrous. Any of these animals that have been calved more than 28 days will then be presented for veterinary examination a week before the start of the breeding season. The only slight disadvantage of this practice is that it extends the period of oestrus detection, which can potentially lead to poorer observation of repeat services.

The pressure to achieve compact calving patterns has also led to the adoption of a number of regimens that are designed to maximise the numbers of cows that conceive in the first few days of the breeding period. Most of these are based upon the strategic use of $PGF_{2\alpha}$, either with or without the detection of pre-mating period heats. An example of such a regimen is to induce oestrus with $PGF_{2\alpha}$ on the day before mating starts in all cows that were in oestrus more than 6 days before the start of the breeding season. The remaining cows are induced 6 days later, when they have a susceptible corpus luteum. Hence, most cows can be mating within the first week of the breeding period.

As well as anoestrous cows, animals that have had dystocia, retained fetal membranes or vaginal discharges are also examined before the start of the breeding season.

There has been a debate concerning the relative merits of one versus multiple insemination sessions each day, with the conclusion that the advantages of increasing the number of insemination sessions have a marginal effect upon conception rates. Indeed, timing of insemination has remarkably little effect upon conception rates, except in the case of low fertility bulls, which have higher conception rates after insemination late in, or after, oestrus (Figure 24.14; Macmillan and Watson 1975). Hence, the decision whether to use AI technicians or to inseminate one's own cows is based upon cost and/or convenience, rather than conception rate.

At the end of the AI period, bulls are turned in with the cows to serve the residual animals that have not conceived to AI. The target is that between 65% and 75% of cows should have conceived to AI (Hayes, 1998), which should minimise the requirements for bulls. However, it is common practice to use an excessive ratio of bulls to cows during the post-AI period, in order to ensure that a single infertile bull does not jeopardise the herd's reproductive performance. Many bulls have a veterinary examination for breeding soundness before they are turned in with the herd, although it is all too common to have to examine infertile bulls after the end of the breeding season.

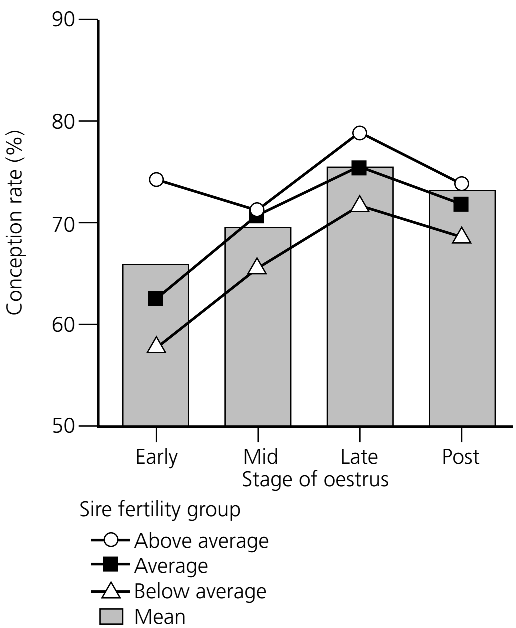


Fig. 24.14 Effect of timing of insemination upon conception rate. Above average fertility bulls achieve high conception rates at all stages of oestrus, but the conception rates achieved by lower fertility bulls decrease as the timing of insemination becomes suboptimal (data from Macmillan and Watson, 1975).

Pregnancy testing

The initial stage of pregnancy testing is the observation of non-return to oestrus. Indeed, given the brevity of the AI period, this is actually a vital stage, since failure to observe returns to oestrus will inevitably mean that the cow has no further opportunities to conceive to AI and therefore, if she does conceive, it will be to a sweeper bull. It has been widespread practice to examine entire herds for pregnancy, about 6 weeks after the removal of sweeper bulls from the herd. This examination has two functions: firstly, to identify the cows that are not pregnant so that, as previously described, they can be culled when the herd is dried off, and, secondly, to identify the cows that have conceived to sweeper bulls.

More recently, this pattern has been changing. It is increasingly common to examine the entire herd, either by a manual examination per rectum 6 weeks after the end of the AI period, or by ultrasonography 4–5 weeks after the end of the AI period, so that the cows that have conceived to AI can be identified (Macmillan, 1998). Examination at this stage of pregnancy allows accurate predictions of gestational age to be made. It also permits the identification of cows that have relapsed into anoestrus in time for them to be treated before the termination of the breeding season. Cows that are not identified as pregnant at this preliminary examination are re-examined 6 weeks after the bulls are withdrawn, again allowing accurate determination of gestational age. In this way, reliable information is generated about the proportions of cows that have conceived at each stage of the breeding season, allowing appropriate decisions to be made about the cows' future management.

Management of anoestrous cows

The majority of anoestrous cows are in true, nutritional anoestrus (Macmillan et al., 1975; Fielden et al., 1977; Rhodes et al., 1998). On examination per rectum, these animals are found to have inactive ovaries. Most cows that lack significant ovarian structures (i.e. a corpus luteum or a preovulatory follicle) are treated with some form of progesterone-releasing intravaginal device (see Chapter 22).

Monitoring herd fertility

The key parameters of herd fertility for the seasonally calving herd are (apart from the calving interval) the interval between the start of calving and the mean calving date of the herd, and the proportion of cows that are culled for failure to conceive.

Computer-aided herd fertility analysis for the seasonally calving herd requires the use of rather different assessment parameters from those that are used for the year-round or weakly seasonal calving herds of Europe and North America. To meet this need, a program based upon the requirements of seasonally calving herds has been developed. This program (DairyWIN©) uses the following key criteria to assess herds' reproductive performance (Table 24.9):

1. submission rate during the first 3 weeks of the breeding season
2. conception rate to first service
3. interservice interval analysis
4. proportions of the herd conceiving 4 and 8 weeks after the start of the breeding season
5. proportion of the herd culled for failure to conceive.

The proportion of cows conceiving 4 and 8 weeks after the start of mating and the percentage of cows culled for failure to conceive are important retrospective summaries of herd performance. The former information describes the expected calving pattern for the forthcoming calving season. The latter is regarded by farmers as a crucial indicator of reproductive performance, for it not only subsumes all of the aforegoing information, but represents a key financial outcome, given the high ratio between the value of replacements and culls. Where reproduction has been well managed, culling rates of as little as 5% can be achieved. However, recent years have seen a steady increase in the proportion of cows culled for infertility, a trend that has been variously correlated with the increase in herd size, increase in yield and substitution of Holstein genetics for more traditional Friesian and Jersey breeds.

Submission rates and oestrus detection efficiency

Determining the causes of failure to meet targets for any of the key criteria of herd reproductive

Table 24.9 Key targets used by DairyWIN© for assessing the reproductive performance of spring-calving, pastoral dairy herds (reproduced with permission)

• Proportion of cows calved 4 weeks after planned start of calving	67%
• Proportion of cows calved 8 weeks after planned start of calving	95%
• Proportion of cows calved < 40 days at planned start of mating (PSM)	10%
• Proportion of cows submitted for service by 21 days after PSM	90%
• Proportion of cows submitted for service by 28 days after PSM	92%
• Proportion of interservice intervals < 17 days	13%
• Proportion of interservice intervals 18–24 days	69%
• Proportion of interservice intervals 39–45 days	7%
• 49-day non-return rate (NRR) to first service	61%
• Pregnancy rate to first service	60%
• Services per conception	1.7
• Proportion of cows pregnant 4 weeks after PSM	57%
• Proportion of cows pregnant 8 weeks after PSM	86%
• Proportion of cows non-pregnant 165 days after PSM	7%
• Calving to conception interval	83 days
• Proportion of cows aborting	< 5%

performance can involve a considerable amount of probing into herd management. For example, it is possible to have a low submission rate for a number of reasons. Most obviously, there may be a high incidence of anoestrous cows or a failure to detect oestrus. The main causes of anoestrus have already been considered, while the causes of failure of oestrus detection are many. But a low submission rate can also be caused by a poor calving pattern, such that cows may still be in the period of physiological (rather than pathological) postpartum anoestrus at the start of the breeding season. In this situation, a conscious decision may have been made by the herd manager not to attempt to breed cows until an appropriate period has elapsed after calving. Indeed, if a significant proportion of the herd has calved late, high submission rates are not actually desirable and may be accompanied by low pregnancy rates due to the insemination of cows at too early a stage postpartum (Xu and Burton, 1996).

An example of such a herd is given in Figure 24.15. The Reproductive Monitor Report from Dairy WIN© (Figure 24.15a) indicates that there was a poor submission rate (55%) in the first 3 weeks of the mating period, a problem that was complicated by inadequate oestrus detection (23% short interservice intervals) and, not unsurprisingly, a low conception rate (53% 49-day nonreturn rate to first service). Further analysis of the causes of this problem showed that there was an excessive number of animals that were calved less than 40 days at the start of the mating period, animals that had a poor submission rate throughout the mating period (Figure 24.15b). In parallel with the poor submission rate, the conception rates of animals that actually were served were poor, especially in the animals that were calved less than 60 days at first calving (Figure 24.15c). Hence, the poor calving pattern became a self-perpetuating problem that was exacerbated by the poor condition scores of the entire herd at the time of calving. A similar problem was shown by the herd illustrated in Figure 24.16, in which very low submission rates were achieved in the animals that were calved less than 40 days at the start of mating.

Determining whether low submission rates are due to a high proportion of anoestrous cows can often be facilitated by examining the submission rates of individual age-classes of cows (Macmillan et al., 1975). In pastoral management systems, it is usually the animals calving for the first time that are under the greatest nutritional stress (Burke et al., 1995; McDougall et al., 1995), with the second calvers less at risk, although still vulnerable if they are not well managed during their first lactation. Hence, a separate analysis of younger cows will often reveal deficiencies in their management that have led to anoestrus.

Inadequate growth rates and poor condition scores of first-calved heifers at the time of mating were the cause of the problem shown in Figure 24.17. First-calved heifers were insufficiently grown to compete for feed with adult animals and, since they could not obtain enough food for lactation, they were certainly unable to obtain enough for growth. Hence, they lost excessive condition, leading to the poor submission rates. Many first calvers failed to conceive and so were culled as non-pregnant animals at the end of their first lactation. However, it can also be seen that the problem persisted into the second lactation of the heifers that did conceive, for the submission rates of 3-year-old animals were only marginally better than that of the 2-year-olds. Not until the third lactation had the surviving animals reached mature body weight and acceptable levels of reproductive performance.

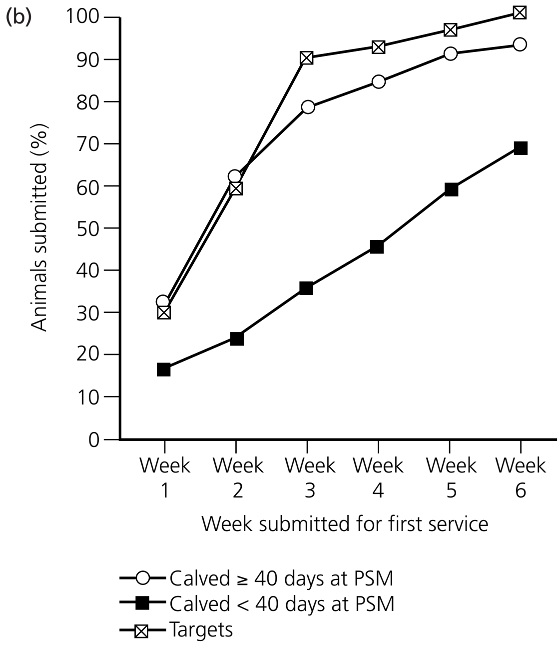
Failure of oestrus detection as a cause of low submission rates is a less frequent problem. Analysis of interservice intervals may help to explain such problems. When oestrus detection is simply inaccurate, there is usually a high incidence of short (i.e. less than 17-day) and long (25 to 35-day) intervals, and of missed heats (37 to 48-day intervals). Clinical examination of supposedly anoestrous animals will, in this situation, reveal a high incidence of animals that are cyclic and have active luteal structures within their ovaries. An example of such a problem is shown in Figure 24.18. At first glance, the high 3-week submission rate (91%) seems excellent, but the high level of short interservice intervals (35%) and the low conception rate (42%) indicate that many of the animals that were presented for insemination at the start of the mating period were not, in fact, in oestrus (Figure 24.18a). Moderate levels of

(a)

Reproductive Monitor Report

Stock Class: Adult Cow

	Period 1	Target
CALVING PERFORMANCE		
4-week calving rate	61%	67%
8-week calving rate	86%	95%
SUBMISSION RATES		
Percent calved < 40 days at PSM	15%	10%
21-day submission rate	55%	90%
28-day submission rate	64%	92%
RETURN INTERVALS		
Return intervals %2–17 days	23%	13%
Return intervals %18–24 days	57%	69%
Return intervals %39–45 days	2%	7%
CONCEPTION RATES		
1st service 49-day NR	53%	61%
1st service pregnancy rate	42%	60%
IN-CALF RATES		
4-week in-calf rate	38%	57%
8-week in-calf rate	73%	86%
% not in-calf by PSM + 165 days	10%	7%



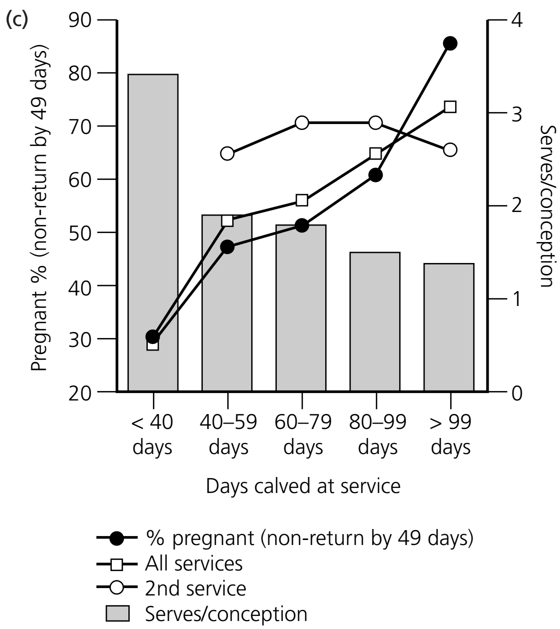


Fig. 24.15 DairyWIN© analysis of dairy herd with low submission rate. (a) Main parameters of Reproductive Monitor, showing low submission rates, poor conception rates and a high proportion of short interservice intervals. NR, not recorded (reproduced with permission). (b) Relationship between time after calving and submission rate for herd shown in Figure 24.15(a). (c) Relationship between time after calving and conception rate to first, second and all services, and upon services per conception, for the herd shown in Figure 24.15 (a).

overdetection of oestrus in the early part of the mating period do not represent a serious problem, but, in this case, the herd's reproductive performance had suffered seriously, leading to low percentages of the herd being pregnant by 4 and 8 weeks into the mating period.

Interservice interval analysis is, thus, of greatest value as a means of investigating low submission rates or poor conception rates. The presentation of interservice interval information in a simple histogram form (Figure 24.18b) is sometimes a useful introduction to discussing the delicate

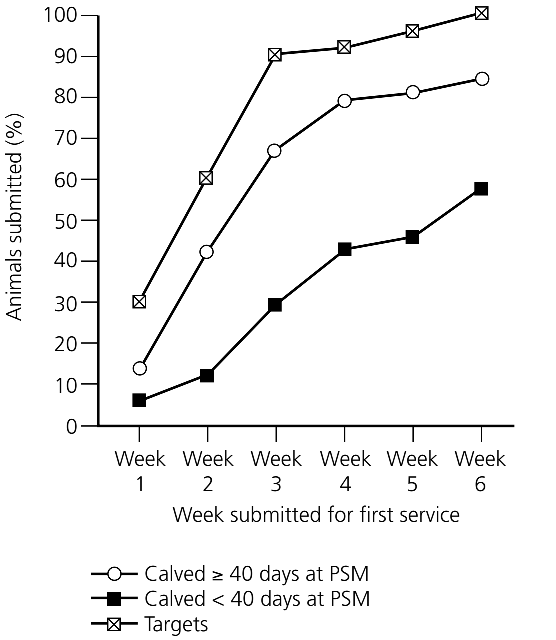


Fig. 24.16 Relationship between time after calving and submission rate for a herd with a poor overall 3-week submission rate.

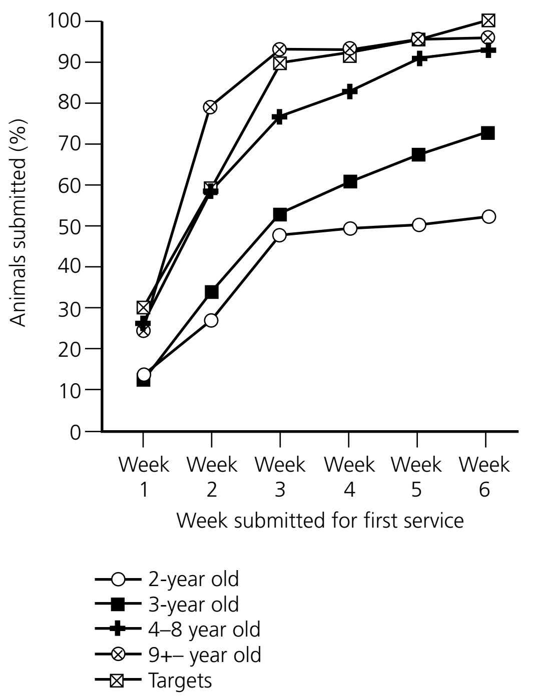


Fig. 24.17 Relationship between age and submission rate for heifers calving for the first time in inadequate body condition.

(a)

Reproductive Monitor Report

Stock Class: Adult Cow

	Period 1	Target
SUBMISSION RATES		
Percent calved < 40 days at PSM	11%	10%
21-day submission rate	91%	90%
28-day submission rate	94%	92%
RETURN INTERVALS		
Return intervals %2–17 days	35%	13%
Return intervals %18–24 days	44%	69%
Return intervals %39–45 days	2%	7%
CONCEPTION RATES		
1st service 49-day NR	44%	61%
Total service 49-day NR	51%	61%
1st service pregnancy rate	42%	60%
Total service pregnancy rate	39%	60%
Services per conception	2.6	1.7
IN-CALF RATES		
4-week in-calf rate	49%	57%
8-week in-calf rate	75%	86%
% not in-calf by PSM + 165 days	11%	7%

(b)

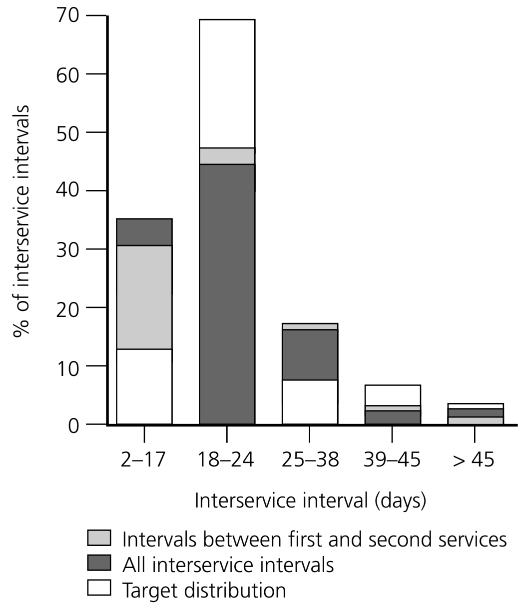


Fig. 24.18 DairyWIN© analysis of a dairy herd with a low conception rate. (a) Main parameters of Reproductive Monitor, showing high submission rates, but poor conception rates due to poor oestrus detection (reproduced with permission). (b) Analysis of interservice intervals of the herd shown in Figure 24.18(a). The proportions of interservice intervals of 18–24 days are well below target, but all other categories (especially intervals of 2–17 days) are above target.

question of poor oestrus detection efficiency with the relevant farm workers.

Other causes of infertility (see Chapter 22)

The causes of low pregnancy rates are similarly multifactorial. Primary causes of poor pregnancy rates include poor insemination technique, poor semen quality (especially where semen used for farmers' own inseminations has been poorly stored) or infertile bulls. Low pregnancy rates are also considered to occur in the face of some micronutrient deficiencies, especially copper and selenium (Lean et al., 1998), although the evidence for these remains somewhat controversial. Likewise, poor pregnancy rates may occur in the face of a poor plane of nutrition, although it is more common for anoestrus, rather than low pregnancy rates, to characterise the infertility that follows malnutrition.

As described above, poor pregnancy rates also occur in the face of inaccurate oestrus detection. A common syndrome is to find mediocre pregnancy rates during the first week of the breeding season. This results from the common fault of overdetection of oestrus in the first 3 weeks of the mating period, particularly in its first week, when herd managers are often excessively zealous in their attempts to achieve high submission rates. Such a situation is generally regarded as of relatively little significance, since the overdetection of oestrus results in most cows being correctly inseminated at some point, with the main economic loss coming from wastage of semen. However, the desire to achieve high submission rates also results in cows being presented for service too soon after calving. The ability of DairyWIN© to compare pregnancy rates at various stages after calving facilitates the diagnosis of this problem (Figure 24.16).

Unfortunately, the brevity of the breeding season and the retrospective nature of pregnancy rate analysis mean that, by the time one becomes aware of a problem, it is often too late to take effective remedial action. It may, however, be possible to extend the AI period, or to use $PGF_{2\alpha}$ to shorten the luteal phase in those that are known not to have conceived, to allow them extra opportunity. Many farmers use oestrus synchronisation at the start of the breeding season to ensure that as many cows as possible are mated within the first week. This is often an effective tool in the compaction of calving patterns, but it needs to be backed up by efficient oestrus detection. For example, in the herd illustrated in Figure 24.19, oestrus was synchronised in many cows so that they were in heat in the first few days of that mating period (Figure 24.19(b)). Hence, a very high 3-week submission rate was achieved (Figure 24.19(a)). However, observation of repeats was poor, as there was a high proportion of 39–45-day interservice intervals (indicating missed heats) and a significant discrepancy between the 49-day non-return rate and the pregnancy rate (indicating failure to observe returns to service). Hence, a large number (17%) of animals failed to conceive by the end of the mating period.

Infectious diseases should not be ignored as a cause of poor conception rates. Diseases that were once thought to be eliminated, such as campylobacteriosis and trichomoniasis, are still found in herds in some marginal areas, while the significance of BVD as a cause of apparent low pregnancy rates is discussed in Chapter 23. Ureaplasmosis also appears to be a significant cause of conception failure. A characteristic hallmark of most of these diseases is the presence of extended interservice intervals, chiefly in the 25–35-day and 36–48-day categories.

Causes of poor pregnancy rates are determined by progressive elimination of possibilities. DairyWIN© can be used to determine whether problems can be attributed to an individual AI sire, or to identify the date from which the problem arose. Such analyses may then lead to examination of straws of semen, comparison of different AI technicians' conception rates, observations of herd managers' insemination technique, examination of cows that are presented as being in oestrus, or breeding soundness examination of bulls. Unless there are very clear indications of venereal disease, within a herd, all other possibilities should be eliminated before investigating this possibility.

A final example of the effects of a management decision upon herd fertility is shown in Figure 24.20. In this herd, calving was induced in two groups of cows, in an attempt to tighten the calving pattern. In the first group, calving was induced at the start of the calving period, so the animals had

(a)

Reproductive Monitor Report

Stock Class: Adult Cow

	Period 1	Target
SUBMISSION RATES		
Percent calved < 40 days at PSM	10%	10%
21-day submission rate	84%	90%
28-day submission rate	93%	92%
RETURN INTERVALS		
Return intervals %2–17 days	8%	13%
Return intervals %18–24 days	61%	69%
Return intervals %39–45 days	12%	7%
CONCEPTION RATES		
1st service 49-day NR	65%	61%
Total service 49-day NR	70%	61%
1st service pregnancy rate	44%	60%
Total service pregnancy rate	42%	60%
Services per conception	2.4	1.7
IN-CALF RATES		
4-week In-calf rate	55%	57%
8-week In-calf rate	65%	86%
% not In-calf by PSM + 165 days	17%	7%

(b)

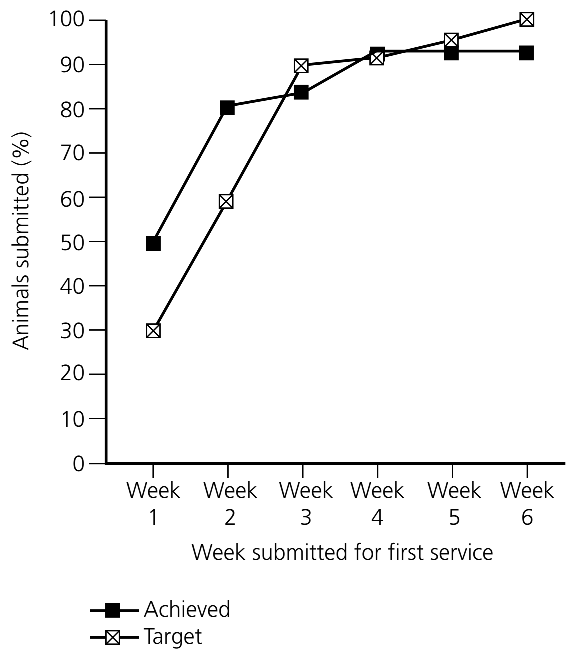


Fig. 24.19 DairyWIN© analysis of a dairy herd in which oestrus synchronisation is used to obtain a high initial submission rate, but after which there is a failure to detect oestrus and returns to service. (a) Main parameters of Reproductive Monitor, showing high initial submission rates, high non-return rate but poor conception rate, and a high proportion of 39–45 day interservice intervals (reproduced with permission). (b) Changes in the proportion of cows submitted for service with time after the start of mating. Note that initial submission rates are well above target due to the use of oestrus synchronisation before the start of mating, but end up below target, due to poor oestrus detection.

plenty of time to recover before mating. They had also been selected for induction long before the event, so had had preferential management in the pre-induction period. These animals had good reproductive performance and, hence, good conception rates. By contrast, a second group were induced at the end of the calving period. As the decision to induce was not made in good time, the cows had no preferential management before induction. Thus, these animals were still in negative energy balance and were still undergoing uterine involution at the start of the mating period, so very few of them eventually conceived.

MANAGING FERTILITY AND ROUTINE VISITS IN BEEF SUCKLER HERDS

Veterinary involvement in assisting the herd manager in the management of fertility in beef suckler herds is often minimal. However, since good fertility, together with correct nutrition, is a major influence on the profitability of suckled calf production, there is a need for veterinary input with the implementation of fertility control schemes and routine visits, although it will not be at the level required for dairy herds.

Apart from the obvious differences, suckler cows have a greater longevity than dairy cows, 9 years compared with 6 years, and produce 6–7 calves in a lifetime. In addition, natural service rather than artificial insemination is generally used, which means that the male has a greater direct influence on fertility and could transmit venereal diseases.

The requirements for good reproductive performance in a suckler herd are as follows:

- A calf per year, thus a 365-day calving index. The calving indices for some of the best herds

Reproductive Monitor Report

Stock Class: Adult Cow

	Whole herd	Early induced	Late induced
CALVING PERFORMANCE			
4-week calving rate	62%		
8-week calving rate	90%		
SUBMISSION RATES			
Percent calved < 40 days at PSM	21%	0%	78%
21-day submission rate	57%	65%	13%
28-day submission rate	68%	87%	13%
RETURN INTERVALS			
Return intervals % 2–17 days	26%	29%	13%
Return intervals % 18–24 days	43%	64%	69%
Return intervals % 39–45 days	6%	0%	0%
CONCEPTION RATES			
1st service 49-day NR	45%	59%	33%
Total service 49-day NR	53%	61%	50%
1st service pregnancy rate	42%	64%	17%
Total service pregnancy rate	34%	53%	25%
Services per conception	2.9	1.9	4.0
IN-CALF RATES			
4-week in-calf rate	35%	57%	13%
8-week in-calf rate	67%	91%	43%
% Not in-calf by PSM + 165 days	17%	9%	44%

Fig. 24.20 DairyWIN© analysis of the effects upon subsequent fertility of induction of calving early or late in the calving period. Calving was induced with the intention of tightening a poor calving pattern. (reproduced with permission).

in the UK exceed this figure (Meat and Livestock Commission Report, 1992).

- A compact calving period of 2 months. This ensures that calves are of similar age and weight at weaning, improves their overall health and reduces calf mortality by ensuring that late-born calves do not acquire infection from older, earlier-born animals. In addition, cows are at a similar stage in their production cycle; thus their feeding and other aspects of management will be the same.
- Cows should calve at the best time of year to utilise the available feed, thus in spring, summer and autumn but not winter.
- Cows should calve at a condition score of $2\frac{1}{2}$–3.
- It is important to use fertile bulls, running with reasonable numbers of cows and heifers. Particularly in heifers, a sire that produces easy calvings should be used.
- Ideally, heifers should be served so that they calve 2–3 weeks before the cows in the herd, to provide the opportunity for a longer calving–conception interval.
- Primipara may lose excessive weight; hence they should be fed separately from and additionally to the rest of the herd. It may be necessary to wean their calves slightly earlier.

A scheme for managing the fertility of a suckler herd to satisfy the requirements previously listed is as follows, and can be modified depending on the time of the proposed calving season:

- The herd is calving during September and October. Details of dystocia, retained placenta, uterine infection and other non-reproductive diseases should be recorded.
- Early to mid-December: assess the condition score of all cows – they should be at least $2\frac{1}{2}$ – and examine per rectum all cows that had reproductive disorders and disease. Ideally all cows should be examined for return of cyclical ovarian activity.
- Early December: examine the bull or bulls for health, bodily condition and fertility. Ensure that there are adequate numbers and that they are free from venereal disease.
- Mid-December: introduce bull or bulls for 8 weeks and remove in mid-February.
- Examine per rectum all cows for pregnancy from the time that the bull was removed and for the next 6–8 weeks. Estimate gestational age and predict the calving date for each animal.
- Calves weaned in June: assess the condition score of cows and modify feeding if necessary.
- Calving during September to October at condition score 3.

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25 Infertility in the ewe and doe (female goat)

SHEEP

The level of fertility in sheep is usually expressed as the reproductive performance of the flock. This can be defined as the number of lambs born per 100 ewes put to the ram (i.e. true lambing percentage). The breeding season commences with the introduction of rams, and all physical and financial performance should be calculated from this point, taking into consideration ewes that die, those that are culled and those that abort or are barren. The Meat and Livestock Commission (MLC) (1988) recognise two categories of reproductive wastage: namely, ewes that die during gestation (dead ewes) and those that fail to lamb (empty ewes). Empty ewes are a cost to the flock, but are often not included in costings, making between-flock comparisons difficult and in some cases giving an over-optimistic impression of reproductive performance (Maund and Jones, 1986). Three factors influence the numbers of lambs sold: fertility, i.e. whether the ewes are pregnant and lamb; fecundity, i.e. the number of lambs born per pregnancy; and survival rate to weaning. In the UK, efforts to maximise numbers of lambs sold have concentrated upon the use of more prolific breeds and improving ewe nutrition. However, despite increased veterinary input and considerable improvements in awareness of disease, and its diagnosis and treatment, the proportion of ewes failing to lamb in the UK has stood consistently at approximately 6% for the past hundred years (Heape, 1899; MLC, 1984, 1988). Improvements in our understanding of neonatal lamb losses, and control of disease in both ewes and lambs, have also contributed to an increase in numbers of lambs reared.

Figures for ewe productivity in lowland and upland flocks in 1998 published by the MLC showed that in 91 lowland flocks the percentages of lambs born, born live and weaned were 177%, 168% and 152%, and for 110 upland flocks, the values were 157%, 150% and 143%, respectively. In both lowland and upland flocks, there were 4% empty ewes. In a detailed study of 5488 ewes in 34 flocks involving pure breeds or crosses, Smith (1991) found that of the 6.4% (348) ewes that suffered true reproductive losses 3.4% were barren, 2.4% aborted, 0.3% were multiply mated but failed to conceive, and 0.3% were anoestrous. Before the advent of accurate and inexpensive methods of pregnancy diagnosis, especially B-mode ultrasound, barren ewes were frequently not identified until they had failed to lamb. Barren ewes are usually culled, and as a consequence there is some genetic selection against poor fertility. Fecundity is influenced by genetic selection, age of the ewe, nutritional status and environment. Lamb survival rate will be influenced mainly by management factors, the environment and also genetic selection for such traits as good mothering behaviour.

The better level of fertility of sheep compared with cattle is a reflection of the more natural breeding environment to which the former are subject. Ewes are generally allowed to run with the ram during the breeding season and not segregated; thus oestrus detection problems are not encountered. Furthermore, most breeds of sheep have a longer period of acyclicity after parturition than the cow, thus allowing the reproductive system time to recover from the effects of pregnancy. Published information on normal conception rates in British lowland ewes is vague. However, the fertility of ewes, as measured by pregnancy (conception) rates to first service by Smith (1991) was 91.6%. In the same study, 99.4% had conceived by the third mating. The main factors responsible for infertility in sheep are specific infectious agents that usually result in abortion. Much veterinary research into sheep reproduction concentrates on these problems. Structural, functional and management factors are of limited importance.

Structural defects

Structural defects of ovine genital organs are uncommon. In an abattoir survey of 2081 sheep genitalia, Emady et al. (1975) found 0.72% with macroscopic abnormalities. In a more recent and extensive survey, involving 33 506 ovine genital tracts (9970 parous) from two UK abattoirs, Smith (1993) identified 6.57% of parous and 1.95% of nulliparous tracts with pathological lesions. Most involved the ovaries and their associated bursae, with fibrin tags and paraovarian cysts being most frequently identified. However, it is unlikely that these lesions alone would cause infertility. There is no doubt that many of the other lesions identified in this survey would have caused infertility or sterility (e.g. ovarian aplasia, ovarian hypoplasia, bilateral hydrosalpinx, aplasia of the paramesonephric ducts, freemartinism, hermaphrodism and pseudohermaphrodism). Owing to the rarity of anastomoses of the adjacent allantoic vessels of twins, the freemartin condition is likely to be rare, but incidences of 0.23–1.22% have been recorded (Dain, 1971; Long, 1980; Smith, 1993). Even higher levels have been detected in the more prolific breeds: for example, 6.85% in Booroola F ewes (Cribiu et al., 1990). Cases of intersexuality are seen, mainly at lamb castration. They are male pseudohermaphrodites referred to by shepherds as 'wilgils'. The fact that several may be seen at once in a flock tends to point to a possible hereditary cause. Other developmental defects of the genital organs of sheep are rare, although there is good evidence of an association between ovarian hypoplasia and breeds with high fecundity (Davis et al., 1992; Vaughan et al., 1997).

Functional factors

Except in the case of unthrifty ewes (which are usually culled), anoestrus is uncommon in sheep; Smith (1991) identified the condition in 0.3% of 5488 lowland ewes. In fact, when the rams are turned out with the flock it is usual for most of the ewes to be mated within a month. The first oestrus of the breeding season in some ewes is anovulatory and, according to Dutt (1954), more frequently ewes fail to become fertilised at these early matings compared to later ones. Ovarian follicular cysts, commonly encountered in cattle, are of limited importance in sheep. Smith (1996) identified follicular cysts in 2.9% and 10.02% of abnormal parous and nulliparous genital tracts, respectively. Luteal cysts were rare. Embryonic death, or resorption, is a conspicuous feature of sheep infertility and is more often associated with multiple than with single conception. It is possible that a greater degree of embryonic death follows early matings. By comparing the number of corpora lutea with the number of fetuses the incidence of the condition has currently been estimated at 20 to 33% (Wallace and Ashworth, 1990; Bruere and West, 1993).

Early embryonic death has been associated with infectious diseases such as toxoplasmosis and Border disease (see below). In a survey by Johnston (1988), 35.2% of barren ewes had elevated antibody titres to *Toxoplasma*, compared with 19.2% of fertile ewes.

Sporadic cases of obvious abortion and of fetal mummification are occasionally seen. A specific environmental cause of sheep infertility, due to grazing on pastures of subterranean clover, was described by Bennetts et al. (1946) in Australia. This clover contains large amounts of the oestrogenic substance genistein, the ingestion of which leads to cystic degeneration of the endometrium and permanent sterility. Although small amounts of oestrogenic substances have been identified in other plants, no comparable degree of infertility due to such substances has been seen outside Australia. Asynchrony or imbalance of the hormonal changes that occur around the time of oestrus and during the early luteal phase probably results in embryonic death. In an experimental study involving ovariectomised ewes as recipients for sheep embryos, a rigid regimen of steroid hormone replacement is necessary to ensure embryo survival (Wilmut et al., 1985). The sequence is: (1) progesterone supplementation to simulate the previous luteal phase; (2) oestradiol to simulate oestrus; (3) low levels of progesterone supplementation to simulate early dioestrus; followed by (4) high levels of progesterone to simulate the normal luteal phase.

Management factors

Oestrus detection and artificial insemination

The best method of oestrus detection is with a raddled, vasectomised ram.

Artificial insemination in sheep has not assumed the popularity achieved in cattle. A number of factors have been responsible, notably the disappointing results using frozen/thawed semen deposited intracervically. The spermatozoa are unable to colonise or traverse the length of the cervix and are rapidly lost from the ewe's reproductive tract. However, the use of intrauterine insemination by laparoscopy has been much more successful, with pregnancy rates of over 70% using both fresh extended semen and frozen-thawed semen (McKelvey, 1999). The penetrability of the cervix of the ewe is currently under investigation in order to devise a method that will produce similar results to those obtained by laparoscopic AI, and reduce the number of pathological lesions frequently detected following the intracervical technique (McKelvey, 1999). Artificial insemination is best used in midoestrus, or 12–14 hours after its onset.

Teasing

The introduction of vasectomised teasers into the flock, before fertile rams, had no effect on pregnancy (conception) rates (Smith, 1991). However, in his study, they had a profound effect upon the onset of cyclical activity and hence a compact lambing season. Of teased ewes, 84.8% exhibited oestrus in the first 16 days after exposure to the fertile ram, whilst two cycles were required for the unteased ewes to show comparable activity. The author also demonstrated the necessity of adequately isolating ewes and rams before teasing from sight, sound and smell of each other.

Ram:ewe ratio

The number of rams per ewe will vary depending upon a number of factors: age of the ram; age of the ewes; whether more than one ram is to be used with the group of ewes; and terrain and size of the enclosure. Ram:ewe ratios of 1:25 to 1:40 are suitable in non-synchronised flocks. However, where synchronisation is attempted, a ratio of at least 1:10 should be available.

Nutrition

It is important that ewes are in good bodily condition at tupping. Increasing the energy intake several weeks before tupping, so that the ewes are gaining weight (flushing), will increase the fecundity in those ewes with the genetic potential. Provided the level of feeding is maintained for a month after mating this should ensure good pregnancy rates. Some reduction in food intake is reasonable during the second and third months of gestation, but feeding should be increased in the last 6–8 weeks before lambing.

Increasing fecundity

Increased ovulation rates can be achieved by the administration of equine chorionic gonadotrophin (eCG) on the 12th or 13th day of the oestrous cycle. Good results have been obtained by immunisation against androstenedione. A commercial product is no longer available in the UK.

Infectious agents

Non-specific infections of the genital tract, especially the uterus, are of minimal importance in ewes, probably because in most breeds of sheep there is a long period of anoestrus following lambing. In the small number of ewes in which bacterial contamination occurs at lambing or postpartum, which is less than 20%, they are rapidly eliminated within a week (Regassa and Noakes, 1999), and thus before the genital tract can be exposed to a period of progesterone influence; this will occur at the next dioestrus which will normally be many months away. In the cow, retention of the fetal membranes (RFM) postpartum is quite common, and this is a major risk factor in the development of endometritis and subfertility. RFM is relatively uncommon in ewes; where it does occur, attempted removal by applying traction to the exposed portions of the membranes can be attempted. If left, they will usually separate and be shed within 5–6 days. If an affected ewe shows signs of systemic illness due to the development of metritis, then she should be treated with an appropriate broad-spectrum antibiotic. However, there are a number of specific infectious agents that can have a profound effect upon fertility, particularly by causing abortion and perinatal mortality.

A survey of diagnoses recorded at Veterinary Laboratory Agency (VLA) regional laboratories in England and Wales and Disease Surveillance Centres in Scotland, listing the infectious causes of ovine fetopathy identified in material submitted to their laboratories from 1977 to 1998, is summarised in Table 25.1.

Enzootic abortion of ewes (EAE)

EAE is also known as ovine enzootic abortion or kebbing. Infection is caused by *Chlamydia psittaci* immunotype 1 (recently reclassified as *Chlamydophila abortus*), which has a predilection for the pregnant uterus. It may also infect goats, cattle, deer and humans, and is the commonest cause of ovine abortion in the UK. For many years, in Scotland and the English border counties shepherds and veterinarians were familiar with an enzootic abortion in flocks. The causal organism was identified by Stamp et al. (1950). The disease is now widespread in Britain, and common in Europe and the western USA. *Chlamydia psittaci* immunotype 1 has a highly specialised life-cycle that involves alternate intra- and extracellular phases that confer advantages for evasion of host immune responses and facilitates the maintenance of low-grade asymptomatic infection (Aitken, 1986).

Epidemiology. The major source of infection, responsible for over 80% of new outbreaks in clean flocks, is the purchase of infected ewes of any age (Greig, 1996). Spread may also be by wildlife, e.g. foxes, gulls and crows. Sheep-to-sheep spread is the commonest route, and lambing time is the greatest time of risk when infected ewes shed large numbers of infectious particles into the environment. Susceptible ewes inhale or ingest chlamydiae from infected placentae, uterine discharges, dead lambs and contaminated bedding. Infective

Table 25.1 Percentage frequency of isolation of pathogens from ovine fetopathies examined by Ministry of Agriculture Veterinary Investigation Centres (Source VIDA-II)

	1977	*1984*	*1988*	*1989*	*1992*	*1993*	*1994*	*1995*	*1996*	*1997*	*1998*
Brucella abortus	0	0	0.02	0	0	0	0	0	0	0	0
A. pyogenes	0.5	1.6	1.0	0.8	1.1	1.3	1.4	1.2	1.2	1.3	1.1
Campylobacter spp.	13.2	14.3	7.3	6.9	6.7	4.9	7.6	8.8	11.0	9.4	10.5
Chlamydia	32.2	39.5	40.1	41.9	46.9	49.3	49.3	53.4	50.0	50.9	37.8
L. monocytogenes	0.7	2.1	2.6	3.2	3.0	2.5	3.2	2.7	2.3	2.3	2.4
S. abortus ovis	0.5	0.1	0.07	0.05	0	0	0	0	0	0	0.04
S. dublin	1.7	0.3	0.6	0.6	0.4	0.3	0.4	0.5	0.3	0.3	0.40
S. typhimurium	0	0.3	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.16
Other *Salmonella* serotypes	2.2	1.8	1.7	1.3	1.2	1.3	2.6	2.4	2.6	2.8	2.3
Toxoplasma spp.	36.2	31.3	40.3	38.4	35.4	34.6	28.9	25.2	26.4	24.4	33.2
Coxiella burnetti	NR	0.1	0.05	0.03	0.2	0.1	0.1	0.03	0	0.12	0.16
Fungi	0.7	0.1	0.3	0.4	0.2	0	0.1	0.03	0	0.04	0
Other pathogens	12.0	8.4	5.9	6.3	4.7	4.3	4.7	4.7	6.0	8.3	11.9
Total identified	583	2419	4116	3774	2529	2475	2527	2907	2833	2460	2447
Total submitted	1349	4790	7292	6712	4184	4072	4330	4667	3676	4560	4195
Percentage diagnosed	43.2	50.5	56.4	56.2	60.4	60.8	58.4	62.3	77.1	53.9	58.3

NR = Not recorded

particles (elementary bodies) may survive for weeks at low environmental temperatures. Ewes infected early in pregnancy usually abort; otherwise the chlamydia lie dormant until the next pregnancy. Chlamydia are not transmitted in the milk of infected ewes. However, lambs may acquire infection from uterine discharge on the teats. Of the lambs infected by the ewes, field evidence suggests that around 30% may develop placentitis in their first pregnancy (either as lambs or shearlings), a proportion of which may abort (Greig, 1996).

Chlamydia acquired outwith pregnancy lie dormant. However, they can be reactivated from their 'latent' state during pregnancy. Neither the site of latency nor the precise triggers of reactivation have been identified. Latently infected flocks are unrecognisable by immunological methods. The work of Buxton et al. (1990) demonstrated that ewes were susceptible to infection from early gestation onwards. The tonsil and lymphoid tissue of the pharynx has been shown to be a primary site of infection, with subsequent blood-borne spread to major organs and lymph nodes. Thereafter, until 60 to 90 days of pregnancy, the site of chlamydial persistence has not been ascertained and although infection of the placentae and fetuses occurred from 60 days gestation, pathological changes were not observed until after day 90. Rapid replication of *C. psittaci* leads to local necrosis and contiguous spread of infection involving the cotyledonary and intercotyledonary placenta and apposing endometrium, resulting in abortion that usually occurs in the last 2 weeks of pregnancy. The macroscopic signs of a placentitis are similar to that following *Brucella abortus* infection in cattle. The intercotyledonary allantochorion is oedematous, thickened and leathery in appearance; there is degeneration and necrosis of the fetal cotyledons and a thick yellow deposit on the chorion.

Abortion occurs 40–50 days after being infected; however, those ewes infected late in pregnancy do not abort until the following pregnancy. In split lambing flocks, late lambing ewes may acquire infection from infected ewes in the earlier lambing flock and abort in the same season (Blewett et al., 1982). Infected bought-in sheep may abort in the first year spreading infection at lambing time to susceptible ewes and lambs, resulting in an abortion storm the following year.

Most aborted lambs are well developed, fresh and show no autolytic changes, indicative of recent death in utero; some infected ewes may produce both dead and live lambs. However, lambs born alive may be weak, fail to survive and in spite of good nursing contribute to the overall losses from EAE. A small number of ewes may develop post-abortion metritis (Aitken, 1986). Abortion rates vary from 5% to 30%, the upper level most likely to occur in the first or second year following the introduction of infection, and thereafter at a rate of 5–10%. However, these figures do not take into account losses in the neonatal period which may be as high as 25% (Greig, 1996).

The disease is extremely rare in hill flocks, unless housed for lambing in facilities previously used by infected lowland flocks.

Although rams can become infected and may develop epididymitis, there is no evidence that they play any significant role in the transmission of EAE (Appleyard et al., 1985). In the UK, rams rarely run with ewes during lambing/abortion times and therefore are not exposed to chlamydial infection.

Diagnosis

Clinical signs. There are no premonitory signs of impending abortion. Ewes are not ill. However, a few ewes may show evidence of a vaginal discharge for several days beforehand and possibly behavioural changes. There may be abortions, premature lambs, weakly live lambs and normal lambs with infected membranes. Ewes may retain fetal membranes leading to metritis, but no other clinical signs are seen.

Placental lesions and staining. The placenta is usually acutely inflamed, thickened and necrosed showing typical signs of a placentitis (Plate 4). Smears from infected intercotyledonary areas, and the wet skin of the fetus, can be stained by the modified Ziehl–Neelsen method to detect intracellular inclusion bodies, which occur as small acid-fast cocci; they may be seen intracellulary as clumps, or singularly scattered throughout the smear; these may be confused with *Coxiella burnetti* organisms, which are larger.

Serology. The demonstration of specific chlamydial antibody in fetal fluids or precolostral lamb serum with a fluorescent antibody test is specific evidence of chlamydial infection.

The complement fixation test is the routine diagnostic test used, a titre of over 64 generally being accepted as positive. Paired samples should be taken, at the time of abortion and 3–4 weeks post-aborting; in positive ewes samples show a significant rise in antibody titres. Vaccinated ewes will have lower titres with no evidence of a rise. An enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescent antibody test are also available.

Treatment. Antibiotics that will reduce rather than eliminate abortions can be used in flocks with extended lambing seasons. For the best results, treatment should be given as soon after 95–100 days of gestation as possible, at which time possible cases of placental infection will have commenced. Although it is expensive, long-acting oxytetracycline, at a dose of 20 mg/kg repeated every 10–14 days until lambing, has been used (Aitken, 1986). This treatment will reduce the number of organisms shed, but does not eliminate infection. Nor can it reverse pathological changes already present in a heavily infected placenta; hence some abortions will still occur despite treatment.

Control. Control (Aitken et al., 1990) should aim at keeping the flock clean by buying all replacement stock from EAE-accredited flocks within the Premium Health Scheme (under the control of the Scottish Agricultural College).

(a) Following diagnosis of EAE

- Isolate, for up to 3 weeks, and mark all ewes that abort.
- Send dead lambs and membranes to a laboratory for diagnosis.
- Reduce risk of spread to other ewes.
- Burn or bury dead lambs and membranes not needed for diagnosis.
- Clean lambing area and cover with clean straw.
- Discourage use of ewes to foster lambs, as infection may be picked up from vaginal discharges and infected fleeces. If lambs are fostered they should not be used for breeding.
- In following years consider vaccination policy and/or strategic use of oxytetracycline.

Ewes that have aquired infection do not develop positive titres until they abort; therefore it is not possible to screen a flock to detect latent infection.

Protection by vaccination. Enzovac (Intervet UK), which contain a temperature-sensitive strain of *Chlamydia psittaci*, requires a 2-month period after injection to develop ‘protective’ antibody levels. Vaccination can be used from 5 months of age, and also in older animals between 1 and 4 months pre-tupping. The vaccine will protect lambs from transplacental infection. High-risk flocks, viz. with >5% abortions per year, and sheep bought from non-accredited flocks should have vaccination repeated yearly or biannually. Low-risk flocks, viz. where <5% abortions occur, and sheep bought in only from accredited flocks need to be vaccinated once only. An inactive preparation, Mydiavac (Novartis UK Ltd), has also been developed, and experimental data indicates that it may reduce the level of abortion in previously infected ewes during on outbreak of chlamydial infection.

(b) If EAE is not present. Strenuous efforts must be made to prevent the disease gaining entry using the following management strategies:

- Maintain a closed flock and purchase rams from known sources, or replacements from EAE-monitored flocks.
- Purchased ewes should be lambed separately from the indigenous flock in the first year, and all abortions and barren ewes should be investigated.

Zoonotic risks. *C. psittaci* from sheep can be extremely dangerous to pregnant women, growing rapidly in the unborn baby’s placenta. Initial mild influenza-like symptoms become progressively more severe, and abortion occurs within a week. Disseminate intravascular coagulation may develop in the mother causing critical illness. Intensive nursing normally results in complete recovery, but regrettably, to date all the babies have died (Buxton, 1986).

Toxoplasmosis

Toxoplasma gondii infection is the second most common cause of abortion in the UK; it has been shown to cause abortion, stillbirths and weakly offspring in many domestic species, including sheep, and has a world-wide distribution in animals and humans. Infection in non-pregnant sheep is typically mild and inapparent (Blewett and Watson, 1983), but in pregnancy it is essentially a disease of the conceptus.

The causal organism has a complex life cycle, involving an asexual cycle that can occur in any species of mammal or bird, and a sexual cycle that can only be completed in cats and wild *Felidae*. In the cat family, the parasite multiplies within the epithelial cells of the intestine and, as a consequence, oocysts will be excreted in the faeces for about 8 days, during which time tens of thousands of oocysts can be shed. These sporulate within a few days and are then ingested by sheep (Frenkel, 1973).

Epidemiology. The principal vector in the spread of toxoplasmosis is the cat and its related wild species. Infective toxoplasma oocysts may survive up to 2 years on pasture, feed or bedding. They are passed in the faeces of young cats, who become infected when they first begin to hunt. Although toxoplasms have been demonstrated in the semen of experimentally and naturally infected rams (Spence et al., 1978), infection of the ewe at tupping would be unlikely to cause abortion. Toxoplasms can also be passed in milk during acute infections. Lateral spread within a flock from aborting ewes is likely to be relatively unimportant. However, lambs born alive that survive from infected ewes can be congenitally infected. As few as 200 oocysts will infect one ewe, and as many as 1 million may be present in 1 g of cat faeces (Buxton, 1989). Once ingested sporozoites are released, they penetrate the intestines and are distributed to many organs where tissue cysts form, a febrile response occurs after 5–12 days in conjunction with a parasitaemia. Toxoplasma can be detected in the uterine caruncular septa 10 days after oocyst ingestion, and placental trophoblast cells after 10–15 days; toxoplasma-specific fetal antibody is present after 30 days (Buxton, 1989).

Clinical signs. The effect upon reproduction depends upon the stage of pregnancy when infection occurs. If early in gestation, i.e. before 60–70 days, fetal resorption usually occurs, with ewes returning to oestrus or remaining barren. Unlike EAE, numerous barren ewes, which have not been seen to abort, may be detected in the flock at scanning or lambing time. With early embryonic mortality, providing a ram is still present and the breeding season has not ended, ewes are capable of conceiving, now with a good immunity. Infection in mid-gestation results in abortion or mummification; in the latter case, only one member of a set of twins or triplets may be involved. Infection after 120 days usually results in stillbirth, or weakly or normal lambs.

The gross appearance of the placenta, particularly the cotyledons, is fairly typical of the disease. Cotyledons are bright-to dark-red in colour with multiple small white nodules 1–3 mm in diameter. These nodules may be sparse or so numerous that they become confluent; sometimes normal cotyledons are present (Plate 5). The intercotyledonary areas of the allantochorion appear normal (unlike infection with *C. psittaci*).

Diagnosis. The condition is characterised by barren ewes, abortions, stillbirths, and mummified and weakly lambs. The appearance of the placenta is diagnostic.

Confirmation can sometimes be made using Giemsa or Leishmann-stained smears of those cotyledons containing the white nodules. Alternatively, histological sections of the cotyledons may be required to demonstrate the presence of the parasite. Examination of the brain, especially in those lambs that die soon after birth, may reveal foci of glial cells and leucoencephalomalacia, which are characteristic of the infection (Buxton et al., 1981a). Immunofluorescent staining of cotyledon sections can also be used.

A number of satisfactory serological tests on the maternal serum have been used including the dye test of Sabin and Feldman, the indirect fluorescent antibody (IFA) test, radioimmunoassay and the ELISA test (Buxton, 1983). The ELISA test has been modified to detect anti-toxoplasma immunoglobulin G (IgG) (Buxton et al., 1988) in body fluids (Buxton, 1983). The indirect haemagglutination test (IHA) has been developed so that kits are available for use by veterinarians in practice laboratories. A single serum sample with an elevated titre may well only indicate past infection.

Serology of ewes is difficult to interpret as antibodies remain elevated for years. However, paired samples may prove beneficial. Those taken at a 14-day interval and showing a rising titre are indicative of an active infection.

Infected lambs will have precolostral antibodies, and serology can be performed on pleural, pericardial, or peritoneal fetal fluids or lamb serum, providing that the lambs have not received colostrum. If postcolostral samples only are available, it is necessary to demonstrate IgM and IgG antibody (Buxton, 1983).

Treatment and control. Chemoprophylaxis with monensin given in the food at the rate of 15 mg/animal/day during pregnancy can significantly suppress toxoplasma infection in sheep (Buxton et al., 1988), but it is not licensed in the UK for this purpose. Decoquinate, the anticoccidial drug fed daily at 2 mg/kg body weight, also significantly reduces the effect of *T. gondii* oocysts ingested by pregnant sheep (Buxton et al., 1996) and is licensed in the UK for this use. Both these products work best if they are being fed at the time sheep encounter infection rather than after infection has been established.

Ewes can be treated during the acute phase of the disease with sulfonamides and potentiated sulfonamides such as trimethoprim, but it is expensive. A recent study has demonstrated the efficacy of a combination of sulfamethazine and pyrimethamine, which is used to treat the disease in humans (Buxton et al., 1993).

Although there is virtually no danger of lateral spread from aborting ewes, isolation in the early stages of an abortion outbreak should be implemented, since there is a possibility that other infectious agents may also be present.

Ewes that have aborted will be immune and should be retained in the flock, and new additions should be exposed to possible infection with oocysts from contaminated food as early as possible before the start of the breeding season.

Recently, an effective vaccine has been developed using living tachyzoites of the S48 strain of *T. gondii* (O'Connell et al., 1988; Buxton et al., 1991) (Toxovax, Intervet, UK). Ewe lambs should be vaccinated from 5 months of age while ewes and shearlings should be vaccinated during the 4-month period prior to tupping; pregnant animals must not be vaccinated. Recent studies show that the degree of protection produced by the S48 tachyzoites was as good at 18 months as 6 months after vaccination. The manufacturers state that other live vaccines should not be administered within 4 weeks of the administration of Toxovax.

Transmission and pathogenesis. Without doubt, the principal vector in the spread of toxoplasmosis is the cat and its related wild species. They excrete oocysts in their faeces and contaminate pasture, forage and other foodstuffs. Young cats and older breeding cats are the main problem. Old neutered cats on a farm are helpful, not only in reducing levels of vermin, but in keeping feral cats away. Foodstuffs should be protected from faecal contamination by cats. Wild rodents, especially mice, are the main source of infection for cats.

Infection of ewes outside pregnancy produces a good immunity. Replacement ewes should be exposed to the farm environment as soon as possible after purchase, so that they can ingest oocysts and acquire immunity before tupping.

Toxoplasma gondii can affect humans but usually does not cause clinical disease. The exceptions are women who become infected for the first time during pregnancy. Placental and fetal infection results in severe damage to the unborn child. Other people at risk are those that are immunosuppressed, and they should not assist at lambing time (Buxton, 1986).

Campylobacteriosis

Campylobacter is the third most common cause of abortion in the UK and although both *C. fetus fetus* and *C. jejuni* cause abortion in ewes, the former is the main organism isolated.

Epidemiology. Infected and often symptomless animals excrete these organisms in faeces. *C. jejuni* is mainly from a wildlife source and *C. fetus fetus* from carrier sheep. Unlike in cattle, where the route of infection is mainly venereal, in sheep it is by ingestion and mainly intestinal. Once abortions occur, there is lateral spread to other susceptible pregnant ewes. The organism survives well in cold moist conditions but soon perishes in hot, dry weather. The only clinical signs of the disease are abortion, usually in the last 6 weeks of gestation; lambs at full term may be born dead or in a weak condition. Apart from some vulval swelling, and the presence of a reddish-coloured vulval discharge, ewes rarely show any other clinical signs. Metritis may develop after abortion, some ewes becoming ill and even dying. Aborted material is infective. Ewes infected <3 months into pregnancy are not affected. If ewes are infected >3 months into pregnancy, a bacteraemia develops with placentitis being the main lesion. In late pregnancy, abortion occurs between 1 and 3 weeks after infection. Flocks infected for the first time may have levels of abortion varying from 5% to 50%. There is a strong immunity after infection, but this is serotype-specific. Symptomless carriers may excrete the organism for up to 18 months.

Diagnosis

- There are signs of placentitis with oedema and necrosis of the fetal cotyledons. However, these are not pathognomonic. The aborted fetus looks fresh with no specific gross pathology, and in approximately 25% of the aborted fetuses, there are characteristic necrotic foci (Plate 6) of 10–20 mm diameter in the liver (Dennis, 1991).
- Disease is commonest in young ewes, or older ewes that have lost their immunity.
- The organism can be identified in Gram- or modified Ziehl–Neelsen-stained smears from the placenta and fetal stomach contents and cultured from the placenta, fetal stomach or liver.
- Serology is not useful.

Treatment and control

- As soon as campylobacteriosis is suspected, aborting ewes should be isolated from pregnant ewes. If extensive lateral spread is a possibility, pregnant ewes should be treated with intramuscular injections of 300 000 IU of penicillin and 1 g of dihydrostreptomycin on 2 consecutive days.
- Dispose of aborted materials as the disease is zoonotic. Avoid infecting sheep over 3 months in lamb, or wildlife that can later act as reservoirs of infection.
- When campylobacteriosis has been confirmed, mix aborted ewes with those already lambed to stimulate production of a strong immunity. The disease is self-limiting, and most ewes from an infected flock acquire an immunity in the first year, irrespective of whether or not they have aborted. This acquired immunity lasts about 3 years, in most circumstances, equal to the expected breeding life of the ewe.
- Turn over feed troughs, preventing birds from feeding at them.
- Try to keep the flock closed. Bought-in ewes should be mixed with the resident sheep for as long as possible before mid-pregnancy, then separated in late pregnancy and lambed separately.

In the USA, New Zealand, Australasia and Europe, a formalin-killed adjuvant vaccine incorporating the most prevalent serotypes, I and V, has been used. Two injections, 15–30 days apart, are given either before the breeding season or during the first half of pregnancy. The immunity lasts for approximately 3 years, but replacement ewes must be vaccinated. No such vaccine is available at present in the UK. There is some evidence that in the early stages of an outbreak of campylobacteriosis in a flock, vaccination of all remaining pregnant ewes might be worthwhile. Since there is a 10–14-day delay before immunity develops after vaccination, early diagnosis is imperative.

Salmonellosis

Several serotypes cause ovine abortion including *S. abortus ovis, S. typhimurium, S. dublin and S. montevideo*. Occasionally exotic strains are isolated, and are usually associated with imported foreign protein.

Epidemiology. Many species of animal, including humans, may act as sources of infection, which, in early pregnancy, may result in barren ewes and, in late pregnancy, abortions, and dead and weak lambs. Affected ewes are often ill, losses may be catastrophic in both ewes and lambs, and those that recover may act as symptomless carriers.

Clinical signs. These will vary with the serotype of organism and are summarised below.

S. abortus ovis. This is a host-specific strain, once common in the southwest of England but now rarely isolated. Few systemic clinical signs occur in ewes other than abortion, which usually occurs in the last 6 weeks of pregnancy. Symptomless carriers are often a problem.

Two distinct clinical pictures may be observed in the lambs

- They may be born weak and die within a few hours of birth.
- They may be born healthy, suddenly become ill and die in the first 10 days of life.

S. montevideo. This strain has been a problem in southeast Scotland for several years. Apart from abortion, there has been little evidence of systemic illness. During the severe winter of 1982, an abortion rate of 10.1% was noted in 11 500 breeding ewes. Severe weather, with prolonged cold stress, necessitated close congregation of these sheep for the early provision of foodstuffs. These arrangements may have compounded the problem and led to water courses being polluted, resulting in animals downstream becoming infected. However, it was eventually thought that seabirds had a major part to play. The disease is not as severe as cases of *S. typhimurium* and *S. dublin* infection, and diarrhoea is not a feature in lambs born alive (Linklater, 1983).

S. typhimurium. The clinical picture is totally different to those previously described. Anorexia, pyrexia (up to 106°F) and profuse scour are usual. A foul-smelling vaginal discharge may be present. Death may occur from septicaemia or dehydration in 6–9 days. Lambs may be born dead, and those that are born alive may show signs of severe illness with diarrhoea and a high mortality rate. Severe losses amongst the ewes are not unusual. Numerous observers have commented that the disease often follows times of stress, e.g. after gathering, winter shearing, housing and vaccination.

S. dublin. The clinical signs are not normally as severe in both ewes and lambs as with *S. typhimurium* infection. Death is usually from septicaemia or dehydration, and mortality rates are usually much lower.

Diagnosis. It is important to stress that *Salmonella* spp., other than *S. abortus ovis*, are zoonoses, hence care should be exercised when dealing with infected material.

- The clinical picture is that of scouring and pyrexic ewes. Before, or in association with, abortion a foul-smelling vaginal discharge may be present.
- Lambs born alive may become ill with fatal septicaemia or pneumonia, especially in cases of *S. typhimurium* and *S. dublin* infection.
- Identification of the organism follows culture of fetal stomach contents, placental tissue or vaginal discharges. In addition, fluorescent antibody techniques may also be used for the rapid diagnosis of organisms in the same tissues.
- Serological tests can be used to diagnose infection with *S. abortus ovis*.

Transmission and pathogenesis. *S. abortus ovis* is host-specific and is usually introduced into a flock by infected sheep. Other species of salmonella are not host-specific, and can be introduced through contaminated food, water, wild birds or other infected livestock. Once the disease has become established, it will readily spread by ingestion of contaminated food or water. There is always a danger that symptomless carriers may remain in the flock, providing a persistent reservoir of infection.

S. montevideo appears to have a predilection for sheep and could become endemic in the sheep population of the UK (Linklater, 1983). It has been isolated from mesenteric lymph nodes of sheep at abattoirs all the year around, and thus the carryover of infection from year to year can occur in sheep themselves.

Treatment and control. A number of general principles have been suggested for the control of *S. montevideo* that are applicable to other *Salmonella* species (Linklater, 1983):

- Isolate affected sheep that have aborted, or scoured profusely, and treat with an antibiotic to which the salmonellae are sensitive, thus limiting excretion of the organism.
- Keep aborted ewes separate from those yet to lamb.
- If birds are possibly the source of infection, then turn over feed troughs when not in use, regularly change the feeding area and avoid feeding on the ground.
- Avoid stress situations arising within the flock, such as frequent moving, and make sure that foodstuffs are freely available with sufficient trough space to avoid competitiveness.
- Try to prevent sheep drinking from streams and open ditches by using piped fresh water.

Listeriosis

Listeria have a world-wide distribution in several domestic species, especially ruminants, and accounts for approximately 2% of ovine abortions in the UK. Two strains of *Listeria* produce disease in sheep, *L. monocytogenes* and *L. ivanovii*, and the disease may be presented in one or more of the following forms:

- encephalitis
- abortion
- diarrhoea and septicaemia
- keratoconjunctivitis and mastitis
- septicaemia and death in young lambs.

The incidence of listeriosis has increased in the UK in recent years, with encephalitis being the commonest form. Neurological signs characteristic of listerial encephalitis are circling with evidence of unilateral facial paralysis, head tilting, and turning. Abortions in sheep are produced by both *L. monocytogenes* and *L. ivanovii*, and although they may occur at any stage they are most frequent in late pregnancy. Initially, sheep may be pyrexic. There are no distinguishing characteristics to the abortion, and usually no typical complications following expulsion of the fetus, which may be autolysed. Weakly lambs are often born, and grey/white focal necrosis may be seen in the fetal liver (so-called 'sawdust liver'). The placental villi are necrotic and the chorion is covered with a brownish red exudate; there is a heavy brown vaginal discharge (Low and Linklater, 1985), and rarely death of the ewe occurs from metritis or septicaemia.

Several forms of the disease may be present in the flock at the same time. In one outbreak, diarrhoea and septicaemia occurred 2 days after the commencement of silage feeding, and although the silage was removed, encephalitis was seen 4 weeks later followed by abortions. These signs were not seen in the same ewes, and it is rare for the nervous disease and abortions to occur together.

Diagnosis. Diagnosis is based on isolation of the organism from vaginal swabs, fetal membranes or the fetus, and fetal liver lesions.

Smith et al. (1968) have used fluorescent antibody techniques, whilst inoculation of mice with necrotic liver and the production of keratitis in rabbits are additional tests.

Treatment. The organisms are sensitive to a wide range of antibiotics that may prove beneficial if given to ewes that have aborted and are discharging.

Transmission and pathogenesis. *L. monocytogenes* is ubiquitous, frequently found in soil but also isolated from foodstuffs and faeces of healthy animals. Soil is the most likely source of infection, especially following the feeding of soil-contaminated silage where poor fermentation has occurred. Sheep are probably frequently exposed to infection, but presumably it requires some other factors to precipitate clinical listeriosis. Following ingestion in late pregnancy, the organism penetrates the gut mucosa and infects the fetus, causing a septicaemia and placentitis, both of which may kill the fetal lamb. As a consequence, abortion occurs.

Control. All aborted ewes should be isolated and the abortion site cleaned up.

In Iceland, listeriosis is called 'silage disease', and it has been suggested that the feeding of silage appears to make ewes more susceptible to listeriosis.

The incidence of listeriosis in all its forms has increased dramatically with the practice of feeding silage to sheep, especially that stored in round bales. In a survey carried out in Scotland by Fenlon (1985), *L. monocytogenes* was isolated from 2.5% and 5.9% of clamp silage examined in 1983 and 1984, respectively, and from 22% and 4% samples of big bale silage. Attempts should be made during silage production to develop conditions that are not favourable for the multiplication of the organisms. Low and Linklater (1985) made the following recommendations:

- Use additives to reduce the pH of the silage; make high-quality silage with a pH below 5, avoiding gross soil contamination (where the ash content exceeds 70 mg/kg dry matter), i.e. avoid mole hills and having forage harvesters set too low.
- Compact and seal silage the same day as made, making sure that round bales are securely tied and not punctured.
- Avoid feeding obviously mouldy or poor-quality silage that can be smelt or has come from the top or sides of the pit.
- Remove any silage that has not been eaten by sheep after 48 hours.

- On farms where listeriosis occurs annually it would be advisable not to graze any fields with livestock that were intended for silage-making.

Border disease

This disease was first recognised in flocks along the English–Welsh border in the 1950s (Hughes et al., 1959), affecting newborn lambs that showed neurological symptoms such as tremor and a coarse fleece (so-called 'hairy shakers') and were generally weak with a high mortality rate. Since then, it has been recognised in many other places in the UK (Barlow and Dickinson, 1965; Acland et al., 1972; Sweasey et al., 1979; Nettleton, 1990). It has also been shown to cause reproductive failure.

Aetiology. The disease is due to infection with a pestivirus similar to that which causes bovine viral diarrhoea (BVD) in cattle and European swine fever.

Clinical signs. The clinical signs in lambs are well documented elsewhere (Barlow and Gardiner, 1983; Nettleton, 1990). In adult ewes, infection results in a mild pyrexia that would probably go undiagnosed. However, if ewes are pregnant, the virus will affect the fetus causing fetal death with mummification, abortion or, if early on in fetal life, death with resorption, or the birth of weakly affected lambs. Abortion can occur at any stage of gestation, although it is most common around 90 days with the voiding of a brown, mummified or swollen anasarcous fetus (Barlow and Gardiner, 1983). The conceptus is most susceptible to experimental infection between 16 and 80 days of gestation (Nettleton, 1990). Hence, if infection occurs at an early stage, the only clinical sign will be barrenness.

Diagnosis. This can be made on clinical signs in lambs, supported by histopathological examination of the brain and spinal cord, virus isolation from the lamb, or a fluorescent antibody staining technique. Serological tests on ewes that abort, or are barren, have to be examined in relation to antibody levels in other ewes in the flock.

Transmission. Pestiviruses from other species can also, under experimental conditions, cause Border disease in sheep, so that among domestic animals, cattle in particular (Carlsson, 1991), but also goats, represent potential sources of infection.

However, the most likely source of infection is from ewe lambs that have recovered from Border disease being introduced into the flock. These individuals remain chronic excretors of the virus for a long period of time, yet appear healthy. Although likely to have reduced fertility, they can give birth to infected progeny that themselves are a source of infection. Some ram lambs can excrete the virus in their semen. They may have poor fertility associated with small soft testes (Barlow and Gardiner, 1983).

Treatment and control. There is no treatment and, as yet, there is no commercially available vaccine, although one may become available eventually. The disease is best controlled by ensuring that the flock remains closed and hence the disease does not gain entry. Once it is present in a flock it is important, in the early stages of the outbreak, to attempt to segregate pregnant ewes from those that have given birth to clinically affected lambs. At the same time, to ensure that non-pregnant ewes develop an immunity, they should be exposed to infection, and thereafter, any surviving lambs from the infected flock should not be retained for breeding and be sent for slaughter, so that symptomless carriers do not remain.

Leptospirosis

The disease occurs in late gestation and the immediate postpartum period, resulting in severe losses for the farmer. A total of 6% of adult sheep in England have antibodies to *Leptospira interrogans* serovar *hardjo*.

It usually occurs during two short periods of time when sheep are physiologically immunocompromised, viz. in ewes 2 weeks before lambing, and in neonatal lambs in the first week of life.

Epidemiology and transmission. The disease is not seen in traditionally managed hill flocks, but it occurs when they are bought as replacements for intensively reared and housed lowland flocks. Reproductive wastage occurs during the first lambing season, but not in subsequent years. There is still debate over whether or not sheep are a maintenance host for the infection, or whether there is a requirement for cattle as an established maintenance host to be closely involved (Cousins et al., 1989). Nevertheless, it is generally recognised as good practice, in the control of the disease, to minimise contact between the two species.

Clinical signs. The clinical sign in adult ewes is reproductive wastage in the form of late-term abortion, stillbirth and the birth of weak lambs. Of 872 aborted lambs examined at Stormont in Northern Ireland between 1981 and 1987, a total of 17% were infected with *Leptospira*, the majority with *hardjo. L. hardjo* has also been isolated from the brains of lambs with meningitis. Agalactia, as seen in cows (see Chapter 23), has also been observed in *L. hardjo*-infected ewes in Northern Ireland.

Diagnosis. In cases of abortion, stillbirth or weakly lambs diagnosis is based on the isolation of the organism from aborted fetuses or fetal membranes, its demonstration, by a fluorescent antibody technique, in the same tissues or placenta, or the presence of a rising antibody titre in paired blood samples.

Control. Control is by vaccination, using a quarter of the cattle dose of *L. hardjo* vaccine before tupping, with a repeat dose in 2–4 weeks (Ellis, 1992).

Those ewes that have not aborted when an abortion storm occurs can be treated with 25 mg/kg of dihydrostreptomycin as a single dose.

Brucellosis

Sheep can be infected with both *Brucella melitensis* and *B. ovis*. The former is endemic in many Mediterranean countries, Africa and Central America; it does not occur in the UK. The latter has been reported in parts of Eastern Europe, South Africa, western states of the USA, New Zealand and Australia; it does not occur in the UK.

B. melitensis is primarily a disease of sheep and goats, but can affect other species, including man (Malta fever). Transmission is by direct ingestion of the products of abortion or drinking infected milk. It causes abortions, stillbirths or weak lambs in late pregnancy. The placental lesions are similar to those identified with *B. abortus* infection in cattle, and diagnosis is by direct examination or culture of placental smears, fetal stomach contents or vaginal discharges. Serological tests, such as the complement fixation test, are used. The disease can be controlled by using *B. melitensis* or *B. abortus* S19 vaccines.

B. ovis is host-specific. Its main effect is upon the ram, where it causes an epididymitis and subsequent infertility or sterility (see Chapter 30). Following experimental infection of the ewe, the organism causes a placentitis followed later in gestation by abortion or the birth of small weak lambs. However, under field conditions, abortion rarely occurs, and according to Hartley et al. (1954), if it does occur, the incidence is usually low (7–10%). Whilst a ram can be infected after serving a ewe previously served by an infected ram, the ewes themselves are not infected venereally. The method of infection of ewes is not fully understood.

Q fever

This is due to infection with the rickettsia, *Coxiella burnetii*. Although of little significance in sheep farming, it has been associated with a small number of outbreaks of ovine abortion in the UK (Marmion and Watson, 1961). Its main importance is its public health implications, producing influenza-like symptoms, pneumonia and cardiac lesions in humans, and thus it is important that the agent is recognised when fetal material is examined to avoid the possibility of human infection. Microscopically, it may easily be mistaken for *B. abortus* or *Chlamydia*.

Placenta and vaginal discharges are heavily contaminated, allowing spread of infection at parturition or later by aerosol from the fleece or dust in the lambing area, acting as a source for human infection (Watson, 1973).

Ureaplasmosis

Ureaplasma spp. have been isolated from normal ewes (Ball et al., 1984) and from ewes with granular vulvitis (Doig and Ruhnke, 1977). There is some suggestion that they are common inhabitants of the urogenital tract of sheep. Ureaplasmas have been identified as a cause of infertility and abortions in cattle and in other non-domestic species, and may have a possible role in infertility and abortion in sheep (Livingstone et al., 1978). The ram has been implicated as a major distributor of infection (McCaughey and Ball, 1981).

Tick-borne fever

This is limited to those areas where the sheep tick *Ixodes ricinus* occurs, since almost all ticks harbour the infective agent *Cytoecetes phagocytophilia*. The disease is known in the southwest of England, Scotland, Scandinavia, the Netherlands and South Africa. Abortion in adult sheep, previously unexposed to ticks, occurs naturally following tick-borne fever infection in late pregnancy, usually 2–8 days after the commencement of a fever, which can be as high as 107°F. A proportion of pregnant ewes may die. Some fetuses may die in utero, become mummified and be expelled weeks later. Recovery in non-pregnant ewes is generally uneventful.

Diagnosis can be confirmed by identifying the organism in the leucocytes of ewes that have aborted, or during the septicaemic phase.

Treatment. Oxytetracycline can be used for the rest of the naïve flock.

Control. Infected ewes that survive develop an immunity, whilst the majority of all sheep from tick-infected areas will have acquired an immunity at an early age. Newly purchased non-acclimatised sheep should be introduced to farms before tupping, preferably when tick numbers are at their highest. Ticks can also be controlled by adopting the appropriate dipping routine.

It is noteworthy that rams during the breeding season, when exposed to tick-borne fever for the first time, can have reduced fertility for several months due to poor sperm quality (Watson, 1964).

GOATS

In the absence of any major infectious cause of abortion, infertility in the goat is generally not a major problem, normally with only a small number of barren does remaining at the end of the breeding season.

Structural defects

Abnormal sexual differentiation during embryological and fetal development, resulting in intersexes, is relatively common in the goat, especially in breeds such as the Alpine, Saanen and Toggenburg. It is much more prevalent in polled individuals, where 'polledness' is a simple dominant character with full penetrance, but it is also associated with a recessive hermaphrodite effect with incomplete penetrance (Baxendell, 1985). Intersexes can also occur as a result of freemartinism, where placental fusion occurs in twins of dissimilar sex; examples of goat freemartinism have been reported by Smith and Dunn (1981). However, the incidence of hermaphroditism appears to be higher than that of freemartinism in this species.

Hermaphrodites that have been described in goats are mainly male pseudohermaphrodites, having testes and the accessory reproductive organs of the female; they are genetic females.

Intersexes vary in the degree of external structural abnormality. Most are generally female-like in appearance at birth but, as they grow and mature, there will be evidence of an enlarged clitoris, perhaps testes in the inguinal region and the development of male secondary sex characteristics, including the typical male odour (see Chapter 4).

Functional factors

The goat is a seasonal breeder responding to the effects of declining day length. It is not unusual to have irregular oestrous cycles at the beginning and end of the breeding season, especially in goatlings, with short cycles of between 5 and 7 days.

Anoestrus may be due to starvation, parasitism or mineral deficiencies. In the case of the latter, phosphorus and the trace elements copper, iodine and manganese as well as vitamin E have been implicated. It can also be influenced by chronic debilitating diseases.

Hydrometra or pseudopregnancy ('cloudburst')

Hydrometra is the accumulation of sterile secretions within the uterine lumen. The aetiology of the condition is not known precisely, but it is always associated with high progesterone levels secreted by a persistent, functional CL, cessation of cyclical activity, and variable degrees of abdominal distension. The incidence of the disease varies between herds and within the same herd from year to year. Studies involving 71 dairy herds in France found an overall incidence of 2–3% (Mialot et al., 1991), although on one farm in the study it was 20%. In Holland, Hesselink (1993a) found a mean incidence in three herds, totalling 550 does, of 9%. The disease occurs more frequently in older does and is uncommon in yearlings. In Hesselink's study (1993b) there appeared to be an association with the use of progestogen sponges and eCG treatment to advance the onset of cyclical activity before the start of the normal breeding season.

One possible cause of hydrometra is early embryonic loss. However, not all animals will have been mated by the buck prior to pseudopregnancy.

Two types of pseudopregnancy occur:

1. After mating there is fertilisation, followed by early embryonic death, the CL persists and the doe acts as if pregnant. The abdomen becomes enlarged, and in some there is a degree of udder development if not the onset of lactogenesis. Those that are lactating may have a fall in yield. This type of false pregnancy generally lasts for the duration of the gestation period, or even longer, until the CL has regressed spontaneously. The term 'cloud burst' is used to describe the voiding of large volumes of fluid from the uterus as the pseudopregnancy is terminated. Following this, the abdominal distension disappears; some does may search for the 'missing' kids.

2. Following oestrus, when the doe was not mated, there is cessation of cyclical ovarian activity but there is no marked hydrometra. At the end of the period of acyclicity, affected does expel a bloody discharge (Smith, 1986). Therefore, any unbred does that do not return to oestrus after their first oestrous cycle in the autumn, should be treated with $PGF_{2\alpha}$ for possible pseudopregnancy.

Differentiation of hydrometra from normal pregnancy can be made using transabdominal B-mode ultrasound imaging, demonstrating the presence of a fluid-filled uterus in the absence of a fetus or placentomes. After 50 days of anoestrus, pregnancy and pseudopregnancy can be differentiated on the basis of serum oestrone sulphate levels (see pregnancy diagnosis in goats in Chapter 3). Treatment with 2.5 mg $PGF_{2\alpha}$ will be followed by expulsion of the fluid and oestrus will occur in approximately 4 days. By using a second injection 12 days after the first, good levels of fertility can be achieved with 85% conceiving, compared with 95% of unaffected animals (Hesselink, 1993b).

Cystic ovarian disease

Lyngset (1968) found an incidence of 2.4% ovarian cysts at necropsy. Most were unilateral, single and varied in size between 1.2 and 3.7 cm.

Cystic ovarian disease has been described in dairy breeds and has been particularly evident where they have grazed oestrogenic clovers and legumes (Baxendell, 1985). A history of nymphomania may suggest follicular degeneration, and the typical clinical signs are those of continuous oestrus and short interoestrus intervals with a failure to conceive. They should be treated with 1500–2500 IU of human chorionic gonadotrophin (hCG); gonadotrophin-releasing hormone (GnRH); or progesterone treatment for 18 days (Smith, 1986).

Abortion in Angora goats

Abortion has been recognised as a problem in the Angora goats in eastern Cape Province in South Africa since the turn of the century, and it has reached such proportions that it has threatened the viability of the mohair industry.

Recent studies have identified two types of abortion:

1. The most common is a 'stress-induced' abortion that occurs in poorly grown and immature does. The abortus is fresh and may be born alive. This is the major type of abortion loss in production operations.

High metabolic requirements for fibre production in the Angora predisposes the breed to nutritional and other forms of stress to a greater degree than in goats of other breeds (Shelton, 1986). It has been suggested by Wenzel et al. (1976) that hypoglycaemia in pregnant does stimulates the immature fetal adrenal to produce oestrogen precursors, which result in the placental synthesis of oestrogens and subsequent abortions; blood glucose concentrations are influenced by short-term interruptions in food intake. Most abortions occur between 90 and 120 days of gestation, at the time of rapid fetal growth. It may be seen as a storm 1–2 days after stress.

2. 'Habitual abortion' is probably a genetically determined hyperactivity of the maternal adrenal cortex, prematurely initiating the process of parturition (Shelton, 1986). Fetuses that are usually born dead are often oedematous. Habitual aborters should be culled, together with any live offspring that might have survived. Does abort in the absence of stress, and levels as high as 5% may occur in flocks that do not practise culling.

Management factors

Timing of service or artificial insemination

Optimum pregnancy rates are obtained when does are mated towards the end of oestrus (which lasts 12–36 hours) and just before ovulation. Some goat owners serve their does only in the first 12 hours of oestrus supposedly to increase the number of female kids but with a consequential reduction in pregnancy rates (Baxendell, 1985).

Nutrition

Vitamin A, certain minerals (manganese and iodine) and energy deficiencies reduce fertility, and may be associated with abortion when the deficiency is chronic.

Stress

Stress-induced abortion has been described above in Angora goats. However, there is evidence that other breeds of goat will abort if subjected to stress. This can result from being chased by dogs, inadequate feeding, transportation and adverse weather, particularly during the fourth month of gestation (Shelton, 1986).

Infectious agents

Non-specific infections appear to play a minor role in causing infertility in does, probably for similar reasons discussed above for the ewe. However, specific infectious agents are important in causing abortion. Unfortunately, little information on their relative importance in the UK is available. Many of these specific infectious agents are also important in sheep. A survey of diagnoses recorded at VLA Regional Laboratories in England and Wales and Disease Surveillance Centres in Scotland listing the infectious causes of goat fetopathy identified in material submitted to their laboratories from 1984 to 1998 is summarised in Table 25.2.

Brucellosis

Brucella melitensis is the organism most frequently involved and is endemic in many Mediterranean countries, Africa and Central America; it does not occur in the UK. *B. abortus* occasionally causes abortion but *B. ovis* has not been isolated from goats.

B. melitensis causes abortion in late pregnancy, stillbirths or weakly kids; following the first exposure, abortion may be in the form of a storm. Few abortions occur in other years, and some does may become sterile from uterine lesions. Kids may become infected from does' milk (Smith, 1986). The disease can be diagnosed by bacterial culture from the fetus, fetal membranes or vaginal discharges, and can be controlled by routine vaccination. It is important to remember that *B. melitensis* is a zoonosis.

Campylobacteriosis

This is uncommon. It is caused by infection with *Campylobacter* spp., probably *C. jejuni* and possibly *C. fetus fetus*. Does may or may not show evidence of a systemic illness, and may abort in late gestation or produce stillborn or weakly kids, and have a post-abortion muco- or sanguino-purulent discharge. Multiple necrotic foci up to 2 mm in diameter may be seen in the liver of the aborted kids. Diagnosis, treatment and control are similar to those described for sheep.

Table 25.2 Percentage frequency of isolation of pathogens from caprine fetopathies examined by Ministry of Agriculture Veterinary Investigation Centres (Source VIDA-II)

	1984	*1988*	*1992*	*1993*	*1994*	*1995*	*1996*	*1997*	*1998*
A. pyogenes	7.1	3.5	0	0	0	0	0	0	0
Campylobacter spp.	7.4	3.5	5.0	0	0	7.1	3.7	0	0
Chlamydia	28.6	19.3	0	15.8	42.9	14.3	14.8	21.4	25.0
L. monocytogenes	0	8.8	35.0	15.8	7.1	21.4	33.3	14.3	12.5
Salmonella serotypes	0	0	0	0	0	0	0	0	0
Toxoplasma spp.	42.9	43.9	30.0	57.9	35.7	21.4	14.8	28.6	37.5
Coxiella burnetti	0	3.5	0	0	7.1	0	3.7	14.3	18.8
Fungi	0	1.8	0	0	7.4	0	0	0	0
Other pathogens	14.3	15.8	30.0	5.3	0	28.6	25.9	21.4	6.3
Equine herpes 1	NR	NR	NR	5.3	0	0	0	0	0
Total identified	14	57	20	19	14	14	27	14	16
Total submitted	62	161	61	61	49	49	49	46	38
Percentage diagnosed	22.6	35.4	32.8	31.1	28.6	28.6	55.1	30.4	42.1

NR = Not recorded

Chlamydial (enzootic) abortion

This is an important cause of infertility in goats in many countries, and is the commonest cause of infectious goat abortion in the USA (East, 1986). Infection is due to *Chlamydia psittaci* (recently reclassified as *Chlamydophila abortus*), which is similar to, or identical with, the strain responsible for enzootic abortion in sheep.

Abortions usually occur in the last 4 weeks of gestation, with levels as high as 25–60% in does kidding for the first time; stillborn and weakly kids can also occur. Diagnosis and treatment are similar to that described above for sheep. Does infected in late pregnancy usually abort during the subsequent pregnancy and can produce infected kids which, after a latent phase, abort during their first pregnancy. The disease is best controlled by good hygiene to prevent lateral spread to susceptible animals, especially kids and young does, and the use of a vaccine, which has been made compulsory in some countries where the disease has become widespread (Polydorou, 1981). Enzovax, which is used in sheep, is not licensed for use in goats in the UK (Mahwinney, 1999).

C. psittaci is a zoonosis.

Leptospirosis

Although it is not a frequently diagnosed cause of abortion there are reports in the literature (Van der Hoeden, 1953; Baxendell, 1985), particularly associated with *Leptospira grippotyphosa* infection. There is usually systemic illness preceding the abortion associated with septicaemia, but clinical signs of icterus are not often present.

Diagnosis is based upon identification of the organism and serological tests. The disease can be treated in the acute phase with streptomycin, but it is doubtful if this will prevent abortion occurring. Control measures, involving the use of vaccines, might be tried in an outbreak.

Listeriosis

Encephalitis, due to *Listeria monocytogenes* infection, is quite common in goats. The same organism can cause abortion in late gestation or stillbirth. The does may show no signs before aborting, but may develop a necrotic metritis and vaginal discharge shortly afterwards.

As in sheep and cattle, poorly fermented, soil-contaminated silage is a likely source of infection. The pathogenesis, diagnosis, treatment and control are similar to those described for sheep.

Salmonellosis

There are no host-specific *Salmonella* species in the goat. However, the ubiquitous salmonellae have been reported to cause abortion (Baxendell, 1985).

Toxoplasmosis

Toxoplasma gondii is the commonest cause of infectious goat abortion in the UK. It causes fetal death with resorption if infection occurs early in gestation, or abortion of kids, which may be stillborn, alive but weak or normal, depending upon the time in pregnancy that the doe was exposed to infection. However, unlike in sheep, fetal death is preceded in some cases by a period of severe illness with pyrexia, anorexia, diarrhoea and muscle weakness (Dubey et al., 1980). During an acute infection, toxoplasm may be excreted in the milk (Dubey, 1980) and be a source of human infection if drunk unpasteurised (Skinner et al., 1990). Also, experimentally at least, toxoplasms may be present in goat semen for a variable time after infection (Dubey and Sharma, 1980) but the epidemiological significance of this, as in sheep (Blewett et al., 1982), may be very slight.

The placental lesions are very similar to those described in sheep. Diagnosis is dependent upon identification of the organism in placental tissue or serological tests. Aborting does develop an immunity, and should be retained within the herd, whilst young, non-pregnant does should be exposed to infection before they become pregnant.

Domestic cats and wild *Felidae* play a critical role in the spread of the disease as in sheep.

Treatment and control measures are similar to those described for sheep (see above). Toxovax is not licensed for use in goats (Mahwinney, 1999).

T. gondii is a zoonosis and thus care should be taken in handling possibly infected material.

Q fever

Q fever, caused by *Coxiella burnetii*, can cause abortion and stillbirth in goats, without previous clinical signs or following a few day's illness involving dullness, depression and anorexia. The abortion rate can be very high in some infected herds (5–50%, Miller et al., 1986). Large numbers of organisms are expelled into the environment from placental tissue, uterine fluids, colostrum and milk (see sheep section). Ticks have also been implicated in the spread of Q fever, and may be the initial means of introduction into the herd.

Diagnosis is made upon identification of the organism in smears of the placenta or the organs of the abortus, and serological tests demonstrating a rising antibody titre (see sheep section).

There is no vaccine, and does can remain chronic carriers of the organism; *C. burnetii* is a zoonosis and is excreted in milk.

Mycoplasmosis

A number of *Mycoplasma* species have been identified as causing abortion.

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26 Infertility in the mare

The objective of the veterinarian working in any horse-breeding enterprise, regardless of size, should be to produce the maximum number of live, healthy foals from the mares bred during the previous season; we could add to this 'as early as possible' in many breeding programmes. Perhaps the biggest obstacle to achieving this aim is the infertile or problem breeding mare. Very few mares are permanently and completely infertile, but subfertility of varying degrees is a major problem. There are many causes of subfertility that warrant a mare to be categorised as a 'problem breeding mare' and whilst it is important to recognise the underlying cause, the implementation of a successful treatment strategy is equally important. Mare owners frequently have problem mares from which they want to breed and the veterinarian must be able to help maximise the chance of this wherever possible. It might take several cycles to establish a pregnancy, and even then, there is an increased possibility of pregnancy failure. Commitment from both mare owner and veterinarian is needed; the owner should be made aware of this at the outset and be given a realistic expectation as to the chance of success. The aim of this chapter is to consider the problem breeding mare and how to provide an effective management policy that can be applied in daily clinical practice.

There are two major ways in which we have negatively influenced fertility:

1. Reproductive performance is usually not the prime criteria in breeding horses
2. Since the early 19th century, when 1 January was declared the official birthdate for thoroughbred foals, irrespective of their actual birth dates within that year, horse breeders have been plagued with problems attempting to breed mares in the winter and early spring outside their most fertile period. The promotion of yearlings in the autumn sales also contributes to the pressure for early breeding, since well-grown older yearlings tend to sell for higher prices.

To understand subfertility, some grasp of normal expectations of fertility is useful. Assessment of reproductive efficiency is an extensive subject, and for an excellent detailed study, the reader should refer to Hearn (2000). Possibly the two most important, or at least the two most-quoted, parameters in connection with reproductive efficiency are end-of-season pregnancy rate and live foal rate. From the veterinarian's point of view, pregnancy rate per oestrous cycle is a more up-to-date indicator of the efficiency of the breeding operation. A well-managed stud farm should typically achieve pregnancy rates (diagnosed at 15 days of gestation) per oestrous cycle of 65%, an end-of-season pregnancy rate of 85% and a live foal rate in excess of 75%. These figures may be lower than owner expectations, and it is a good idea to discuss them with mare owners at the beginning of the breeding season. The figures need to be reviewed in the light of the history and past breeding performance of a particular mare. Pregnancy rates at the end of the season will depend on the fertility of the stallion; the fertility of mares; and management. This last factor is often related to the value of the horses involved, i.e. frequent veterinary attention in cases where it is justified by the potential value of the foal results in better fertility. Very expensive stallions tend to attract more fertile mares, or the stud may accept only young, fertile mares. Well-managed studs tease mares regularly and individually; this is very time-consuming. An experienced stud manager knows, for example, the reasons why some mares fail to exhibit oestrous behaviour. In turn, the length of time that a mare fails to show oestrus

Table 26.1 Outline of a step-by-step protocol for the clinical examination of an infertile mare

1. Obtain the mare's previous breeding history
2. Assess her physical condition, general health and perineal conformation
3. Culture swab samples collected from the vestibule, clitoral fossa and sinuses
4. Examination per vaginam using a speculum, and collection of endometrial swabs for bacterial culture and stained cytological smear
5. Manual vaginal examination
6. Examine the reproductive tract by rectal palpation
7. Transrectal realtime ultrasound examination of the reproductive tract
8. Endometrial biopsy
9. Endoscopic examination of the endometrium
10. Peripheral venous blood sample for hormone analysis
11. Peripheral venous blood sample or hair follicle for chromosome analysis

before being presented to the veterinary surgeon for examination depends on stud policy and the owner's wishes.

The clinician should be aware of how to investigate the problem breeding mare. A protocol for such an investigation of an infertile or subfertile mare is outlined in Table 26.1.

Causes of infertility and subfertility

Many factors, acting either alone or in combination with others, can cause infertility or subfertility. Broadly, they can be categorised into infectious or non-infectious factors, with the latter being further divided into structural abnormalities and functional aberrations. This format will be used to discuss the various causes in this chapter. The factors are outlined in Table 26.2.

STRUCTURAL ABNORMALITIES OF THE FEMALE REPRODUCTIVE TRACT

Vulva

In the normal mare, the vulva provides the first effective barrier to protect the uterus from ascending infection. The 'normal' mare has three functional genital seals forming a barrier between the external environment and the uterine lumen: the vulva, the vulvo-vaginal constriction and the cervix. During oestrus, the vulva and cervix relax, leaving the vulvo-vaginal constriction as the only seal.

The vulval lips should be full and firm and meet evenly in the midline with 80% or more of the vulval opening below the brim of the pelvis. If the vulval seal is high (more than 4 cm of length dorsal to the pelvic floor) in relation to the pelvic brim, the vestibular seal is incompetent and there will be aspiration of air with bacteria and contaminated material into the vagina (pneumovagina; also called 'windsucking'). The initial vaginitis may lead to cervicitis and acute endometritis resulting in subfertility. Contamination of the caudal reproductive tract with bacteria during pregnancy can result in embryonic death, and in late pregnancy can result in the development of placentitis and lead to abortion. Furthermore, the pneumovagina may lead to a urovagina (urine pooling within the vagina) when the vestibule and urethral opening are displaced cranially. The more severe conformational abnormalities are more likely to result in failure of the vulval seal, and to increased faecal contamination since the vulva forms a shelf on to which faeces may collect. The vulval lips may be angled at 25 or even 50° to the vertical in these cases.

Caslick (1937) first pointed out the importance of this condition in relation to genital infection in thoroughbred mares. Interestingly, it is most commonly found in thoroughbreds and, in the author's experience, is almost unknown in Shires and native ponies.

Defective vulval conformation can be congenital, which is very rare, or acquired, which is due to (1) vulval stretching following repeated foalings, (2) injury to perineal tissue, or (3) poor bodily condition (old, thin mares).

Table 26.2 Summary of the causes of mare infertility

Non-infectious		
Structural	*Functional*	*Infectious*
Defective vulva Defective vestibulovaginal constriction Vesicovaginal reflux Vaginal bleeding Persistent hymen Abnormal cervix Uterine tumour Uterine haematoma Uterine abscess Uterine adhesions Uterine cysts Partial dilatation of the uterus Abnormal oviduct Ovarian tumour Ovarian haematoma Gonadal dysgenesis Developmental abnormalities	1. No oestrous behaviour a. Anoestrus caused by ovarian quiescence (i) Winter anoestrus (ii) Poor body condition (iii) Disease (iv) Chromosomal abnormality (v) Pituitary abnormality (vi) Ovarian tumours (vii) Lactation-related b. Anoestrus caused by prolonged luteal function (i) Prolonged dioestrus (ii) Dioestrous ovulation (iii) Pyometra (iv) Pregnancy/pseudopregnancy c. Anoestrus caused by behaviour (i) Silent heat (ii) Erratic postpartum behaviour 2. Shortened luteal phase – endometritis 3. Irregular or prolonged oestrus a. Transitional ('spring') oestrus b. Ovarian neoplasia c. Chromosomal abnormalities 4. Ovulatory dysfunction 5. Multiple ovulation 6. Gestational failure a. Early embryonic death b. Fetal death/abortion (i) Infectious: viral/bacterial/fungal (ii) Non-infectious: twins, uterine body pregnancy (iii) Placental/developmental abnormalities	Endometritis: bacterial/fungal Metritis Pyometra

Older multiparous mares are more commonly affected with pneumovagina. However, young mares that are in work and have little body fat and/or poor vulval conformation can develop pneumovagina. In some mares, pneumovagina may only occur during oestrus when the perineal tissues are more relaxed. A 'Caslick index' has been described in an attempt to determine which mares require treatment (Pascoe, 1979), but its use is not widespread. Some mares make an obvious noise whilst walking, but in other mares the diagnosis may be more difficult. The presence of a frothy exudate in the anterior vagina on examination with a speculum is pathognomonic. Rectal palpation of a ballooned vagina or uterus from which air can be expelled confirms the diagnosis. Realtime ultrasound examination of the uterus may reveal the presence of air as hyperechoic (white) foci sometimes seen as a line at the opposed luminal surfaces (Figure 26.1). Cytological and histological examination of the endometrium may demonstrate significant numbers of neutrophils indicative of an endometritis. Rarely, eosinophils are also found in association with pneumovagina.

Treatment: Caslick's vulvoplasty operation

Treatment should be directed at correcting the cause of pneumovagina, and concurrently treating the resulting acute endometritis. The former can be done surgically by Caslick's operation, although in some cases increasing the physical condition and fat status of the mare may be sufficient. This is the most common surgical procedure performed in studfarm practice, but in the author's opinion some mares are subjected to Caslick's operation unnecessarily. The operation should be reserved for mares with a true vulval

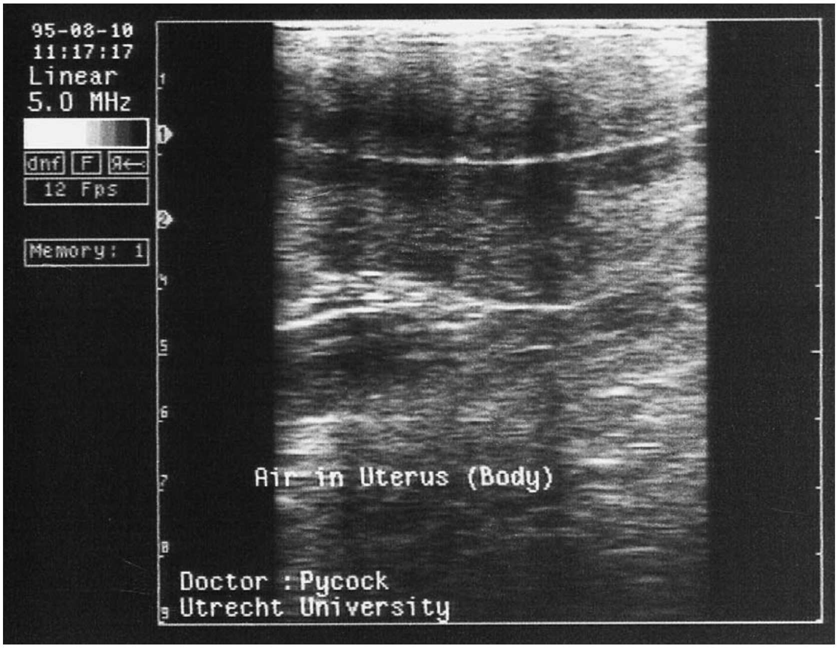


Fig. 26.1 Hyperechoic reflections appearing as a line at the opposed lumenal surfaces of the uterine body. The reflections are caused by air in the uterine body.

defect, rather than just because the mare has failed to become pregnant.

The mare should be suitably restrained and her vulva thoroughly cleaned and dried. With a gloved hand, the level of the floor of the pelvis is determined. This allows you to ascertain the level to which the dorsal commissure of the vulva must be sutured. Beginning at this level, the mucocutaneous junction of the vulva is infiltrated with local anaesthetic through a 21-gauge 1-inch needle. It is important to use plenty of local anaesthetic and in many cases at least 20 ml will be needed. The distension induced by the volume of local anaesthetic helps to evert the mucocutaneous junction. Both sides of the vulva are infiltrated in a stepwise fashion proceeding dorsally up to the dorsal commissure, being sure that enough is placed right at the dorsal extent of the vulva. For mares operated on previously it is important to infiltrate deeply. Using rat-toothed forceps and scissors a *very thin* (no more than 4 mm) strip of mucosa should be removed from the anaesthetised area. For older mares that have had the operation performed several times, more radical dissection may be necessary before healthy (bleeding) tissue is reached. In some cases, where there is not much vulval mucosa remaining, it is best just to debride the junction with a scalpel blade to induce slight haemorrhage and not actually remove any further tissue. The exposed submucosal tissues are sutured together using simple interrupted sutures (mares which have had the operation performed before) or a locking pattern. The suture material may be permanent or absorbable. The gauge of the suture material should not be too thick as this encourages faecal material to attach to the sutures. Skin staplers can be used, but the author has not found it any quicker compared to conventional suture techniques and it was harder to obtain a good alignment. Antibiotics are not given, but tetanus prophylaxis is needed if the mare is not vaccinated.

The aim of the operation is to reduce the vulval aperture and so prevent pneumovagina and faecal contamination of the vestibule. The time of suture removal is not crucial, and is normally done approximately 2 weeks after surgery. However, the vulva must be re-opened by performing an episiotomy before the next foaling, otherwise major injury can result. Mares that require natural mating subsequently may also need to have an episiotomy if the size of the vulva has been greatly reduced. If not, the vulva may tear and/or injury to the stallion's penis may occur. The episiotomy wound should be repaired soon after foaling or mating to prevent pneumovagina. If there has been severe trauma to the vulva at foaling, it may be necessary to wait for the tissue swelling to subside before attempting repair.

If repeated episiotomy followed by closure is not done very carefully, there can be considerable loss of vulval tissue, poor healing and major problems for the mare.

When the angle of the vulval surface relative to the vertical is the primary defect, Caslick's operation is often ineffective, and perineal resection should be used to achieve a satisfactory vulval conformation (Pouret, 1982). Caslick's operation has no effect on urovagina.

Vulvo-vaginal constriction

Immediately in front of the external urethral opening is the vulvo-vaginal constriction or vestibular seal. In genitally healthy mares this forms the second line of defence against aspirated air and faecal material.

Hymen

Manual vaginal examination of maiden mares often reveals the presence of hymen tissue which generally breaks down with pressure. A complete persistent hymen can also occur, which can result in the accumulation of fluid within the vagina and uterus due to impaired natural drainage. Sometimes the hymen may be so tough that it can only be ruptured using a guarded scalpel blade or scissors. The small incision can then be enlarged using the fingers and hand. Rarely, failure of proper fusion of the Müllerian ducts may result in the presence of dorsoventral bands of fibrous tissue in the anterior vagina and fornix. They do not interfere with fertility and are easily broken down manually.

Vagina

Vesicovaginal reflux, also known as urovagina or urine pooling, is the retention of incompletely voided urine in the cranial vagina due to an exaggerated downward cranial slope of the reproductive tract. Pneumovagina from a defective vulval conformation also predisposes to the condition. Transient urine pooling, which is sometimes found in postpartum mares, usually resolves after uterine involution has occurred. Clinical signs can include urine dripping from the vulva, urine scalding and a history of failure to conceive. Diagnosis is easiest using a speculum examination during oestrus to detect urine in the cranial vagina. Uterine infection with an accumulation of exudate in the vagina can be confused with the condition. In severe cases, urine pooling should be surgically corrected by vaginoplasty (perhaps more correctly termed caudal relocation of the transverse fold, as surgical intervention is in the vestibule) (Monin, 1972), urethral extension (McKinnon and Belden, 1988) or perineal resection (Pouret, 1982). Vaginal bleeding from varicose veins in the remnants of the hymen at the dorsal vestibulovaginal junction is occasionally seen in older mares, particularly during oestrus and the second half of pregnancy. Although diathermy can be used, treatment is not usually necessary as the varicose veins normally shrink spontaneously.

Third-degree perineal lacerations and recto-vaginal fistulas

Surgery for perineal lacerations is described in Chapter 18. It is important to be familiar with the procedure to be followed when this is encountered as a sequel to foaling. Both conditions are most often seen in young, primiparous mares, although the overall occurrence is less than 0.1% of all foalings. The rigidity of the birth canal, especially the vulvo-vaginal junction, is important in its pathogenesis.

In most cases, the veterinarian becomes involved only after the foal is born and the damage already exists. For treatment in the acute situation, you should realise that it is difficult to estimate the amount of devitalised tissue. Even though the edges of the wound may look fresh and clean, much more tissue is damaged and bruised. This is why immediate repair is not performed, unless you are present within 2 hours of the injury, and even then most clinicians advise delaying surgery.

First aid treatment should include:

- debridement of non-viable tissue
- provision of haemostasis and general cleaning of the area
- parenteral broad-spectrum antibiotics for 5 days
- NSAIDs and tetanus prophylaxis
- daily cleaning
- monitoring of uterine involution.

Elective surgery is performed after at least 10 weeks, and if the foal survives, the operation is best performed after weaning.

Cervix

The cervix forms the important third (and last) protective physical barrier to protect the uterus from the external environment. The cervix must also relax during oestrus to allow intrauterine ejaculation and drainage of uterine fluid. An inflammation of the cervix is usually associated with endometritis and/or vaginitis.

Anatomically, the cervix is a thick-walled sphincter. Expansion and contraction are possible due to the action of the longitudinal and circular smooth muscle, which is rich in elastic fibres. A distinctive feature of the equine cervix is its dilatability, and the absence of rigid, annular constricting rings seen in farm animals. This means that the uterine body can be entered by a relatively large-diameter instrument. The cervix undergoes changes in size, consistency and shape according to hormonal changes during the oestrous cycle. During anoestrus the cervix is flaccid, dry and may be partially open. During dioestrus and pregnancy, elevated concentrations of plasma progesterone cause the cervical os to close, although its lumen can still be entered. The cervix is pale, tonic and dry and projects into the cranial vagina. The cervix during oestrus is usually moist and sometimes hyperaemic. The cervical os is usually open and oedematous, and rests upon the ventral floor of the cranial vagina.

Often an older maiden mare has an abnormally tight cervix due to fibrosis. The cervix fails to relax properly during oestrus, so that fluid is unable to drain and accumulates in the uterine lumen (Pycock, 1993). In many cases this fluid is sterile and contains no neutrophils. Once the mare is bred, the fluid accumulation will be exacerbated due to poor lymphatic drainage and impaired myometrial contraction compounded by the tight cervix. The amount of intrauterine fluid will vary in individual mares, ranging from a few millilitres to over a litre in extreme cases. Frequently these mares are susceptible to post-breeding endometritis even though they have never been bred before. To maximise their fertility it is vital that the veterinarian is aware of the possibility of this type of cervical lesion.

Failure of the cervix to open during oestrus can lead to unwillingness of the stallion to complete mating or ejaculate intravaginally. Artificial insemination has been used successfully in mares with an abnormally narrow cervix. Mares with a fibrosed cervix that become pregnant do not normally have any difficulties at foaling.

Failure of the cervix to close during dioestrus can lead to persistent endometritis and failure to conceive, or early embryonic death. Failure to maintain closure during pregnancy can lead to gestational failure. Both surgery and exogenous progesterone have been tried in an attempt to encourage closure of the cervix.

Assessment of the cervix must form a part of the routine prebreeding examination of a mare, either directly using a speculum per vagina and/or by digital exploration, preferably during dioestrus when it is more tightly closed under the influence of progesterone.

Injury, resulting in cervical incompetence or fibrosis, most often occurs during parturition. This is especially the case if fetotomy is performed by an inexperienced clinician, or without adequate instrumentation. Injury can also occur during vigorous mating by an oversized stallion, especially if the mare was not in full physiological and behavioural oestrus, although usually it is not too severe. The cervix can also be damaged by irritant chemicals such as povidone-iodine. Adhesions of the cervix can be broken down manually, but this must be done daily to prevent recurrence. If severe, adhesions may contribute to the development of pyometra. A recent study reported that older, multiparous mares may be predisposed to cervical lacerations (Miller et al., 1996).

If severe, cervical lacerations may need surgical repair to restore normal cervical shape and function. Post-breeding repair of cervical lacerations has been documented (Foss et al., 1994). Since many cases of cervical lacerations are only detected at the prebreeding examination or at the time of AI, post-breeding repair is preferable since it avoids a 4–6-week healing period, resulting in a reasonable chance of pregnancy.

Developmental abnormalities of the cervix have been described; these include aplasia and a double cervix.

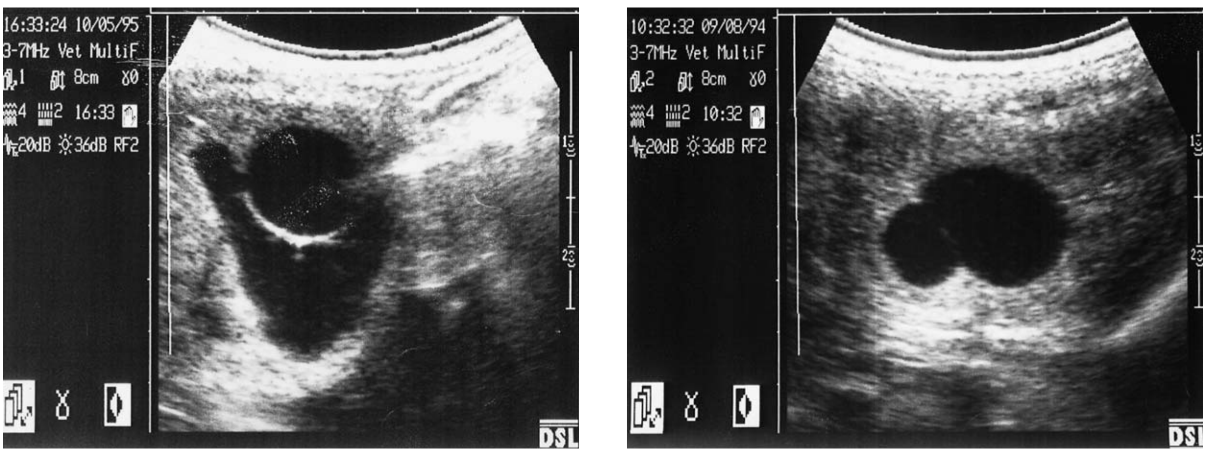
Uterus

Uterine cysts

Uterine cysts are the most common type of uterine lesion identified in the mare. Two distinct morphological types are recognised: (1) endometrial cysts, which are usually 2 cm or less in diameter, and (2) lymphatic cysts, which are generally larger. Although they can be diagnosed at post-mortem examination, the use of ultrasonography has shown that the incidence is much greater than was originally suspected. One detailed study found an overall prevalence of 26.8%, with mares over 11 years 4.2 times more likely to have cysts (Eilts et al., 1995).

The relationship between subfertility and uterine cysts is not clear. Some authors suggest that uterine cysts can reduce pregnancy rates (Adams et al., 1987). However, a large field study concluded that there was no evidence to suggest that they adversely affect the establishment or maintenance of pregnancy (Eilts et al., 1995). Their effect on fertility could be by restricting early conceptus mobility, whilst later in pregnancy they may interfere with nutrient absorption because of contact between the cyst wall and yolk sac or allantois. However, it is difficult to substantiate their primary role, as they are a common sign of uterine disease in general, including senility and previous endometritis. The author's experience is that only mares with multiple large cysts have a 10–20% reduced pregnancy rate.

Cysts can be confused with an early conceptus and give rise to false positive early pregnancy diagnosis or the incorrect diagnosis of twin pregnancies during ultrasound scanning. Differentiation is based on previous cyst mapping, but also the early mobility of the conceptus, the presence of specular reflections, the conceptus's spherical appearance and growth rate. Some cysts can be very difficult to distinguish from pregnancies (Figure 26.2). Often, there is an opportunity to scan the mare before, or immediately after, breeding, but this is not always possible. In the case illustrated, it is impossible to be certain whether there are twin unilateral vesicles of approximately 20 and 16 days, or a single pregnancy and a 19 mm cyst, or a cyst with adjacent free fluid, or a 19 mm conceptus with adjacent free fluid. In these cases, it is vital



(a) (b)

Fig. 26.2 (a) Ultrasonographic image of the right uterine horn of an 18-year-old mare bred 20 days ago. (b) Ultrasonographic image of the right uterine horn of a mare with unilateral twins of 14 and 17 days of gestation.

to study the ultrasonographic image carefully. When one looks closely at the wall between the two structures in Figure 26.2(a) it is relatively thick and hyperechoic when compared with the twin vesicle shown in Figure 26.2(b). This would confirm that at least one structure is a cyst. Reassessment of the irregular structure will confirm that the fluid is contained, and does not extend up or down the horn as would be found with free fluid. The appearance of an embryo around 22–24 days of pregnancy provides a definitive diagnosis. Thorough identification of cysts at the beginning of the breeding season minimises the chance of false positive pregnancy diagnosis.

Larger lymphatic cysts may interfere with the mobility phase of the early conceptus and thus prevent luteolysis (failure of maternal recognition of pregnancy). Later in pregnancy, the absorption of nutrients and the development of chorionic villi may be diminished in places of contact between cysts and fetal membranes, leading to an increased risk of embryonic death. The need for treating endometrial cysts is uncertain. If a mare is found at the beginning of the breeding season with a large number of cysts, it is generally best to continue to attempt to get the mare in foal that season. If she fails to become pregnant, some form of treatment should be attempted and an endometrial biopsy should be taken to help determine the likelihood of her carrying a foal to term. Because of risks such as uterine haemorrhage, mechanical uterine curettage is rarely used. Larger cysts can be punctured using an endometrial biopsy instrument or manually if the cervix allows passage of one hand. Chemical curettage has equivocal results; the cysts may disappear but scar tissue may form. Thermocautery, in conjunction with endoscopy involving looping and subsequent burning of cysts, is possible. Wounds after cautery appear to heal very quickly, usually within 4–6 weeks. Because endoscopy should be done while the mare is in dioestrus when the cervix is relatively closed, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) should be given after cauterisation and the uterus should be lavaged with saline.

Most uterine cysts involve the endometrium, but occasionally an extraluminal uterine cyst lying external to the endometrium can be identified on ultrasound examination. Its location should be verified by identification of the uterine lumen. Extraluminal cysts usually have no adverse effects on fertility.

Partial dilatation of the uterus

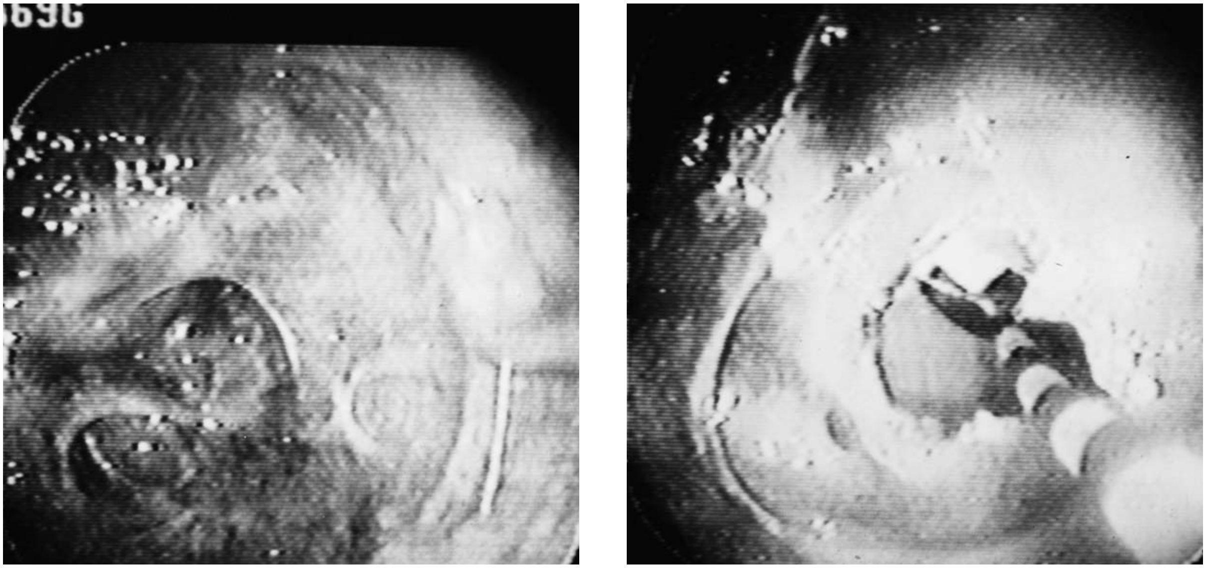
The discrete collection of fluid in permanent ventral dilatations at the base of one or (rarely) both horns of the uterus, which can be palpated per rectum, was first reported by Knudsen (1964). Ventral uterine enlargements have subsequently been discussed by Kenney and Ganiam (1975), who suggested that they originated by one of four mechanisms: mucosal atrophy, myometrial atony, lymphatic lacunae or endometrial cysts. Their precise relationship to subfertility is not clear, but mares that fail to eliminate the fluid and debris that accumulate in these sacculations after mating are susceptible to the establishment of chronic endometritis (see later); treatment is the same as for mares with defective uterine clearance.

Uterine adhesions

Uterine adhesions are most frequently diagnosed on endoscopic examinations of the uterus. It has been suggested that the incidence may be greater than was previously thought (Stone et al., 1991). Multiple adhesions adversely affect fertility by causing fluid accumulation or by affecting the mobility of the conceptus. Severe adhesions can completely obstruct one or both of the uterine horns. It is possible to remove the obstruction endoscopically by either cautery or laser techniques, starting at the thin membranous parts of the obstruction (Figure 26.3). It is important not to 'burn' too deeply in the uterine wall, as in these cases more severe damage to the uterine wall might occur. After removing the obstruction, the uterus should be flushed to remove any debris and the mare treated with $PGF_{2\alpha}$. In addition to an assessment of an endometrial biopsy, the prognosis for future breeding also depends on the severity of the obstruction and to what extent the obstruction could be removed.

Uterine foreign bodies

Uterine foreign bodies (e.g. fetal remnants), which may act as a nidus for the establishment of



(a) (b)

Fig. 26.3 (a) Adhesion almost completely obstructing the left uterine horn. (b) Removal of the obstruction by endoscopic cauterisation. (see colour plate 1).

chronic endometritis, have been documented (Ginther and Pierson, 1984) but are uncommon. In one case, the mare had a history of failure to conceive and short interoestrus intervals following a dystocia. Ultrasound examination revealed hyperechoic fetal bone, part of the scapula (Figure 26.4) which was removed manually via the cervix. The mare conceived after appropriate intrauterine lavage and antibiotic treatment for the endometritis. Other foreign bodies that have been reported include straws following AI, and the tips of uterine swabs.

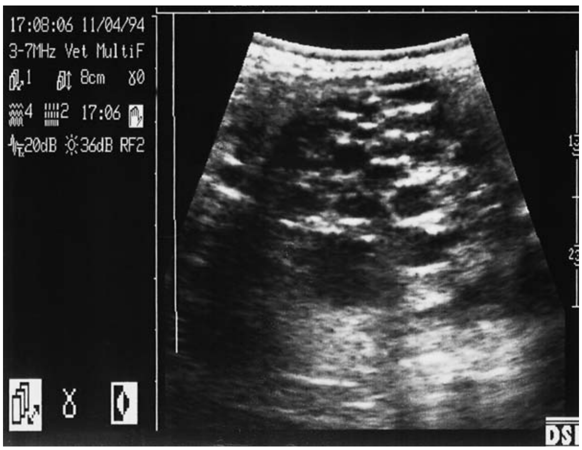
Fig. 26.4 Hyperechoic fetal bone visible on ultrasonographic examination of the uterine body.

Uterine neoplasia (Madwell and Theilen, 1987), abscesses (Van Camp, 1993) and haematomata (Shideler et al., 1990; Pycock, 1994a) are rarely reported in the mare.

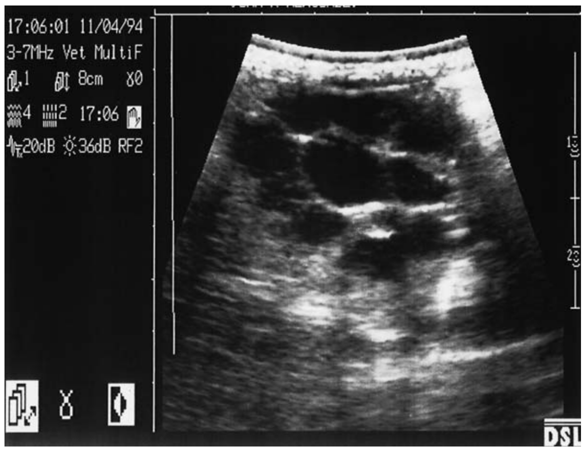
Uterine tubes and periovarian structures

Uterine tube abnormalities are usually due to remnants of embryological structures, and are rarely reported in the mare. A recent report concluded that although evidence of salpingitis could be detected in problem breeding mares, the incidence was no different from that noted in normal, fertile mares (Ball et al., 1997). The presence of collagenous masses within the uterine tube that might occlude the lumen has been documented (Liu et al., 1990). Dye tests are used in cattle to test tubule patency, but this is difficult in the mare, and occlusion is very rare. Cysts lying within the ovarian stroma near the ovulation fossa of the ovary arise from the surface epithelium and are often seen in older mares during examination of the ovary. They are known as ‘retention’, ‘inclusion’ or ‘fossa’ cysts and generally have no adverse effect upon fertility (Figure 26.5(a)). They are termed ovarian cysts by some authors, which may account for the diagnosis of 'cystic ovaries' in the mare. Sometimes the disease can be falsely diagnosed due to:

- Vernal transition, in which a single follicle or multiple large ones persist without ovulating.
- Fossa cysts, normally only a few millimetres in diameter and not usually as large as those in Figure 26.5(b). Care must be taken not to confuse them with a normal ovary with several small follicles (Figure 26.5(c)) or conceptuses. Careful examination allows accurate identification of their position. It is possible that sometimes, particularly if they are large and numerous as can occur in older mares, they could impede the release of the oocyte from the ovulation fossa. Small nodules located within the loose connective tissue covering of the ovary (adrenocortical nodules) have also been identified.
- Periovarian cysts, which are fairly common, especially in Shires and Clydesdales. They are not endocrinologically active, do not usually interfere with the process of ovulation and do not generally affect fertility. Occasionally, large cysts may be palpated or imaged with ultrasonography, and may be confused with follicules. However, the lack of change in size or appearance of these structures is usually diagnostic.



(a)



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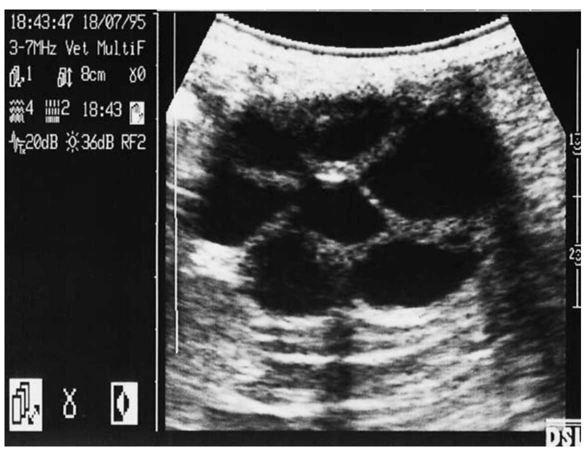


(c)

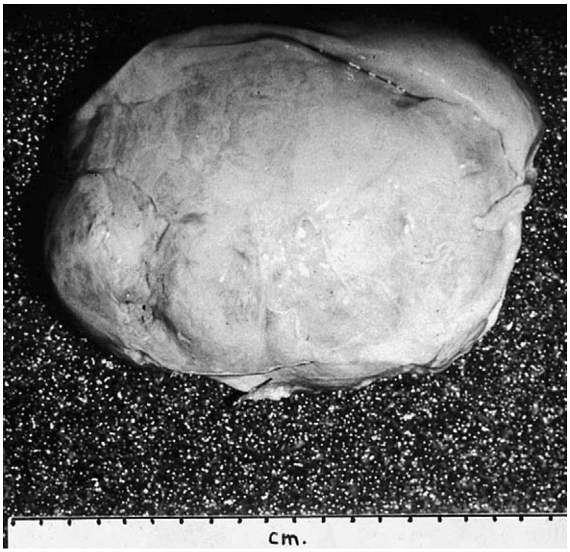
Fig. 26.5 (a) Ultrasonographic image of the ovary of a 15-year-old mare showing retention cysts each a few millimetres in diameter. (b) Ultrasonographic image of the normal ovary of a 17-year-old mare showing large retention cysts. (c) Ultrasonographic image of the normal ovary of a 16-year-old mare with seven small follicles.

Ovarian neoplasia

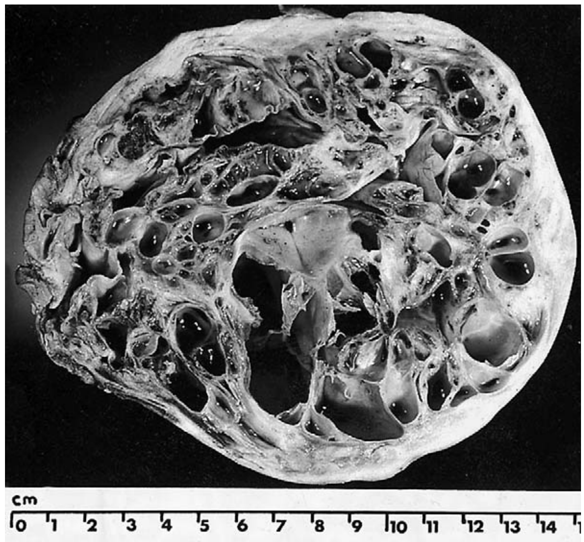
There are several reasons for the presence of a large ovary in a mare: (1) a normal ovary during the transition or breeding season with large follicles, as frequently detected during early spring, persistent luteal phase and early pregnancy; (2) a solid neoplastic lesion, such as a granulosa theca cell tumour, teratoma, dysgerminoma, cystadenoma and carcinoma; (3) haematomata of the ovary; (4) abscesses; and (5) haemorrhagic and luteinised follicles.

Ovarian neoplasia is uncommon in the mare although many types of tumour have been described, with the granulosa theca cell tumors (GTCTs) (Figure 26.6) being by far the commonest (Meagher et al., 1977).

GTCTs arise from the sex cord stromal tissue within the ovary and may be hormonally active, producing variable amounts of steroids that cause behavioural changes and alteration to normal cyclical activity. Mares can exhibit nymphomania, anoestrus or aggressiveness with signs of virilism (clitoral enlargement, stallion-like conformation).



(a)



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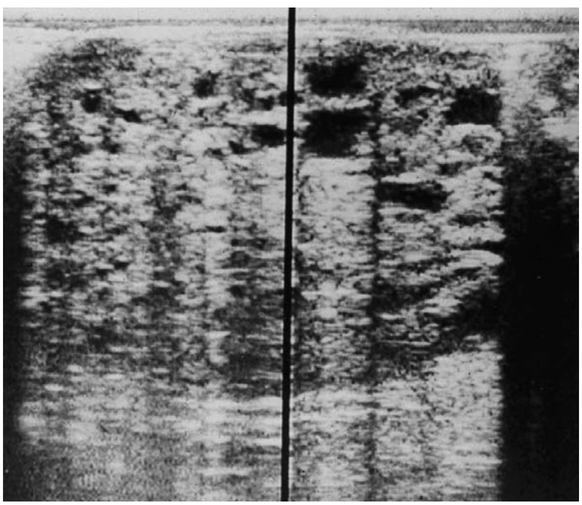


(c)

Fig. 26.6 Granulosa theca cell tumour. (a) Gross characteristics. Note the large size and smooth surface. (b) Cross-section of an affected ovary showing the 'honeycomb' or multiocular appearance. (c) Ultrasonographic image showing the 'honeycomb' appearance with multiple, small, non-echogenic areas separated by large areas of dense stroma.

There appears to be no breed predisposition for GTCTs, and there is a wide range of age distribution.

The tumours are often large before they are diagnosed, and one grossly enlarged ovary >10 cm diameter) with the opposite ovary small and firm on palpation with no visible follicles above 1 cm (resembling an ovary of a mare in deep anoestrus) is indicative, but not diagnostic, of a GTCT.

In mares with GTCTs, behavioural changes alone can be misleading since many affected mares do not show virilism or any other behavioural changes, and tumours other than GTCTs can also result in elevated plasma testosterone values. It has been the author's experience that occasionally owners express the opinion that their mare is 'awkward' when in oestrus, and request veterinary treatment. Frequently such mares are required to perform to a high level, e.g. advanced dressage. If examination during a reported period of abnormal behaviour reveals marked follicular development, it is tempting to diagnose 'cystic ovaries' as the cause of the behavioural changes. On other occasions, the mare may even be in dioestrus when examined. In any case, owner pressure to perform an ovariectomy on suspicion of a GTCT should be resisted, at least until the mare has been monitored throughout several cycles to determine whether her behavioural problems are related to oestrus.

When it is thought that the behavioural problems are truly linked to oestrus, daily treatment with progesterone or a synthetic progestogen should prevent cyclical ovarian activity and oestrus. Although rare in an unbred mare, there is a possibility of an increased risk for endometritis in a mare on long-term progesterone supplementation and she should be monitored for this. In addition, the problems may well recur following cessation of treatment.

Another possibility is to get her pregnant. Possible disadvantages of this approach are that she cannot be shown or compete in the later stages of pregnancy, and the problems may recur after birth of the foal. There may be some permanent conformational changes due to the pregnancy which could detract from the mare's showing potential.

The presence of a large ovary is not necessarily indicative of a tumour. Removal of the ovaries is not necessarily the answer and is irreversible. Ovariectomy should be done only after thorough client education and discussion. A peripheral blood sample for testosterone, oestrogen and progesterone assays is useful. Increased concentrations of testosterone support the clinical diagnosis; oestradiol concentrations may be raised and progesterone concentrations are usually low. Identification of elevated concentrations of the hormone inhibin may be more reliable than testosterone in confirming the presence of a GTCT. The secretion of high amounts of inhibin by the neoplastic granulosa cells inhibits follicle-stimulating hormone (FSH) secretion, and is thought to be the reason for atrophy of the contralateral ovary (Piquette et al., 1990).

A GTCT often appears ultrasonographically as a large (7–40 cm) spherical mass with a multicystic or 'honeycomb' appearance (Figure 26.6(c)). However, there is no typical appearance of a GTCT on ultrasound; some are uniformly dense and others have a single, large, fluid-filled centre or even several large cysts. The echogenicity of the cyst wall differentiates it from persistent, large anovulatory follicles. Teratomata, depending on their composition, have marked echoic areas in their stroma related to calcified deposits of bone, teeth and hair. However, the ultrasonic appearance of some GTCTs seen by the author can be similar to that of luteinised, unruptured ('haemorrhagic') follicles. Histopathological examination is the only method of obtaining a definitive diagnosis.

It is important to diagnose accurately the reason for the enlarged ovary. For example, in one report, 39% (11 out of 28) of surgically excised enlarged ovaries did not warrant removal (Bosu et al., 1982). Cases of GTCT may be found at routine examination of mares, maybe even after foaling, yet rarely have these mares shown any behavioural changes. Larger tumours that have been present for some time are more likely to cause erratic behaviour and colic signs.

Unilateral ovariectomy is the only satisfactory treatment for GTCTs, since the prospect of breeding from the mare is extremely poor unless the neoplastic ovary is removed. It is important not to be too hasty in removing the ovary, and a mare should always be scheduled for a second examination some weeks later. In the case of a tumour, the ultrasonic appearance would change little in the short term and would certainly appear similar if re-examined several weeks later. The reproductive prognosis of the mare is generally good, depending on the state of inhibition of the other ovary and provided no uterine tissue had to be removed. Most mares return to normal cyclical ovarian activity, although this often takes as long as one breeding season, especially in cases of severe suppression of the remaining ovary.

Most GTCTs are benign and unilateral although a bilateral case has been reported. Metastasis of the tumour is rare, but does occur (Meagher et al., 1977).

Ovarian haematoma

See later under 'Anovulatory haemorrhagic follicles'.

Gonadal dysgenesis (see Chapter 4)

This condition is not common. However, in a maiden mare, once winter anoestrus has been eliminated as a cause of acyclicity, XY ovarian dysgenesis must be considered as a possible cause with small, inactive ovaries and an immature tubular genital tract. Examination of the reproductive system detects very small ovaries (<1 cm in diameter), and a poorly developed tubular genital tract, which is difficult to palpate. This is similar to mares with XO chromosomes (Turner's syndrome). There is no treatment, and the mare is sterile.

FUNCTIONAL INFERTILITY AS A CAUSE OF SUBFERTILITY

Mares are seasonally polyoestrous, and environmental and other factors can exert a profound effect on reproductive function, particularly during the transitional period between winter aroestrus and the onset of cyclical activity in the spring. Although irregularities of follicular development, ovulation and behavioural patterns are also observed during the normal breeding season, they are not as common. However, endometritis can also cause cyclical irregularities.

Anoestrus due to ovarian acyclicity

Winter anoestrus

The onset of cyclical activity is stimulated by increased day length (see Chapter 1). During winter months mares are normally acyclical.

Diagnosis. On rectal palpation or transrectal ultrasound imaging both ovaries will be small (<3 × 2 × 2 cm), and in some mares there will be a number of small follicles. Plasma progesterone concentrations are >1 ng/ml.

Treatment. Although increasing day length is the primary controlling factor, ensuring freedom from disease and good body condition by stabling, adequate nutrition, anthelmintic therapy and attention to dental conditions can hasten the onset of cyclical ovarian activity. Thus, prolonged anoestrus can be prevented by good management. Progesterone/progestogen withdrawal therapy has been used successfully. Progesterone can be administered as an oil-based intramuscular injection, orally as the synthetic progestogen altrenogest (Equine Regumate) or by using a silastic progesterone-releasing intravaginal device (PRID). However, such therapy is effective only in anoestrous mares that are already well into the transitional phase to the resumption of normal cyclical ovarian activity. Repeated daily injections of equine pituitary gland extract to mares in winter anoestrus lead to follicular development, whilst Hyland et al. (1987) have reported success using a mini-pump that infused gonadotrophin-releasing hormone (GnRH) intravenously over a period of 28 days. These last two treatments are impractical for routine use.

In aged mares, the delayed initiation of normal cyclical ovarian activity may reduce the number of oestrous cycles during the breeding season and, therefore, it is particularly important to prevent poor body condition from occurring in such animals.

Pituitary abnormalities

Rarely Cushing's syndrome caused by adenomatous hyperplasia of the intermediate pituitary has been associated with anoestrus in aged mares. This is presumably due to destruction of the cells secreting luteinising hormone and follicle-stimulating hormone.

Lactation-related anoestrus

Lactation-related anoestrus is commonest in mares foaling early in the season. Affected mares may have a normal postpartum oestrus after 6–12 days, but fail to return to oestrus at the end of the first dioestrus. Alternatively they may not even have a normal 'foal heat'.

Diagnosis. The ovaries resemble those of a mare in deep winter anoestrus, i.e. small and inactive; the condition can last for several months. Originally it was thought to be due to prolactin suppressing pituitary gonadotrophin release, but this is now in doubt. Affected mares should be teased and examined weekly per rectum to assess their ovarian status.

Treatment. Treatments similar to those described above for winter anoestrus have been used, but with little success. Twice-daily injections of 0.04 mg (10 ml) of a synthetic GnRH analogue (buserelin; Receptal) have been found to induce the development of a follicle within 7–14 days of commencing therapy. The author has successfully treated seven out of 14 mares using this regimen, but it is expensive, the pregnancy rate at the induced oestrus is reduced and the mare may return to anoestrus following the induced ovulation.

Anoestrus caused by a prolonged luteal phase

Persistence of luteal activity

Persistence of luteal activity in the non-pregnant mare is a major cause of subfertility. Traditionally, the term 'prolonged dioestrus' has been used to describe a condition where the the corpus luteum persists beyond its normal cyclical life span of 15/16 days, resulting in the maintenance of elevated circulating progesterone concentrations for longer than expected. Ginther (1990), in reviewing the condition, has suggested that the term 'prolonged luteal activity' should be used, as 'persistent dioestrus' implies that the corpus luteum persists, whereas it is possible that others are formed sequentially from dioestrous ovulations. These occur in up to 20% of oestrous cycles in thoroughbred mares (less frequently in ponies) and are not accompanied by oestrus; the cervix will remain pale in colour, dry and tightly closed. If dioestrous ovulations occur late in the luteal phase, they will be refractory to the effect of endogenous luteolysins, resulting in a persistent luteal phase.

True persistence of the corpus luteum occurs in approximately 20% of ovulations. These mares present great difficulty to the stud manager as they can be assumed incorrectly to be pregnant.

Diagnosis. Plasma progesterone profiles are indistinguishable from those of pregnant animals. The uterus becomes firm and tubular (tonic) and the cervix is typical of that of pregnancy. Transrectal ultrasound imaging fails to detect a conceptus.

Treatment. Failure of synthesis and/or release of $PGF_{2\alpha}$ at the end of dioestrus is the most likely cause of persistence of the corpus luteum. Ginther (1990) has suggested that it might also be due to failure of the corpus luteum to respond to $PGF_{2\alpha}$, or failure of $PGF_{2\alpha}$ to reach the corpus luteum. Treatment is by the injection of a luteolytic dose of $PGF_{2\alpha}$ or a synthetic analogue. The interval between treatment and ovulation varies considerably depending upon the size of follicles at the time of treatment. Therefore, it is advisable always to examine mares using ultrasonography before treatment in order to assess the status of folliculogenesis.

Pyometra

Pyometra (see also later) is the accumulation of substantial quantities of inflammatory exudate in the uterus causing its distention (Hughes et al., 1979). When the endometrium is severely damaged, there is extensive loss of surface epithelium, severe endometrial fibrosis and glandular atrophy causing a prolonged luteal phase, presumably due to interference with the synthesis or release of $PGF_{2\alpha}$. This is in contrast to mild endometritis with the collection of small amounts of intraluminal uterine fluid, which is more likely to cause premature release of $PGF_{2\alpha}$ and luteolysis .

Pregnancy and pseudopregnancy

Pseudopregnancy is a term used to describe a syndrome in which non-pregnant mares that have been mated do not return to oestrus. It occurs if there is early embryonic death after 15 days of gestation with persistence of the corpus luteum verum resulting in a prolonged luteal phase. The cervix remains tightly closed, and the uterus is tense and tubular. It is differentiated from pregnancy by the absence of a conceptus on ultrasound examination. If early fetal death occurs after endometrial cup formation at 36 days (see Chapter 3), mares will either become anoestrous or come into oestrus. However, in the latter, follicular luteinisation without ovulation is thought to occur and therefore the oestrus is not fertile; this will last until the endometrial cups regress spontaneously at 90–150 days. There is currently no practical way of destroying endometrial cups prematurely.

Behavioural anoestrus – silent oestrus

Some mares either do not show oestrus, or are slow to show detectable signs using standard teasing methods despite the fact that ovulation occurs; this is called silent oestrus. The degree of reduced expression of oestrus varies from partial (suboestrus) to complete (anoestrus). The incidence of silent oestrus has been reported to be 6% (Nelson et al., 1985); it is thought to have a higher incidence in maiden mares early in the breeding season and in mares with a young foal 'at foot'. Other factors that affect oestrous behaviour include being at grass with very dominant mares, and stallion preferences. Fillies that are in training and have been treated with anabolic steroids may be more likely to suffer from the condition due to 'androgenisation'.

Diagnosis. Rectal and vaginal examinations confirm that the mare is in oestrus and has follicles of an ovulatory size. It is essential to distinguish the condition from a prolonged luteal phase in which there is also follicular development.

Treatment. The treatment is based on thorough and careful teasing. Frequent and persistent teasing may persuade the mare to show oestrus. Alternatively, placing the mare in a stable next to a stallion may be helpful. If permissible, artificial insemination can be used. To breed mares naturally during a silent oestrus, some form of restraint may be necessary; many mares approaching ovulation accept the stallion when twitched and hobbled. An intramuscular injection of oestradiol benzoate (10–20 mg) 6 hours before breeding can be tried as a last resort. The veterinary surgeon must ensure that the mare is physiologically ready to be bred. In some cases when the mare is not psychologically prepared for breeding, oestrogens are of little value, and tranquillisers may be more appropriate. In many cases, it is a failure of the oestrus detection system rather than a true reproductive disorder of individual mares. However, it has been associated with reduced oestradiol concentrations in the peripheral circulation and a shorter interval from luteolysis to ovulation (Nelson et al., 1985). There is no suggestion that aberrant morphological abnormalities in follicular development are involved.

Shortened luteal phase – endometritis

At coitus, the mare's uterine lumen becomes contaminated with microorganisms and debris. In most mares there is a transient endometritis that usually resolves spontaneously within 24–72 hours so that the environment of the uterine lumen is compatible with embryonic and fetal life. It is important not to regard this endometritis as a pathological condition. However, if the endometritis persists after day 4 or 5 of dioestrus, in addition to being incompatible with embryonic survival, the premature release of $PGF_{2\alpha}$ results in luteolysis and a rapid decline of progesterone and an early return to oestrus. These mares are referred to as susceptible and they develop a persistent endometritis (Allen and Pycock, 1988). Endometritis will be considered fully later.

Irregular or prolonged oestrus

True persistent oestrus appears to be rare in mares other than during the transitional period from winter anoestrus, or in association with steroid hormone-producing ovarian tumours. Some cases that are presented as having persistent oestrus may actually represent normal behaviour, or other types of behaviour may be misinterpreted as being persistent oestrus. Mares that are anoestrous due to disease or old mares whose ovaries have ceased to function normally may be receptive to a stallion. Frequent urination due to hindlimb or back pain, or a urogenital problem may be mistaken for persistent oestrus.

Transitional 'spring' oestrus

Pressure to breed mares early in the year before the onset of their natural breeding season can cause problems for the veterinarian. Because of the considerable variation in the duration of oestrus during the transitional period, efficient breeding of the mare can be difficult. Shortly after the winter solstice, changes in the pineal/hypothalamic/pituitary axes result in some follicular growth; however, follicles remain small, do not ovulate, and regress. Eventually, after a variable transitional period of up to 2 months, larger follicles (>35 mm) will develop and ovulate, usually heralding the onset of normal cyclical ovarian activity. During the transitional period the behaviour is variable, ranging from total rejection of the stallion, to interest but resistance to him mounting, to normal acceptance. These behavioural signs can be consistent or inconsistent.

Diagnosis. The diagnosis is by thorough ultrasonic examination and rectal palpation, which reveals transitional follicles reaching a preovulatory size of > 30 mm. Visual identification of a corpus luteum or progesterone levels above 4 ng/ml confirm that the first ovulation has occurred and hence the onset of normal ovarian cyclical activity.

Treatment. The treatment of mares in the transitional stage is based on progesterone or progestogens, with or without the addition of oestradiol esters, involving several parenteral routes of administration. Progesterone can be administered as an oil-based intramuscular injection, orally as the synthetic progestogen altrenogest (Equine Regumate) or by using a silastic progesterone-releasing intravaginal device.

Progesterone exerts a negative feedback on gonadotrophin secretion which is followed by an increased release of FSH and luteinising hormone (LH). When the source of progesterone is withdrawn or its effect wanes, because of the withdrawal of the negative feedback effect, there is follicular growth, maturation and ovulation. Progesterone treatment is more effective in mares that are in late transitional stage and is ineffective in mares with minimal follicular activity, particularly during deep anoestrus. Currently, the most effective treatment is the use of in-feed medication with the potent progestogen altrenogest (Equine Regumate). This liquid, which contains 2.2 mg/ml of the active substance, should be added to the food once per day at a dose rate of 0.044 mg/kg body weight for 10 consecutive days; oestrus should occur within 6 days and ovulation between 7 and 13 days after the last treatment. Because of the possibility of ovulation occurring during treatment, an injection of $PGF_{2\alpha}$ on the last day of in-feed medication may be necessary to cause luteolysis of any corpus luteum that may be present. The use of intramuscular injections of progesterone and estradiol-17β in oil for 10 days produces a similar response to altrenogest, but the interval to oestrus is longer due to the suppression of follicular development by the oestradiol.

There has been much interest recently in using GnRH or its analogues, administered by injection, infusion or subcutaneous implant, to hasten ovulation in transitional or even anoestrous mares (Harrison et al., 1990). The author has successfully used 0.04 mg of buserelin (Receptal) given twice daily by intramuscular injection. It is expensive, as treatment is necessary for at least 1–2 weeks – a mean of 15.8 days is cited by Ginther and Bergfelt (1990). It is noteworthy that these authors found a high multiple ovulation rate associated with GnRH treatment. The use of the short-term implant of the GnRH analogue deslorelin (Ovuplant, Peptech Pty Ltd, Australia) has been reported by Meyers et al. (1997) and McKinnon et al. (1997). In the author's experience, there has been no clear advantage of deslorelin over human chorionic gonadotrophic hCG, in inducing ovulation in cyclic mares. However, its value in accelerating the first ovulation of the breeding season following seasonal anoestrus would appear to be a real benefit to the practitioner.

Regardless of the hormones used, mares undergoing treatment early in the season need 16 hours of adequate light and good housing and nutrition to ensure success.

During the transitional period before the first ovulation of the year, mares demonstrate erratic oestrous behaviour of varying intensity. The presence of multiple large follicles, possibly as large as 30 mm, makes detection of ovulation difficult by palpation alone. Even outside this transitional period, misinterpretation of ovulation, even by experienced clinicians, has been shown to be as high as 50%. It is much easier to visualise the corpus haemorrhagicum/early corpus luteum ultrasonographically when the anechoic follicle is replaced by an intensely echoic area representing the early corpus luteum. It is recommended that the interval between matings should not exceed 2 or 3 days, although there have been no critical studies on the survival time of sperm in the mares' genital tract. It is important not to begin breeding too early or this will result in the mare being mated many times. The appearance of uterine oedema (Figure 26.7) is an indication that the follicle should ovulate within a few days. A key factor in the emergence from vernal transition is the development of steroidogenic competence by the follicle, leading to an increase in circulating oestrogen concentrations that cause the release of LH from the pituitary due to a positive feedback mechanism. Oestrogen is responsible for the appearance of uterine oedema (in the absence of progesterone) and so this may be why the detection of uterine oedema is important in signalling the emergence of the mare from the transitional period.

Fig. 26.7 Ultrasonographic image of the uterine horn of a mare showing a marked oedema pattern.

Cystic ovarian disease

Cystic ovarian disease as comparable to the condition described in the cow (see Chapter 22) does not occur in the mare. The persistent follicles that occur during the transitional and other periods are structurally normal; however, their presence may explain why this condition has been diagnosed in the past.

Ovarian neoplasia

This has been considered earlier under structural infertility.

Chromosomal abnormalities

The normal chromosome complement for the domestic horse is 2n = 64. Various sex chromosome anomalies have been described in the horse, but are not common. The incidence of chromosomal abnormalities is difficult to assess, but must be suspected in maiden mares with small, inactive ovaries and an immature tubular genital tract once winter anoestrus has been eliminated as a cause of acyclicity. However, some genetically normal young fillies in training can be acyclic, and thus they must be given more time to mature reproductively; karyotyping must be performed before making a final diagnosis.

The main karyotypic abnormality of such mares is the 63,XO (Turner's syndrome) genotype. Examination detects very small ovaries (< 1 cm in diameter) and a poorly developed tubular genital tract that is difficult to palpate. These mares are usually small for their age and do not cycle, although occasionally they may show passive oestrous signs. There is no treatment and the mare is sterile.

Other chromosome abnormalities include ovarian hypoplasia and testicular feminisation.

These are also rare, but must be considered in female horses with irregular cycles and small ovaries during the breeding season.

Ovulatory dysfunction

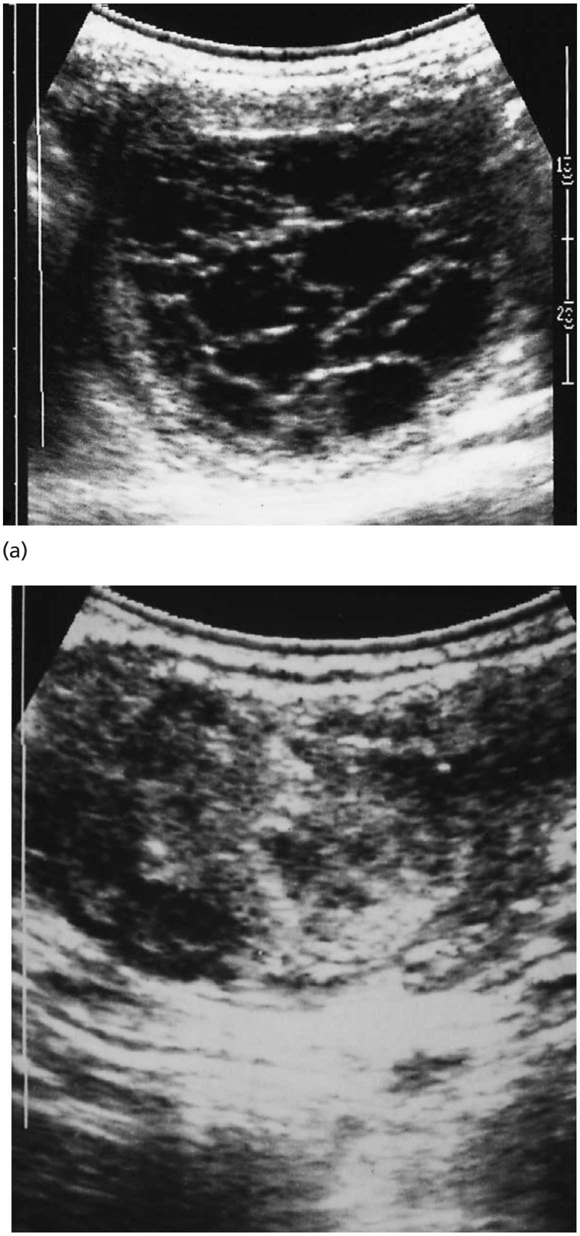
Anovulatory haemorrhagic follicles

A form of apparent ovulatory failure has been described in the mare in which the preovulatory follicle grows to an unusually large size (7–10 cm), apparently fails to rupture and ovulate, but fills with blood (Figure 26.8) and then gradually regresses. These haematomata persist for a variable period of time, often beyond the next ovulation and corpus luteum formation, and normal cyclic ovarian activity continues. They normally resolve spontaneously and no treatment is required. The condition is known as 'haemorrhagic anovulatory follicle syndrome'. In one recent study, 12 cases occurred in eight mares during 213 ovulatory intervals monitored by ultrasound (Ginther and Pierson, 1989). Where this occurs, the preovulatory follicle fills with blood and is initially recognised, using transrectal ultrasound, by the presence of scattered free-floating echogenic spots within the follicular antrum (Figure 26.9). As the blood coagulates, the ultrasonic appearance varies from honeycomb or 'net-like' to a uniformly echogenic mass (Figure 26.10). These structures can be as large as 8–10 cm, occasionally much larger, and develop an outer wall of luteal tissue. Functionally, they gradually regress in the same way as a normal corpus luteum, but they remain visible ultrasonically over subsequent oestrous cycles. No treatment is usually necessary. Sometimes they may also fail to regress around day 14–15 of the cycle and persist.

Haemorrhagic follicles may be difficult to diagnose. The rise in plasma progesterone is not useful for detecting ovulation since most haemorrhagic follicles tend to luteinise, thus producing progesterone and hence their alternative name 'luteinised unruptured follicle'. These structures cannot be detected by the behavioural responses of the mare, since oestrogen concentrations are ini-

Fig. 26.8 Ultrasonographic image of an anovulatory haemorrhagic follicle measuring 90 mm × 70 mm in the right ovary of a mare.

Fig. 26.9 Ultrasonographic image of the initial appearance of an anovulatory follicle measuring 55 mm × 40 mm in the ovary of a mare.



(a)

(b)

Fig. 26.10 (a) Ultrasonographic image of an anovulatory haemorrhagic follicle with a 'net-like' appearance within the follicular fluid. (b) Ultrasonographic image of an anovulatory haemorrhagic follicle with a more echoic, uniform appearance.

tially elevated, and subsequently, progesterone concentrations may increase and terminate oestrous behaviour similar to that following ovulation. On palpation, they are smooth with varying degrees of firmness. This can be confusing, since they may feel like preovulatory follicles or corpora haemorrhagica, or they may increase in size and become very large. The most obvious difference in their appearance is when they are examined ultrasonographically. Commonly, there are multiple echoes from within the follicular cavity, giving a net-like appearance within the follicular fluid. The structures may have a similar appearance to that of a granulosa theca cell tumour (GTCT); the anechoic areas are separated by trabeculae and are similar to those of a multicystic GTCT. The diagnosis of a haemorrhagic follicle may be made on the basis of clinical signs: namely, maintenance of cyclicity, a normal contralateral ovary, the presence of an ovulation fossa and speed of enlargement and regression of the ovary with time. Their significance is that the oocyte is not released but remains within the large unruptured haemorrhagic follicle. The abrupt decrease in follicle diameter normally associated with ovulation is not noted, but rather a steady increase in size and shape; stigma formation due to follicle softening is not seen. However, one cannot unequivocally state that they did not form by rapid filling between examinations.

The cause of these haemorrhagic follicles is not known. Similar structures are seen under continued equine chorionic gonadotrophin (eCG) stimulation during days 40–150 of pregnancy.

Anovulatory follicles in aged mares

While there is no documented menopause in mares, an age-related ovulation failure has been documented (Vanderwall et al., 1993). Some aged mares, particularly over 20 years of age, fail to ovulate despite showing oestrous behaviour. On ultrasound examination their ovaries resemble those of seasonally anovulatory mares with a few small (<10 mm) follicles. Endometrial biopsy shows evidence of gland atrophy. Currently there is no treatment, but identification of such mares is important to avoid unnecessary coverings.

Multiple ovulation

Double ovulations occur during 8–25% of oestrous cycles, the frequency depending upon the breed and type of the mare (thoroughbreds, highest rate; ponies, lowest rate). Accurate detection of such ovulations is important as twinning is highly undesirable: firstly, because it often results in abortion and, secondly, even if both fetuses survive and are carried to term, many are dysmature, resulting in a high neonatal mortality rate. A further complication is that if embryonic/fetal death occurs after the formation of the endometrial cups (see Chapter 3), these latter structures persist until they spontaneously regress as if pregnancy had been maintained, resulting in pseudopregnancy. Studies using transrectal ultrasound imaging have shown that the mare has an embryo reduction mechanism (see the review by Ginther, 1988) so that there is a wide disparity between the number of double ovulations and twin births. Most embryo reduction occurs after fixation at day 17 (see Chapter 3), and it is greatest when it occurs in the same horn and when the conceptuses are of unequal size. A deprivation hypothesis has been suggested (Ginther, 1989).

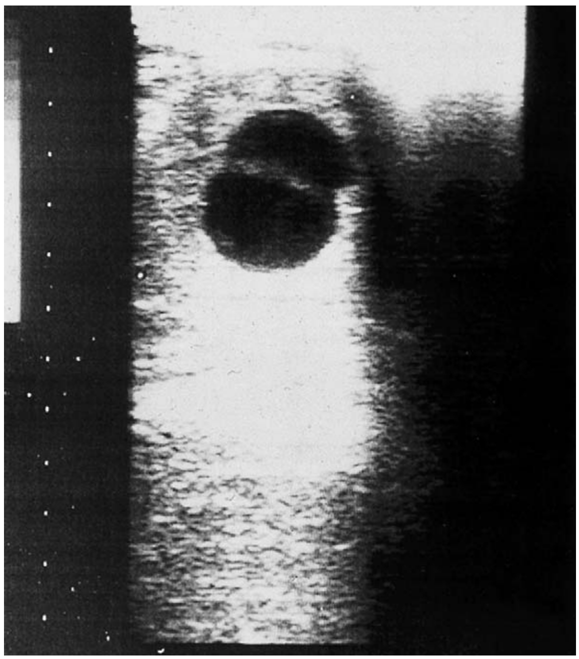
Rectal palpation alone can be misleading in detecting a double ovulation, particularly when the two follicles are on the same ovary. The use of ultrasound examination of the ovaries, which should routinely be performed in conjunction with a thorough transrectal palpation, usually allows detection of a double ovulation. Sometimes the ovulatory area can appear indistinct for the first 24 hours; in these cases the mare should be re-examined 2 days later when it can be seen more easily whether there is more than one corpus luteum (Figure 26.11).

Management of twin ovulation. Multiple ovulation in the mare should not be regarded as a reason for withholding breeding. Instead, pregnancy rates are improved after twin ovulation. Although accurate interpretation of the ultrasound image of early pregnancies in the mare and the technique of crushing a conceptus are skills that require experience, the advent of B-mode ultrasound imaging has provided a method of more readily managing a twin pregnancy in the mare.

There are two approaches to dealing with twins:

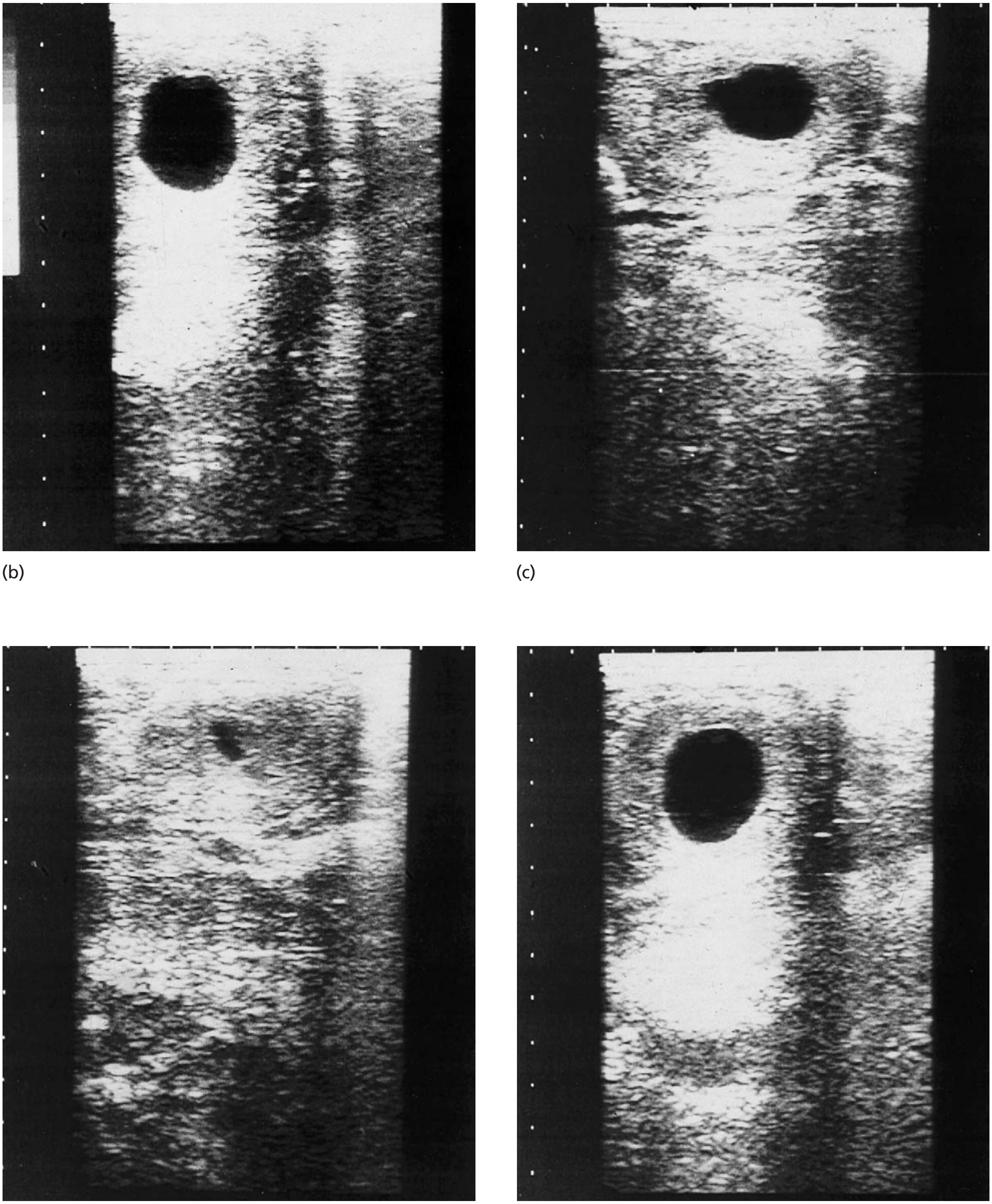
1. If the initial examination of the mare occurs before fixation (day 16/17) the twin embryos are reduced to a singleton by the manual destruction of one, either by pressure with the transducer or by the use of the hand (Figure 26.12). The author

Fig. 26.11 Ultrasonographic image of an ovary with a double ovulation 48 hours previously.



(a)

Fig. 26.12 Ultrasound images of a unilateral set of twin 16-day pregnancies. (a) Unilateral twin pregnancy. (b) By gentle pressure with the transducer, one of the conceptuses has been moved apart from the other. (c) Pressure is put on the selected conceptus using the transducer. The spherical shape is lost and then a distinct popping sensation is felt. (d) The small amount of fluid visible is rapidly resorbed. (e) The normal-looking remaining conceptus.



(b) (c) (d) (e)

Fig. 26.12 *continued*

prefers to separate the conceptual vesicles gently using the transducer to enable the procedure to be imaged. When the conceptual vesicles are of dissimilar sizes, the smaller one should be ruptured. This is easier at days 14–16 when they are 14–20 mm in diameter than days 11–13 when they are 6–11 mm in diameter. The disadvantage of this method is that it is more expensive, in that all mares are scanned before the time of return to oestrus. In addition, if ovulations that occur more than 3 days apart have not been detected, a mistaken diagnosis of a single pregnancy may be made if the second vesicle is too small to detect. However, with experience, this technique is highly effective, and it is the method of choice of the author. Analysis of data over several breeding seasons has shown that there is no increased incidence of mid- to late-term abortion in mares that have had one of twin conceptuses crushed.

2. If initial examination is done after fixation but before day 30, and if both conceptuses are in one horn, one option is to terminate a pregnancy using $PGF_{2\alpha}$. It is advisable to re-examine the mare 5 days later in case reduction has occurred, or transvaginal ultrasound-guided allantocentesis can be attempted. Management of twin pregnancies after this period is complicated by the formation of endometrial cups at approximately day 37/38 of gestation. Endometrial cups remain functional until around days 90–130 of gestation in the presence or absence of a viable fetus. Therefore, if twin pregnancies are not successfully managed before the cups are formed or both embryos die after day 37, the mare usually will not return to a fertile oestrus for a prolonged period of time.

After day 37 of gestation, reduction methods are unreliable. They include dietary energy restriction, surgical removal of one vesicle, intracardiac injection and transvaginal ultrasound-guided needle puncture. The latter would appear to offer the best approach and has been successfully used by the author on several occasions.

Pregnancy failure

Pregnancy failure is a source of major economic loss to the horse industry. Embryonic death occurs before 40 days of gestation when organogenesis is complete, with carly embryonic death (EED) occurring before the maternal recognition of pregnancy (see Chapter 3). Early fetal death occurs before 150 days of gestation, and late fetal death occurs after that. Abortion is defined as expulsion of the fetus and its membranes before 300 days, whereas a stillbirth is expulsion of the fetus and its membranes from day 300 onward.

Embryonic death

In normal fertile mares the fertilisation rate is more than 90%, which is comparable with other domestic species, with estimates of the EED rate at between 5 and 24%. In subfertile mares, the rate is higher. The differences in the estimates are due to varying methods of pregnancy detection, and the animals studied. The period of greatest embryonic death in subfertile mares occurs in the interval before pregnancy can be detected with ultrasound (day 11), particularly at the time the embryo enters the uterus. Between days 14 and 40, the rate of embryonic death varies between 8 and 17%. EED is multifactorial, in which external factors such as environment and management as well as pathophysiological factors are involved. However, the evidence for many of these associations is anecdotal and frequently contradictory.

External factors. External factors involved in embryonic death include stress, nutrition, season of the year, climate, sire effects and transrectal palpation.

Maternal stress due to severe pain, malnutrition and transport has been implicated as a cause of EED. Frequently, mares at stud are transported at various stages of pregnancy; recent work failed to demonstrate any difference in pregnancy rates between transported and non-transported mares. Transporting pregnant mares home from stud a distance of 300 miles (500 km) in less than 9 hours of travelling time can be stressful, but should not result in embryonic death. If a longer journey is necessary, the journey should be broken after 8 hours. Waiting until the fifth week of pregnancy or later to transport brood mares may be advisable when critical events such as descent of the embryo into the uterus and transition from the yolk sac to the chorioallantoic placentation have occurred. The common practice of transporting mares to stud for mating and returning home the same day should not be detrimental to their fertility, as long as the transport is safe and comfortable.

Far from being avoided, regular exercise is important during pregnancy, although during the latter half, forced exercise should be decreased. Rectal palpation and ultrasound examinations should be considered safe procedures when performed correctly, and recent evidence (Vogelsang et al., 1989) gives no indication that ultrasound examination is detrimental to the embryo.

Maternal factors. A number of abnormal maternal factors including hormone deficiencies and imbalances, uterine environment, age and lactation have been implicated.

Hormonal deficiencies and imbalances. Progesterone is critical for the maintenance of pregnancy in mares. The only source of progesterone during the embryonic period is the primary corpus luteum (corpus luteum verum). On the assumption that luteal insufficiency is important in EED, many mares are given exogenous progesterone or progestogens in an attempt to prevent it from occurring. However, the rationale for this widespread practice is highly questionable, although primary luteal insufficiency as a cause of EED has been reported by Bergfelt et al. (1992). Progesterone supplementation has been recently reviewed by Allen (1993), who is sceptical of any benefit.

Many dosage regimens do not effectively elevate or maintain plasma progesterone levels. Withdrawal of supplementary progesterone therapy during mid-gestation may leave the clinician open to criticism if the mare subsequently aborts. In the author's opinion, progesterone therapy is most appropriate in mares that have uterine oedema and an indistinct corpus luteum at the time of first examination for pregnancy (15 days). These pregnancies are usually lost within a few days, but some can be successfully 'saved' by exogenous progesterone and the pregnancy maintained to term. Progesterone in oil (100 mg) or altrenogest (35 mg; Regumate) is given daily until a CL is obvious on ultrasound examination, and all uterine on edema has disappeared. A single injection of 40 μg of the GnRH agonist buserelin has also been shown to reduce the incidence of EED when given 10 days after ovulation (Pycock and Newcombe, 1996a). However, when the same dosage was tested on a larger group of mares, the significance of GnRH treatment to EED was not detected.

Uterine environment. An abnormal uterine environment is detrimental to embryonic survival. Acute endometritis may result in EED by inducing premature luteolysis, or because of its direct embryopathic effect.

Severe periglandular fibrosis of the uterine glands may reduce the chances of embryo survival. Not only is this a response to persistent endometritis, but it also increases with age. This is one of the reasons for the reduced fertility of mares over 12 years of age, for despite similar fertilisation rates, detected pregnancy rates are on average 33% lower.

Foal heat breeding. Mares normally resume cyclical ovarian activity very shortly after parturition so that they are sometimes bred as early as 7–10 days postpartum (at the foal heat). There is conflicting evidence about the level of embryonic death if fertilisation occurs at this time, with some studies showing a higher rate and others no effect. An advantage of breeding at the first oestrus postpartum is that the foaling–conception interval is significantly shorter. The reason for the apparent decreased fertility in mares mated at the foal heat is the hostile uterine environment due to delayed uterine involution or persistent endometritis. However, pregnancy rates are clearly influenced by how strict the selection criteria are for mating at the foal heat. Traditionally, such factors as a normal foaling, placental expulsion, minimal vaginal bruising and absence of infection have been used. Endometrial cytology and ultrasonic scanning of the genital tract of each mare may be more reliable methods on which to base a decision.

Lactation. More pregnancy failures are detected in lactating than non-lactating (maiden or barren) mares; this phenomenon also increases with the age of the mare.

Embryonic factors. Embryonic abnormalities are also important to consider in relation to embryonic death. Embryos recovered from subfertile mares are smaller and have more morphological defects than embryos from fertile mares; however, this may be due to an abnormal uterine environment.

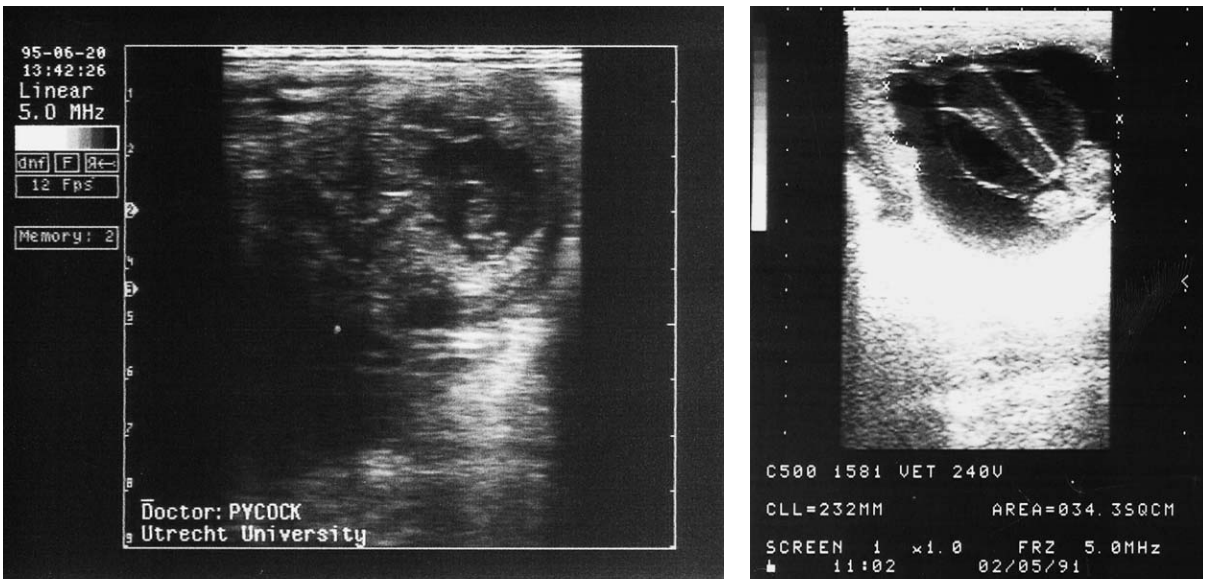
Ultrasonic scanning has provided a valuable tool in studying embryonic death. Because pregnancy is often diagnosed at an early stage, it is important to inform owners that not all pregnancies detected with ultrasound will survive, even in apparently normal mares. There are certain morphological features detected with ultrasound that are typical of mares in which embryonic death is occurring. Some of the consistent features include: (1) presence of fluid within the uterine lumen; (2) prominent endometrial oedema; (3) decreased or prolonged conceptus mobility; (4) undersized or irregularly shaped conceptus; (5) cessation of embryonic heart beat; (6) reduced volume of placental fluids (Figure 26.13(a)); (7) disorganisation of placental membranes (Figure 26.13(b)); (8) hyperechogenic areas in the embryo and membranes.

Fetal death and abortion

An overall abortion rate of 10% after 60 days of gestation is usually cited for the horse. The causes of equine abortion can be broadly divided into non-infectious (70%), infectious (15%) and unknown (15%). In practice, it is important to distinguish infectious from non-infectious causes. Vaginal discharge, premature lactation and colic in pregnant mares may indicate an impending or recent abortion.

When abortion occurs, the mare should be isolated, a history obtained and the fetus sent to an approved laboratory for necropsy. If a veterinary surgeon wishes to perform a post-mortem examination, small but representative samples of liver, lung, thymus, spleen and chorioallantois (two samples, one of which is from the cervical star, which is the irregular, star-shaped avillous area of the chorion that lies over the internal os of the cervix) (see Chapter 3) should be sent in formal saline for histological examination. In addition, frozen samples of fresh fetal liver and lung should be stored in a deep freeze at –20°C should viral isolation investigation be required at a later stage. Paired serum samples from the mare and close companions should also be taken for serological investigation. Swabs from fetal heart or liver and the cervical pole of the chorion are used to screen for bacterial infection.

The fetus and fetal membranes (amnion, chorioallantois and umbilical cord) must be care-



(a) (b)

Fig. 26.13 (a) Ultrasonographic image illustrating gestational failure in a 36-day pregnancy. Note the reduction of expected volume of placental fluid. (b) Ultrasonographic image illustrating gestational failure in a 42-day pregnancy. Note the disruption of the fetal membranes (amnion and allantochorion).

fully examined for the presence of abnormalities and areas of discoloration. Placental evaluation in the field has been described by Cottrill (1991), and a detailed description of abortion in mares can be found in Acland (1993).

Many countries have regulations or codes of practice for the general approach to the management of an aborting mare and these should always be followed.

Non-infectious causes of abortion and stillbirth

Twinning. Historically, twins have been the single most important cause of abortion in thoroughbreds. However, they are now much less common due to the widespread use of ultrasonography. The diagnosis of twin pregnancy can be made even if only one fetus is found, as examination of the placenta reveals an area devoid of villi where the two placentas were in contact. Twins should still be submitted to a diagnostic laboratory as twin pregnancies are not protected from equine herpesvirus (EHV) infection.

Umbilical cord abnormalities. In mares, the umbilical cord is twisted, usually in a clockwise spiral. The normal length ranges from 36 to 83 cm (mean 55 cm). Increased cord length (over 80 cm total length) has been associated with excessive cord torsion, which can cause twisting of the umbilical blood vessels. This twisting causes increased resistance to blood flow in both directions and the resulting poor placental perfusion can lead to fetal death. This can result in abortion of an autolysed fetus. Decreased cord length can cause premature tearing of fetal membranes, leading to fetal asphyxia. Twisting and vascular compromise currently constitute the commonest single cause of observed non-infectious abortion.

Premature placental separation. In mares, the interdigitating microvilli are connected by an unidentified electron-dense material. Placental separation involves dissolution of this substance. Causes of premature placental separation are largely unknown, although maternal stress and endophyte-contaminated tall fescue have been implicated. When placental separation occurs shortly before parturition, the thickened placenta often does not rupture through the cervical star, and the allantochorion bulges out of the vulva ('redbag' delivery). The foal can become hypoxic, resulting in the neonatal maladjustment syndrome.

Body pregnancy. In this condition almost the entire chorionic surface of the placenta contained within the uterine body is without villi, while that contained within the horns is covered with an excessive number of villi. The proportion of the placenta corresponding to the two uterine horns is small, and the fetus is situated entirely within the uterine body. The fetus is frequently aborted completely contained within its placenta; its growth has been retarded. The abortion occurs when the nutritional demands of the fetus exceed the ability of the placenta to meet them.

Fetal abnormalities. Severe developmental anomalies involving the central nervous system or development of body cavities have been reported in aborted fetuses (see Chapter 4).

Maternal disease. Pyrexia and malnutrition during pregnancy have been implicated as causes of abortion.

INFECTIOUS CAUSES OF ABORTION

The main causal agents of infectious abortion are viruses, bacteria and fungi, more rarely mycoplamsa and protozoa.

Viral abortion

Viruses that can cause abortion in mares include: equine herpesvirus, equine viral arteritis and equine infectious anaemia.

Equine herpesvirus (EHV)

EHV is the single most important infectious cause of equine abortion. The disease is caused by EHV-1 and, rarely, EHV-4. EHV-1 is also capable of causing respiratory disease (most noticeable in foals and yearlings), paralysis, neonatal foal disease and uveitis/hypopyon. EHV-4 normally causes respiratory disease but occasionally has caused abortion in single mares. Clinical signs of herpesvirus infection of the respiratory tract are not distinguishable from those caused by other viruses (and secondary bacterial infection):

namely, nasal discharge, transient pyrexia and depression. The source of the virus is:

- clinically affected animals with nasal secretions
- aborted fetuses and their membranes
- infected foals born live at term
- mares that have aborted, although they only shed virus from the genital tract for a short period
- asymptomatic virus excreters.

Naturally acquired immunity after EHV-1 infection is short-lived, so that even after only a few weeks or months, reinfection is possible. Evidence has also been found of latent EHV-1 infection, with the virus remaining dormant in the reticuloendothelial cells of clinically normal animals for an unspecified length of time. Stress can activate the virus.

The majority of EHV-1 abortions occur in the last 4 months of gestation. The mares shows no signs of impending abortion or clinical disease. The fetus is usually fresh and still enclosed in its membranes, and typically has excess serosal fluids, minute spots on the liver, jaundice of the mucous and placental membranes, enlarged spleen, perirenal oedema and pulmonary haemorrhages. Rarely, some foals survive for up to 7 days, but they are weak, jaundiced and have a marked leucopenia. Histological lesions include foci of necrosis and eosinophilic intranuclear inclusion bodies seen in degenerating hepatocytes and/or bronchiolar epithelial cells. Virus isolation is possible from lung, liver and thymus samples that have been submitted in viral transport medium. Fluorescent antibody tests can be performed on frozen sections of liver and lung. Unfortunately, there is no test to detect latent carriers.

Control of EHV-1 infection is considered in detail in the UK Horserace Betting Levy Board Codes of Practice. This booklet contains invaluable advice and is updated annually. Groups of mixed ages and reproductive status are most at risk from virus abortion; thus racehorses, hunters, weaned foals and yearlings should be kept away from pregnant mares. The pregnant mares should be kept in small groups, isolated from each other. All abortions and stillbirths should be investigated and the mare isolated pending the results. Newly arrived animals should never be mixed with pregnant mares on a farm.

A killed-virus vaccine and an attenuated live-virus vaccine are available commercially. However, only the attenuated live-virus vaccine (Duvaxyn EHV 1,4; Fort Dodge Animal Health) is licensed for the prevention of abortion in mares in the UK, and the manufacturer advises that it is given during the fifth, seventh and ninth months of pregnancy. Results following vaccination are conflicting. It would seem most likely that, while vaccination does not prevent an individual animal aborting, if the stud has a vaccination policy, then the likelihood of an abortion storm is much reduced.

Equine viral arteritis (EVA)

Equine viral arteritis is a contagious viral disease of the horse that in recent years has become important for the standardbred and thoroughbred industries in the USA. An EVA outbreak was identified for the first time in the UK in 1993. The principal focus of the infection was an Anglo-Arab stallion imported from Poland.

The two important routes of EVA transmission are venereal from a stallion with infected semen, and aerosol via the respiratory secretions of an acutely infected horse. After an average incubation period of 7 days, EVA is excreted in all bodily secretions, including respiratory secretions and urine for up to 21 days (possibly longer in urine). The virus may persist indefinitely in the accessory sex glands in stallions.

Close or direct contact is required for aerosol transmission to occur. Venereal transmission is believed to be the major cause of widespread dissemination of the virus. Stallions that become persistently infected with EVA shed the virus in the semen, which appears to be the sole route. In breeds that permit the use of artificial insemination, the virus can be transmitted through the use of fresh, chilled or frozen semen. Interestingly, marked changes in semen quality in stallions experimentally infected with EVA have recently been described (Neu et al., 1992). The recent outbreak in the UK was initially suspected after a semen sample from the affected stallion was routinely evaluated prior to its use to artificially inseminate a mare.

At present, there is no effective treatment for a chronically infected stallion. Such animals can remain persistently infected with the virus in the reproductive tract for variable periods of time, from several months to a period of years and, in some cases, the lifetime of a particular stallion. Up to now, there is no evidence that mares, geldings or foals that acquire the infection congenitally become carriers.

Clinical signs of EVA are very variable, and subclinical infections are the most common sequel. The classic clinical signs are an influenza-like illness with pyrexia for 1–5 days, depression, a nasal discharge, conjunctivitis, anorexia, a focal dermatitis and oedema of the limbs, ventral abdomen, scrotum, prepuce and periorbital regions. Abortion may occur during, or shortly after, an acute illness or subclinical infection. Abortion occurs as a result of myometrial necrosis and oedema leading to placental detachment, and hence fetal death. Abortions tend to occur in the latter half of pregnancy.

A definitive diagnosis, based on clinical signs alone, is not possible due to their variable nature. Acute EVA can be confirmed by virus isolation from nasopharyngeal swabs, heparinised blood samples, and urine and semen samples. Serological evidence of EVA exposure can be found by taking an initial serum sample as soon as possible after clinical onset, followed by a convalescent sample 10–14 days later to detect a rise in the EVA antibody titre. Diagnosis of abortion due to EVA is largely dependent on virus isolation from the placenta or fetal tissues; there are no pathognomonic gross lesions. Mares infected with EVA will usually abort partially autolysed fetuses, in contrast to fresh fetuses aborted by mares infected with herpesvirus.

Since virtually all acutely infected horses recover uneventfully after EVA, any treatment is symptomatic. In the UK, the Code of Practice considers control measures for EVA and provides excellent guidance in the event of an outbreak.

A modified live-virus vaccine is available in North America, whereas a killed vaccine (Artervac, Fort Dodge Animal Health) is available in the UK. It must be remembered that certain countries will not accept the importation of seropositive animals. If an animal is to be vaccinated, a blood sample for serology should be taken prior to vaccination. A second blood sample should be taken 10 days after the second vaccination to ensure a serological response to vaccination. Many studs in the UK are requiring confirmation that mares are seronegative to EVA prior to arrival at stud. Vaccination of stallions has been widely adopted in the UK.

Bacterial abortion

A large number of bacterial species that gain access to the placenta can cause abortion in the mare. The ascending pathway via the cervix is the primary route of infection, and most infections occur in early pregnancy. Rarely, bacteria may be in the uterus at the time of conception or arrive haematogenously. Bacteria that spread rapidly through the allantochorion often infect the fetus, causing acute bacterial septicaemia. More chronic ascending infections are often localised around the cervical star and cause a focal or local placentitis. The placentitis often leads to placental insufficiency with abortion of a growth-retarded fetus, or the birth of a dysmature foal. The placenta is often thickened and covered with exudate and the fetus septicaemic. Bacteria that cause placentitis are similar to the organisms that cause endometritis (see below). They are often opportunist pathogens that can be isolated from the caudal genital tract of normal mares, i.e. *Streptococcus spp.* and *Escherichia coli.* Others are considered to be venereal pathogens, i.e. *Pseudomonas spp.* and *Klebsiella spp*.

Recently, leptospirosis has been diagnosed in association with abortion in Kentucky in the USA and in Ireland. Leptospiral abortion is difficult to confirm because there are no clinical signs in the mares prior to abortion. Demonstrations of leptospires in fetal tissues and the placenta by immunofluorescence, and serology in mares, are needed for a diagnosis. Treatment of carriers with antibiotics is generally considered useful, but may not eliminate the shedding of the organism.

Fungal abortion

Aspergillus spp. are the most common cause of mycotic placentitis and mycotic abortion in the mare. Much less common is placentitis due to *Candida spp.* infection. Fungal disease has a similar pathogenesis to bacterial abortion with inflammation of the chorion beginning at the cervical pole.

Endometritis

Reduced fertility associated with endometritis, both acute and chronic, has been recognised for many years in brood mares. This subfertility is due to a hostile environment for the developing conceptus, and in some cases, the endometritis causes early regression of the CL. The term 'endometritis' refers to the acute or chronic inflammatory process involving the endometrium. These changes frequently occur as a result of microbial infection, but they can also be due to non-infectious causes. One of the main obstacles to producing the maximum number of live, healthy foals from mares bred during the previous season is the mare, which is susceptible to persistent acute endometritis following breeding.

Cause and pathogenesis

The underlying aetiology of the specific cause of endometritis determines the type of treatment to be used, and the following classification system for equine endometritis is useful:

- venereal infection
- chronic infectious endometritis
- endometrosis (chronic degenerative endometritis)
- persistent mating-induced endometritis (delay in uterine clearance).

It is generally assumed that the uterine lumen of the normal fertile mare is bacteriologically sterile or may have a temporary, non-resident microflora. This is despite the fact that the mare's reproductive tract is often contaminated with bacteria from the act of coitus, foaling and veterinary procedures. Mares with a defective vulval conformation can also aspirate air and bacteria into the vagina that can develop into endometritis.

The bacterial species that cause bacterial endometritis are numerous, and can be classified as follows: (1) contaminants and commensals; (2) opportunist, causing an acute endometritis; and (3) venereally transmitted. Normally, the vestibular and clitoral area has a harmless and constantly fluctuating bacterial population. In association with benign saprophytic organisms, opportunistic organisms such as *Streptococcus zooepidemicus, E. coli* and *Staphyloccocus spp.* can be found. The stallion's penis is colonised by similar organisms. *S. zooepidemicus* is the most commonly isolated bacterial species from acute endometritis, particularly in the initial stages. *E. coli* is the next most common isolate. The uterus responds to these bacteria with a rapid influx of neutrophils (Pycock & Allen, 1990). Normally, these neutrophils phagocytose and kill the bacteria rapidly (< 24 hours). The inflammatory byproducts are then mechanically removed and the endometritis resolves itself except when the mare suffers from pneumovagina or is a 'susceptible' mare. Susceptible mares have a delay in uterine clearance, and the inflammatory byproducts accumulate as uterine fluid. Such mares have a reduced pregnancy rate due to a hostile environment for the early developing conceptus.

In addition to opportunist pathogens, there are three bacteria that are venereally transmitted: *Taylorella equigenitalis* (contagious equine metritis organism, CEMO), *Klebsiella pneumoniae* (capsular types 1, 2 and 5) and *Pseudomonas aeruginosa* (some strains).

Symptomless carriers of both sexes allow persistence within the horse population. Carrier mares, which may or may not have shown signs of previous endometritis, harbour the organisms in the vestibular area, particularly the clitoral fossa and sinuses. Mating or gynaecological examination may result in their transfer into the uterus (Hinrichs, 1991). Stallions may harbour the organisms over the entire surface of the penis and in the distal urethra. Control is by routine screening of swabs taken before mating by laboratories experienced in the isolation and identification of these specific organisms. Details of the control procedures for contagious equine metritis (CEM) and the other venereal pathogens are in the UK Horserace Betting Levy Board's Code of Practice. Anaerobic bacteria have been isolated from the mare's uterus, with *Bacteroides fragilis* the most frequent (Ricketts and Mackintosh, 1987). Further work is needed to assess the importance of anaerobes in endometritis.

Diagnosis

Venereal disease screening. Before the breeding season, swabs should be taken from the clitoral fossa, clitoral sinuses (only the central sinus may be obvious) and the vestibule. The perineal area of the mare should not be cleaned except for the removal of gross contamination of the vulva with faeces using a dry paper towel. A protective disposable glove should be worn by the veterinary surgeon on the hand used to evert the ventral commissure of the vulva and expose the clitoris. The swabs should be placed in transport medium, clearly labelled with the mare's name and sent to an approved laboratory. It is important to penetrate the clitoral sinus, and therefore a large swab tip should not be used. Swabs are cultured aerobically on blood and MacConkey agar to screen for the presence of *K. pneumoniae* and *P. aeruginosa*. Microaerophilic culture on chocolate blood agar (with and without streptomycin) must also be done for the detection of CEMO. In addition, in stallions, two sets of swabs must be taken from the pre-ejaculatory fluid (if possible), penile sheath, urethra and urethral fossa.

In the UK, this screening has been successful in virtually eradicating CEMO and vastly reducing the incidence of venereal disease.

Endometrial culture and cytology. A diagnosis of endometritis can be made by collection of concurrent endometrial swab and smear samples during early oestrus for bacteriological culture and cytological examination, respectively. This allows time for resolution prior to mating, and maximises the chances of pregnancy.

Technique. The ideal technique should ensure that the swab enters the uterus and collects bacteria from the uterine lumen only. It is important to ensure that the method of swabbing does not introduce bacteria into a previously normal uterus. Two methods can be used:

- A non-guarded endometrial swab on a sterile extension rod is carefully passed via a sterile speculum through the cervix into the uterine body and, after withdrawal, is placed in transport medium. A second swab is taken immediately afterwards for the endometrial smear.
- A guarded swab is passed into the uterine lumen using a sterile speculum or enclosed in a disposable plastic arm-length glove. The swab tip is exposed only when it is in the uterine lumen. A swab for cytological examination should again be taken. To reduce the risk of contamination, the use of guarded swabs is advised.

Swabs for culture should be plated on blood and MacConkey agar, and incubated at 37°C for 48 hours. Cultures should be examined at 24 and 48 hours. An air-dried smear is made by gently rolling the second swab either on a Testsimplet (Boehringer Corporation), which is a pre-stained slide or a clean dry microscope slide. The smear can be differentially stained with a rapid stain such as Diff-Kwik (American Hospital Supplies). The stained smear should then be examined for the presence of inflammatory and endometrial cells (Figure 26.14), the latter confirming contact of the swab with the endometrium.

The veterinarian must ensure that the mare is not pregnant before passing a swab through the cervix.

Interpretation. A positive culture result, with no evidence of inflammatory cells in the smear (usually neutrophils), is likely to be due to contamination during collection. Diagnosis of acute endometritis is based on the presence or absence of significant numbers of neutrophils in the smear. Mares that have > 5 neutrophils/high power field (×40) on a cytology smear should be considered to have active endometritis.

Endometrial histology. In some cases, endometrial biopsy may be a useful diagnostic aid.

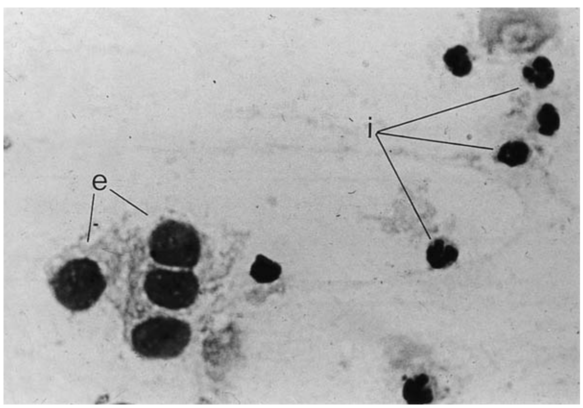


Fig. 26.14 Stained endometrial smear (Diff-K wik, American Hospital Supplies) showing inflammatory (i) and endometrial (e) cells.

For detailed reviews of the clinical application and pathological findings, readers should consult Kenney (1978) and Ricketts (1978). The technique involves the insertion of a biopsy instrument through the cervix and into the uterus. With the biopsy instrument in the uterine lumen, a gloved hand is inserted into the rectum to allow manipulation of the instrument into the desired position. The sample is taken by closing the jaws of the instrument and tugging sharply. To avoid damage, the tissue is carefully transferred into a fixative solution by dislodging it from the jaws of the punch with a fine hypodermic needle. The instrument most commonly used today is the Yeoman (basket-jawed) biopsy forceps, ideally 60–70 cm in length, with which tissue specimens 2 × 3 × 1 cm (about 0.2% of the whole endometrial surface) are obtained. If the uterus appears normal on palpation, the sample should be taken from one of the areas of embryo fixation, i.e. the uterine horn–body junction on either side. Single samples are usually representative of the entire endometrium. If the uterus is abnormal on palpation per rectum, biopsy samples should be taken from both the affected area and a normal area. Biopsy specimens should be fixed in Bouin's followed by sectioning and staining with haematoxylin and eosin. The endometrial biopsy sample should be sent to a laboratory that is experienced in evaluating samples.

Uterine luminal fluid. Since the first description of the identification of the collection of small volumes of intrauterine fluid using ultrasound, which could not be palpated per rectum (Ginther and Pierson, 1984), general awareness of the frequency of this abnormality has increased. The detection of uterine fluid during both oestrus (Figure 26.15) and dioestrus has been reported (Allen and Pycock, 1988). Endometrial secretions and the formation of the small volume of free fluid may be associated with the same mechanism that causes normal oestral oedema. In many cases, the uterine luminal fluid that accumulates before mating is sterile and contains no neutrophils (Pycock and Newcombe, 1996b). The importance of these sterile fluid accumulations is that, although initially sterile, the fluid may act as a culture medium for bacteria that gain entry to the uterus at mating to multiply and may be spermicidal (McKinnon et al 1987; Pycock and Newcombe, 1996b). The amount of fluid that should be considered significant is not clear and it may be that quantity is more important than nature. This is particularly true of fluid appearing during oestrus. The significance depends to some extent on when during oestrus the fluid is observed; fluid detected early in oestrus may have disappeared when the mare is further advanced in oestrus and the cervix relaxes more. Small volumes of intrauterine fluid during oestrus do not affect pregnancy rates, in contrast to mares with larger > 2 cm depth) collections of fluid (Pycock and Newcombe, 1996b). In mares that are susceptible to endometritis there is an accumulation of more fluid than in non-susceptible mares.

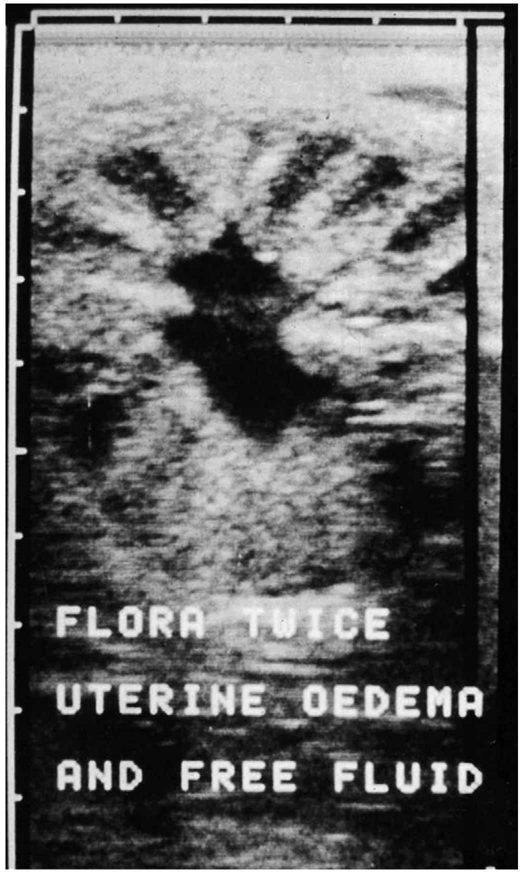
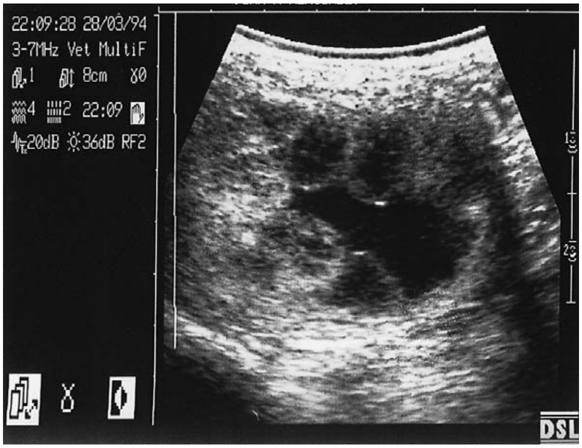


Fig. 26.15 Ultrasound image of fluid in the uterine body during oestrus. The depth is 20 mm, and the fluid is non-echogenic. The mottled appearance of the uterus suggests the mare is in oestrus.

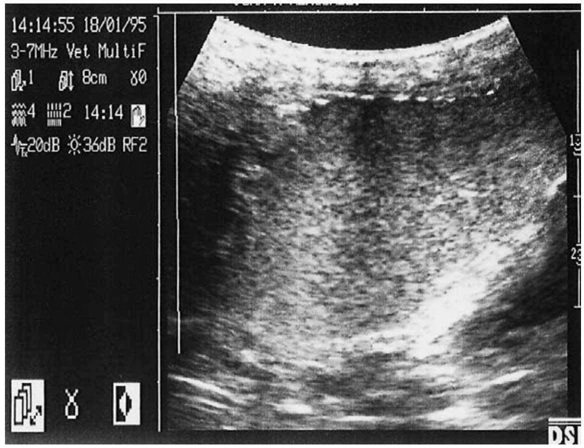
Generally if there is more than 1 cm of fluid during oestrus, some attempt should be made to remove this before breeding using oxytocin treatment. If the volume is above 2 cm, the fluid may need to be drained and investigated for the presence of inflammatory cells and bacteria. The mare may then need to have a large-volume uterine lavage. Intrauterine fluid during dioestrus is indicative of inflammation, and associated with subfertility, due to early embryonic death and a shortened luteal phase (Newcombe, 1997).

Intraluminal uterine fluid can be graded I to IV according to the degree of echogenicity (Figure 26.16). The more echoic the fluid, the more likely the fluid is contaminated with debris including white blood cells. However, fluid containing cells can appear relatively anechoic so care is needed in interpretation. Inspissated pus can be so echoic that it is overlooked. It may be that the actual appearance of the fluid and the ultrasonographic appearance are not as closely linked as once thought. Ultrasonographic appearance may be proportional to the size and concentration of particulate matter within the fluid, rather than the



(a)


(b)



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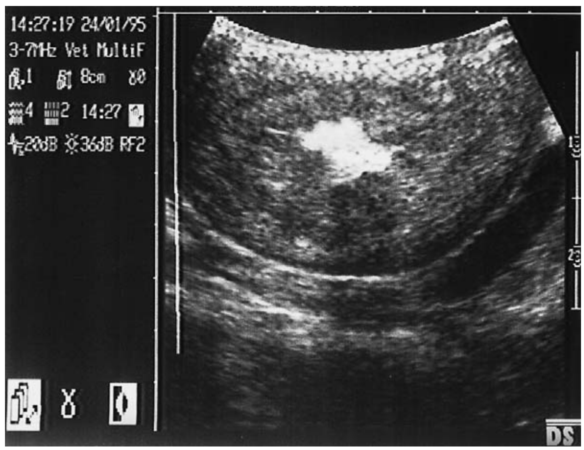

(d)

Fig. 26.16 (a) Ultrasonographic image of grade I uterine fluid: anechoic. (b) Ultrasonographic image of grade II uterine fluid: hypoechoic with hyperechoic particles. (c) Ultrasonographic image of grade III uterine fluid: moderately echoic. (d) Image of hyperechoic fluid in the uterus of an infected mare.

viscosity of the fluid; for example, purulent exudates can appear non-echogenic. Air has hyperechoic foci, and fluid with air bubbles appears cellular. Urine in the bladder can appear echoic, despite being a watery liquid (Figure 26.17).

Detection of intraluminal uterine fluid using transrectal ultrasound imaging. Transrectal ultrasonography provides a rapid, non-invasive method of assessment of the uterus. In a study involving the ultrasonic examination, cytological and bacteriological sampling of the uterus in 380 brood mares before mating (Pycock and Newcombe, 1996b) it was concluded that:

- If no free fluid is detected during oestrus, then acute endometritis as detected in cytology is absent in 99% of cases.
- Free fluid does not indicate inflammation.
- Endometrial cytology and culture fail to detect sterile fluid accumulations.

Therefore, in mares that are particularly susceptible to endometritis and in which vaginal contact should be minimised, endometritis can often be diagnosed on the basis of intrauterine fluid accumulation. This is more meaningful when the mare has already been swabbed and cleared of potential venereal diseases. If fluid is present in the uterus, there is vulvar discharge, or the mare has abnormally short luteal phases, uterine swabs should be taken to determine the cause of these symptoms.

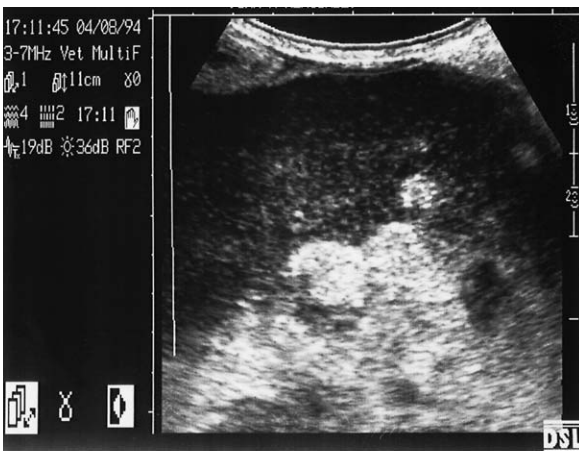

Fig. 26.17 Ultrasonographic image of a full urinary bladder.

Treatment of venereal infections and chronic infectious endometritis

Any mare that is suspected of having a venereal infection must not be bred. In the case of clitoral or vestibular infections, topical treatment is used. This involves thorough cleaning with chlorhexidine surgical scrub followed by the application of 0.2% nitrofurazone ointment for *T. equigenitalis*, 0.3% gentamicin cream for *K. pneumoniae* or silver nitrate and gentamicin cream for *P. aeruginosa*. These pathogens, particularly *P. aeruginosa*, are difficult to eliminate from the clitoris, hence clitoral sinusectomy or clitorectomy may have to be used in refractory cases. A broth culture containing a mixture of growing organisms prepared from the normal clitoral flora can suppress venereal pathogens in some cases. Evidence for the successful elimination of infection is based on three negative sets of clitoral and endometrial swabs, taken at weekly intervals.

Chronic infectious endometritis is found most frequently in older mares that have had several foals. Such mares have compromised uterine defence mechanisms that allow the normal vestibular and vaginal flora to colonise the uterus, thus inducing a persistent endometritis. The most favoured approach to treatment has been the infusion of various antibiotics, dissolved or suspended in water or saline, into the uterine lumen during oestrus. The intrauterine route is preferable to systemic therapy as most acute endometritis cases are localised. Systemic treatment alone, or in combination with local application, is suitable in a few circumstances. Ideally, the choice of antibiotic for local treatment should be based on in vitro antibiotic sensitivity tests. However, in many cases this is not possible and a broad-spectrum combination should be used that is effective against the mixed aerobic and anaerobic infections that commonly occur. A particularly successful preparation has been a buffered, water-soluble mixture of neomycin sulfate (1 g), polymyxin B (40 000 IU), furaltadone (600 mg) (Utrin Wash; Vetoquinol UK) and crystalline benzylpenicillin (5 megaunits) dissolved in 40 ml of sterile water and then instilled through the cervix into the uterus via a sterile irrigation catheter. A larger volume (up to 100 ml) may be better in older, pluriparous mares to ensure distribution throughout the uterus. The use of this extremely broad-spectrum, non-irritant, soluble preparation has not resulted in superinfection with *Pseudomonas spp.*, *Klebsiella spp.*, yeasts or fungi. The number of treatments required depends on individual circumstances, but daily infusions for 3–5 days during oestrus work well in most cases. The success of this treatment can be monitored using ultrasonography to identify the presence of intrauterine fluid. When antibiotics are combined with oxytocin (see later) a single daily treatment for 3 days has, in many cases, proved successful. Repeated endometrial swab/smear examinations may be used to monitor the response to therapy; however, every time the cervix is breached there is the risk of introducing more bacteria. An indwelling intrauterine device has been used that can retain a narrow-diameter infusion catheter within the cervix; however, there is a risk of ascending infection.

In addition to the antibiotic therapy, repeated treatment with $PGF_{2\alpha}$ increases the frequency of the follicular phases, thus allowing intrauterine therapy to be used more readily. In addition, it also reduces the duration of the luteal phase where progesterone increases the susceptibility to infection.

Predisposing causes to the persistent endometritis, such as defective vulval conformation, should also be attended to.

Fungal infections

Mycotic endometritis is not as common as that of bacteriological origin, but recognition of a fungus as the causal agent is important, since commonly used intrauterine antibiotic therapy is ineffective. In cases of fungal endometritis, mares may have a history of normal or abnormal oestrous cycles, they may be anoestrus or barren, and they may have had a recent abortion or a fetal membrane retention; there may be a history of repeated intrauterine antibiotic therapy. Yeasts more frequently cause endometritis than moulds; *Candida albicans* is the most common isolate.

The diagnosis is based upon the presence of fungal elements and inflammatory cells in endometrial smears. In addition, yeasts can also be identified following staining with Diff-Kwik using a magnification of ×400. Fungal elements are more readily identified in endometrial biopsies following staining with Gomori's methenamine silver or periodic acid–Schiff (PAS). Successful culture of endometrial smears for fungi can be difficult because the organisms may be present in low numbers, and furthermore they require a long incubation period.

These infections are very difficult to treat, particularly if they are chronic or deep-seated infections and tend to recur. Intrauterine lavage with 2–3 litres of warm saline, followed by antimycotic preparations such as tamed povidone-iodine (1–2% solution daily for 5 days), nystatin (200 000–500 000 units daily for 5 days) or clotrimazole (400–600 mg every other day for 12 days) has been used with limited success. Selection of the correct treatment should be based on sensitivity results. Uterine irrigation with vinegar or dilute acetic acid has reported anecdotal success, presumably by altering the uterine pH.

The prognosis for the subsequent fertility of mares with mycotic endometritis is poor. If there is no success in eliminating the yeast or fungal infection after three attempts, the owner must be advised of the unlikely chance of success. It is suggested that a normal healthy uterus can eliminate mycotic infection; this means that even if the mycotic infection is successfully treated the mare must be treated as a susceptible mare.

Endometrosis

At the first international symposium on equine endometritis, Kenney (1993) suggested that the term endometritis should not be applied to the degenerative changes within the endometrium often associated with age and parity. The old term 'chronic degenerative endometritis' should be replaced by 'endometrosis'. Endometrosis can, therefore, be defined as the collective term to describe the wide range of degenerative changes (fibrosis and glandular degenerative changes). The condition is diagnosed by endometrial biopsy.

Successful treatment of endometrosis is difficult. Improved fertility after endometrial curettage has been reported. This has involved the use of mechanical and chemical agents (namely povidone-iodine and kerosene) that cause endometrial necrosis. This treatment, apart from being of questionable efficacy, can cause irreversible damage such as adhesions. Repeated daily lavage with 2–3 litres of hot (50°C), sterile, isotonic saline has been suggested as a method of reducing the size of the lymphatics and thereby the whole uterus. The prognosis for fertility remains poor whatever treatment is used.

Persistent mating-induced endometritis

Uterine defence mechanisms. At coitus, the mare's uterine lumen becomes contaminated with microorganisms and debris. Even if mares are bred by artificial insemination, semen is deposited directly into the uterus. In addition, it has recently been shown that spermatozoa without bacterial contamination induce a uterine inflammatory response (Kotilainen et al., 1994; Troedsson, 1995). The former authors showed that the intensity of the reaction was dependent on the concentration and/or volume of the inseminate; concentrated semen, e.g. frozen semen, induced a stronger inflammatory reaction in the uterus than fresh or extended semen. That the intensity of the inflammatory response following insemination depends on the sperm themselves rather than any extender was the conclusion of Parlevliet and her co-workers (1997), who measured the inflammatory response following insemination with raw semen, extended semen and various extenders. The inflammatory response of the uterus is not different for live or dead spermatozoa (Katila, 1997). In most mares, this transient endometritis resolves spontaneously within 24–72 hours so that the environment of the uterine lumen is compatible with embryonic and fetal life. It is important not to regard this endometritis as a pathological condition. Rather it is a physiological reaction to large numbers of sperm, seminal plasma and inflammatory debris from the uterus before the embryo descends from the uterine tube into the uterine lumen 5.5 days after fertilisation. However, if the endometritis persists after day 4 or 5 of dioestrus, in addition to being incompatible with embryonic survival, the premature release of $PGF_{2\alpha}$ results in luteolysis, a rapid decline of progesterone and an early return to oestrus. These mares are referred to as susceptible and they develop a persistent endometritis (Allen and Pycock, 1988).

The concept of susceptibility to endometritis was first suggested by Farrely and Mullaney (1964), who stated that infective endometritis is essentially the failure of an individual mare to limit the uterine and cervical microflora to a non-resident type. Hughes and Loy (1969) developed this concept, and confirmed that resistant mares could eliminate induced infection without treatment, while susceptible mares could not. In general, reduced resistance to endometritis is associated with advancing age and multiparity. Susceptibility to endometritis is not an absolute state, since failure of uterine defence mechanisms need only slow the process of eliminating infection. There is a wide range of susceptibility to endometritis, and mares cannot be neatly categorised into 'resistant' or 'susceptible' (Pycock et al., 1997). Studies on immunoglobulins, opsonins and the functional ability of neutrophils in the uterus of susceptible mares have not confirmed the presence of an impaired immune response (see the review by Allen and Pycock, 1989). Evans et al. (1986) first suggested that reduced physical drainage may contribute to an increased susceptibility to uterine infection. The physical ability of the uterus to eliminate bacteria, inflammatory debris and fluid is now known to be the critical factor in uterine defence. It is a logical conclusion that any impairment of this function, i.e. defective myometrial contractility, renders a mare susceptible to persistent endometritis (Troedsson and Liu, 1991; Troedsson et al., 1993; LeBlanc et al., 1994). The reason susceptible mares have this defective contractility is not known. Recently it has been suggested that the regulation of muscle contraction by the nervous system may be impaired (Liu et al., 1997). The resulting fluid accumulation could be due to failure to drain via the cervix, or decreased re-absorption by lymphatic vessels. Lymphatic drainage could play an important role in the persistence of post-breeding inflammation, and it is interesting that lymphatic lacunae (lymph stasis) is a common finding in endometrial biopsies taken from susceptible mares (Kenney, 1978; LeBlanc et al., 1995).

Detection of the susceptible mare. Detection of the susceptible mare can be difficult, as there may only be subtle changes in the uterine environment, not readily detected by current diagnostic procedures. Many mares show no signs of inflammation before mating, but the inevitable endometritis that follows mating persists.

Whilst the response to bacterial challenge has been used in research studies, history is perhaps the most useful indicator of a susceptible mare in practice. Demonstration of clearance failure using scintigraphic and other methods based on charcoal clearance has been used to make an accurate diagnosis (LeBlanc et al., 1994), but it is difficult to apply in practice. Transrectal ultrasonography to detect uterine luminal fluid has also proved useful in identifying mares with a clearance problem, and would appear the most useful technique in practice. The presence of free intraluminal fluid prior to breeding strongly suggests susceptibility to persistent endometritis (Pycock and Newcombe, 1996b). It has been suggested that excessive production of fluid, due to glandular alterations, may cause intrauterine fluid accumulation rather than a failure of lymphatic drainage (Rasch et al., 1996). However, it is currently not known for certain whether the fluid accumulates due to an excess production, a delay in physical clearance via the open cervix, or decreased reabsorption by lymphatic vessels. It may well involve a combination of all three.

Treatment options for the susceptible mare. The aim of treatment should be to assist the uterus to expel the normal inflammatory products arising from the response to breeding. Since within 4 hours of mating the spermatozoa necessary for fertilisation are present within the uterine tube, and since the embryo does not descend into the uterus for about 5.5 days, mares may be treated safely from 4 hours after mating until 3 days from ovulation, providing non-irritant therapy is used. However, progesterone concentrations rise rapidly following ovulation in the mare, and it is preferable to avoid treatment involving uterine interference beyond 2 days after ovulation. Both natural mating and artificial insemination can be a source of uterine contamination.

The successful management of susceptible mares should logically require some form of post-mating therapy such as intrauterine antibiotic infusion, uterine lavage and intravenous oxytocin; these may be used alone or in combination. The emphasis should be on treatment in relation to breeding and not ovulation. Too often in the past, veterinarians have waited until ovulation before treating these mares. By then, there has usually been a large accumulation of fluid, and the bacteria are in a logarithmic phase of growth.

Uterine lavage. Recognition of the importance of the mechanical evacuation of uterine contents accounted for the introduction of large-volume uterine lavage. The technique involves the mechanical suction or siphonage of 2–3 litres of previously warmed (to 42°C), sterile physiological (buffered) saline or lactated Ringer's solution infused into the uterus via a catheter that has been retained within the cervix via a cuff. The most convenient is a large-bore (30 French) (80 cm) autoclavable equine embryo flushing catheter (EUF-80; Bivona, IN) (Figure 26.18). The cuff is useful as it effectively seals the internal cervical os. The catheter should only be inserted after thorough cleansing of the perineum. The rationale for such an approach is:

- the removal of accumulated uterine fluid and inflammatory debris that may interfere with neutrophil function and the efficacy of antibiotics
- stimulation of uterine contractility
- recruitment of fresh neutrophils through mechanical irritation of the endometrium.

The saline is infused by gravity flow 1 litre at a time, and the washings are inspected to provide immediate information concerning the nature of the uterine contents. The lavage should be repeated until the fluid that is recovered is clear. In most cases, the fluid is evenly distributed in both horns, making transrectal massage of the uterus unnecessary. If a rectal examination is performed whilst the catheter is in the uterus care must be taken to avoid contaminating the catheter. The fluid should be recovered in the same container from which it was infused, thereby preventing air being aspirated into the uterus via the catheter. Measurement of the recovered fluid and ultrasonographic examination of the uterus should be performed after flushing to ensure that all the fluid has been recovered. This is necessary because you are dealing with a mare with an impaired ability to drain the uterus spontaneously.

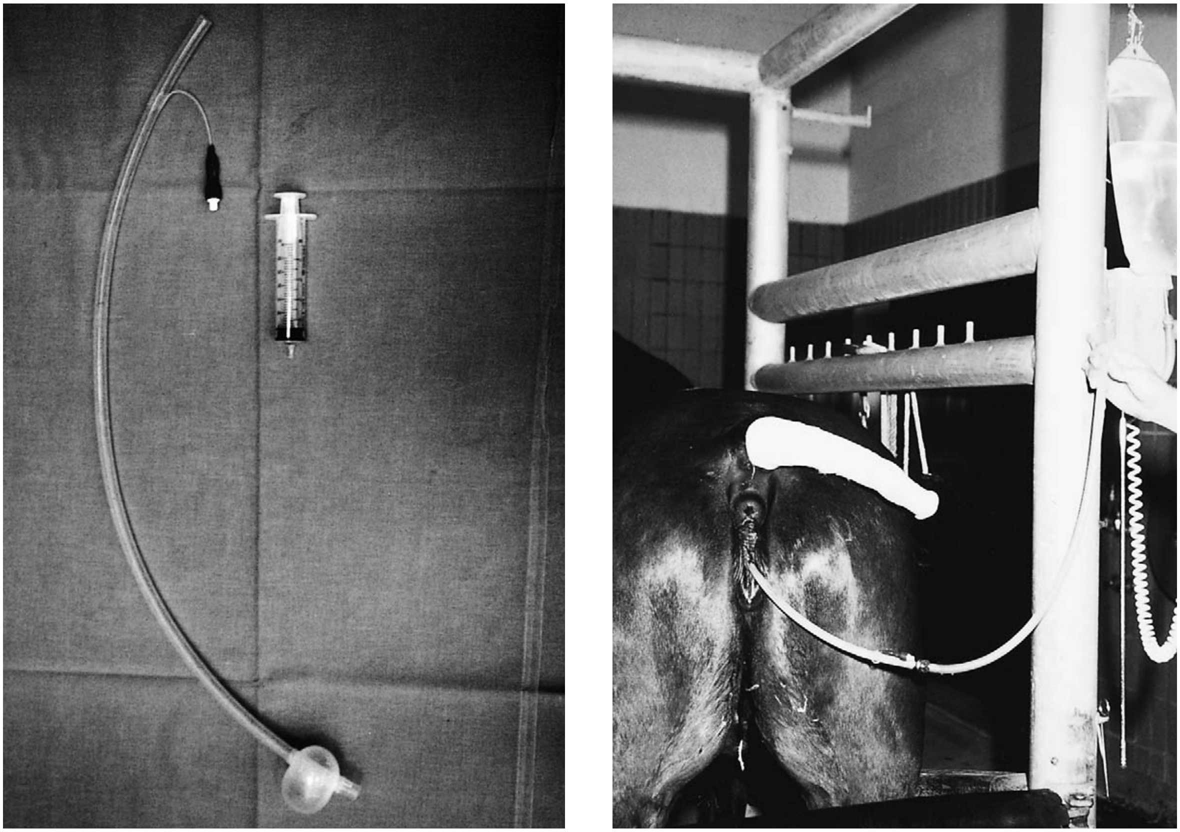
(a) (b)

Fig. 26.18 (a) Large-bore (30 French) embryo flushing catheter, 80 cm in length. Note inflated cuff. (b) Performing large-volume uterine lavage with large-bore catheter in position.

For this reason the process is usually combined with oxytocin injection. Ideally these mares will be bred only once, but if repeated matings are necessary, uterine lavage should be performed after each mating.

Large-volume lavage is beneficial in many cases, particularly the mare with a relatively large (above 2 cm depth) accumulation of fluid after breeding. The process is time-consuming and there is the possibility of further contamination of the uterus by passage of a drainage tube. None the less, where there is more than 2 cm of uterine fluid, or a mare is known to be highly susceptible, the risks are outweighed by the benefit of treatment (Knutti et al., 1997).

It has been shown that saline lavage and uterotonic drugs such as $PGF_{2\alpha}$ are as effective as antibiotics in eliminating bacteria from the uterus (Troedsson et al., 1995). However, this was an experimental study in which a single bacterial species was infused into the uterus, and lavage was within 12 hours of mating. Clinically, there is a mixed bacterial flora, and lavage cannot always be perfomed within 12 hours. This is why the author prefers to continue to use intrauterine antibiotics as part of the treatment protocol.

Oxytocin. The ideal method of treatment will be the use of a non-invasive technique with early and complete elimination of any intrauterine fluid. Oxytocin stimulates uterine contractions in the cyclical, pregnant and postpartum mare and was first suggested as a method to promote uterine drainage in mares with defective uterine clearance by Allen (1991). Until then, oxytocin was not considered to be an appropriate treatment for endometritis, probably because it was assumed that oxytocin induced uterine contractions only in the first 48 hours after foaling. Alternatively use of oxytocin was discouraged because of the worry that it would cause severe colic. However, after the pioneering study of Allen (1991), subsequent clinical experience (Pycock, 1994a; LeBlanc, 1994; Pycock and Newcombe, 1996; Rasch et al., 1996) has allayed early fears that oxytocin would cause severe colic when given as an intravenous bolus. All these workers have reported improved pregnancy rates in susceptible mares after oxytocin administration.

Prostaglandin analogues. Prostaglandins are known to be released very early in mares with endometritis (Pycock and Allen, 1990). The useful role of prostaglandin in increasing myometrial activity and assisting uterine clearance has subsequently been shown (Cadario et al., 1995; Troedsson et al., 1995; Combs et al., 1996). These latter authors showed that the prostaglandin analogue cloprostenol given at a dose rate of 500 μg i.m. caused increased clearance of radiocolloid in susceptible mares, but it was significantly slower than that caused by oxytocin. However, the uterus did contract for a longer time: 5 hours versus 45 minutes. Of the prostaglandins administered ($PGF_{2\alpha}$, cloprostenol and fenprostalene), cloprostenol produced the most consistent response. Cloprostenol would seem to be indicated in mares that have lymphatic stasis as shown by excessive fluid within the endometrium or large lymphatic cysts (LeBlanc, 1997). Cloprostenol should not be given more than 24 hours after ovulation in case of inducing premature luteal regression.

Intrauterine plasma infusions. Based on the research findings of the 1970s and 1980s, which emphasised the immunological aspects of the uterine defence mechanisms, intrauterine plasma has been used in the susceptible mare. Studies following its use have indicated an improvement of fertility (Asbury, 1984; Pascoe, 1995). Both authors suggested that the plasma had an enhancing effect on phagocytosis by uterine neutrophils. Adams and Ginther (1989), in a study that included control groups of mares, found that intrauterine plasma was not efficacious in treating endometritis since there was no improvement in pregnancy rates. In addition, transfer of infectious agents is also possible. Troedsson et al. (1992) suggested that plasma treatment might only benefit certain susceptible mares. This latter point was also alluded to recently by Pascoe (1995) who, whilst remaining enthusiastic about the use of plasma in the management of immune-incompetent mares, conceded that this may only apply to mares without a mechanical clearance problem. Consequently plasma is best used in mares that repeatedly fail to become pregnant, but have no history of fluid accumulation. Since mares that are susceptible to endometritis do not possess a quantitative deficiency of immunoglobulins, it is questionable if such treatment is truly effective.

The old maiden mare syndrome. It is particularly important to recognise and manage appropriately the older maiden mare as in many cases these mares are susceptible to post-breeding endometritis even though they have never been bred before. Often sport or Warmblood mares are not considered for breeding until in their teens and these older maiden mares can be very difficult to get in foal. Many of these mares have some common characteristics that resemble a syndrome. Endometrial biopsy samples reveal glandular degenerative changes and stromal fibrosis (endometrosis) as an inevitable consequence of ageing despite the fact that they have not been bred (Ricketts and Alonso, 1991). Another of the most common characteristics of these mares is uterine fluid accumulation. Often, an older maiden mare has an abnormally tight cervix that fails to relax properly during oestrus so that fluid is unable to drain and accumulates in the uterine lumen (Pycock, 1993). In many cases this fluid is negative for bacterial growth and presence of neutrophils. Once the mare is bred, the fluid accumulation will be aggravated due to poor lymphatic drainage and impaired myometrial contraction compounded by the tight cervix. The amount of intrauterine fluid will vary in individual mares ranging from a few millilitres to over a litre in extreme cases. All too often owners assume that the fertility of these mares is comparable to that of young maiden mares; one of the most important aspects of breeding the old maiden mare is to make the owner aware that there is a high possibility that she will be a problem. These mares must be considered highly susceptible and managed accordingly.

Management protocol useful in the highly susceptible mare. A mare that from past experience/history is known to produce a large amount (several centimetres depth) of luminal fluid after mating should, in the author's experience, be managed using the following protocol. Overall management of such mares must be excellent prior to breeding. Good hygiene at foaling is essential and all mares should be thoroughly examined postpartum for the presence of trauma that might compromise the physical barriers to uterine contamination. Gynaecological examinations, particularly of the vagina, should be performed as aseptically as possible. Thorough digital examination of the cervix can identify fibrosis, lacerations or adhesions that may need treatment before breeding. Since air in the vagina can cause irritation of the mucosa it should be expelled by applying downward pressure with the hand through the rectal wall. Attention should be paid to hygiene at mating by using a tail bandage and washing the mare's vulva and perineal area with clean water (ideally from a spray nozzle which avoids the need for buckets). Breeding should occur at the optimal time, and the number of breedings should be minimised. This means that these mares need very close monitoring of the oestrous period by rectal palpation and ultrasonography. The use of hCG is strongly recommended in such mares in an attempt to ensure they are bred only once. Prediction of ovulation can also be made easier by not breeding these mares too early in the year, i.e. before they have begun to cycle regularly. If feasible, the use of artificial insemination can be helpful to reduce (but not eliminate) the inevitable post-breeding endometritis.

Management involves the following points:

- A single breeding must be arranged 1–2 (or even 3) days before the anticipated time of ovulation.
- Ultrasound examination of the uterus 3–12 hours after mating is performed to assess the amount and echogenicity of any intrauterine fluid.
- After 20 minutes the mare should be re-examined and any fluid pooling in the vagina removed. This is followed by infusion of a low volume (30 ml) of water-soluble, broad-spectrum antibiotics such as already described (Utrin Wash, Vetoquinol UK Ltd, Bicester) into the uterus via a sterile irrigation catheter.
- 2 × 25 IU of oxytocin should be given by the stud farm personnel that evening and again in the morning, by the intramuscular route. In mares with lymphatic stasis, the slower release of prostaglandin (cloprostenol 500 μg i.m.) may be useful in addition to oxytocin. The cloprostenol should be given some 6–8 hours after the first oxytocin injection.
- The mare is re-examined the following day and oxytocin treatment repeated if fluid is still present. Only rarely will a second infusion of antibiotics or lavage procedure be performed due to the risk of uterine contamination. Evaluation of the uterus post-breeding is a crucial time to assess all mares, and too many clinicians fail to do this.

Viral infectious disease – equine coital exanthema

In addition to EHV-1, EHV-4 and equine viral arteritis infection, which cause abortion (see above), EHV-3 causes a relatively benign venereal disease referred to as coital exanthema; it affects both sexes. There have been reports of its transfer during gynaecological examination. The virus can remain dormant until conditions favour its proliferation with the development of the characteristic clinical signs. Normally, following coitus, they develop after an incubation period of 4–7 days. Multiple vesicles appear on the vulval mucosa and perineum, resulting in a short period of local irritation. These rupture, leaving small ulcers 3–10 mm in diameter that are painful to touch. In the absence of infection with opportunist pathogens, healing occurs in 10–14 days, when it ceases to be contagious. There is permanent loss of pigmentation at the site of the healed lesions. Pregnancy rates are not reduced. In the stallion, the vesicles develop on the shaft of the penis and the prepuce; if severe, he may be reluctant to breed. Treatment consists of immediate sexual rest and the application of an antiseptic powder or spray to prevent secondary bacterial infection; this allows the ulcers to heal. The disease is controlled by withholding breeding of all affected stallions and mares and taking hygienic precautions when handling these animals.

Protozoal infections

Dourine

Trypanosoma equiperdum causes a venereal disease called dourine, which is currently prevalent in Africa, the Middle East and Central and South America; it has been eradicated from Europe and North America. The incubation period is 1–4 weeks and the disease has an extremely protracted course that can extend over a period of weeks or months. It affects horses, mules and donkeys of either sex. The initial sign is a non-painful swelling of the external genitalia of both stallions and mares; mares show a vaginal discharge and stallions have a paraphimosis. Some weeks later, depigmented areas and urticaria-like raised plaques 2–10 cm in diameter appear over the body surface. The disease is characterised by a low morbidity, but a high mortality of 50–75%.

Diagnosis of dourine is made from the clinical signs, particularly the skin plaques, together with demonstration of the trypanosome in the discharges and in the skin lesions. A complement fixation test is also available. Treatment using quinapyramine sulfate has been attempted, but stallions that recover may become carriers. Therefore, strict screening using a complement fixation test, with slaughter of positive and affected animals, as well as the institution of quarantine programmes, should be used to control this disease.

PUERPERAL METRITIS

Metritis is inflammation of the entire thickness of the uterine wall. It occurs when there is massive contamination of the uterus, frequently in association with trauma during foaling or RFM. It has a grave prognosis, particularly in heavy horses, since the absorption of toxins from the uterine lumen into the general circulation results in systemic signs including pyrexia, depression, loss of appetite and laminitis. Toxin production is associated with rapid bacterial growth, frequently involving Gram-negative organisms. Treatment involves repeated lavage of the uterus with warm sterile saline (2–3 litres) several times per day until it is free of inflammatory exudates and placental debris. Bacterial growth should be controlled, so as to limit toxin production, with a broad-spectrum antibiotic effective against *E. coli*, which is invariably present. Supportive therapy with parenteral antibiotics, antihistamines (in cases of retained fetal membranes), oxytocin and intravenous fluid therapy is indicated in many cases.

Systemic signs such as pulse rate and mucous membrane colour are used to monitor the response to therapy in conjunction with examination of the uterine fluid.

Despite all efforts, some mares die due to toxaemia or irreversible changes in the foot following laminitis such as pedal-bone rotation.

PYOMETRA

Pyometra is the accumulation of large quantities of inflammatory exudate in the uterus causing its distention (Hughes et al., 1979). It must be distinguished from the smaller, and intermittent, accumulations of fluid that can be detected by ultrasonography in acute endometritis. Pyometra occurs because of interference with natural drainage of fluid from the uterus, which may be due to cervical adhesions or an abnormally constricted, tortuous or irregular cervix. In some cases, the fluid accumulates in the absence of cervical lesions presumably due to an impaired ability to eliminate the exudate. Other predisposing factors are chronic infection with *P. aeruginosa* or fungi.

When the endometrium is severely damaged, there is extensive loss of surface epithelium, severe endometrial fibrosis and glandular atrophy causing a prolonged luteal phase, presumably due to interference with the synthesis or release of $PGF_{2\alpha}$. This is in contrast to mild endometritis with the collection of small amounts of intraluminal uterine fluid, which is more likely to cause premature release of $PGF_{2\alpha}$ and luteolysis.

Some clinicians restrict the term 'pyometra' to cases where, in addition to the accumulation of exudate within the uterine lumen, the corpus luteum persists beyond its normal life span. Some mares with pyometra have normal, regular cyclical ovarian activity. Persistence of the corpus luteum is probably due to failure of the synthesis and/or release of prostaglandins from the uterus. Mares that have prolonged luteal activity have the greatest endometrial damage. The mare with pyometra seldom shows overt signs of systemic disease even when there is up to 60 litres of exudate in the uterine lumen. Very occasionally there is weight loss, depression and anorexia. Pyometra has been classified into two categories in mares: open and closed (Hughes et al., 1979). In a case of closed pyometra, the fluid accumulates due to a closed cervix. In open pyometra, the cervix remains open, but purulent material accumulates because of impaired uterine clearance. A vulval discharge is often observed in open pyometra, especially at oestrus, which may vary in consistency from watery to cream-like. Although the culture of endometrial swabs can sometimes result in the growth of mixed organisms or sometimes no bacterial growth at all, in most cases the organism isolated is *S. zooepidemicus*.

Diagnosis. The diagnosis of pyometra is based upon rectal palpation, ultrasonic examination of an enlarged fluid-filled uterus (Figure 26.19) and analysis of the uterine fluid. Pregnancy must be eliminated together with rare conditions such as mucometra and pneumouterus.

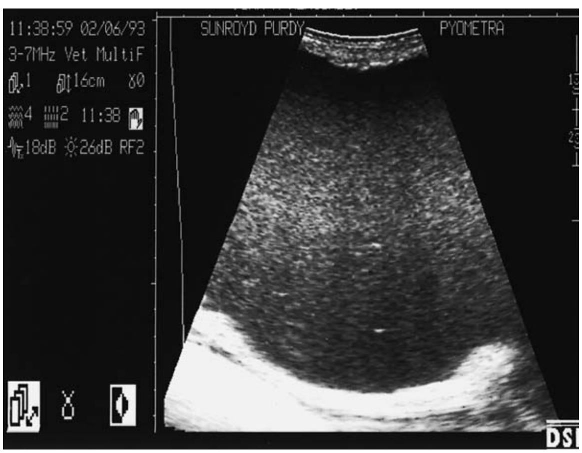

Fig. 26.19 Ultrasonographic image of pyometra.

Due to the lack of systemic illness, cases of pyometra have often become chronic before treatment is sought. In such cases the prognosis is poor because of severe endometrial damage, which is unlikely to be able to sustain a normal pregnancy.

Treatment. The aim of treating pyometra is to expel the purulent material from the uterus. In the absence of systemic illness or an unsightly vulval discharge, treatment of chronic pyometra may not be indicated, although some mares can show signs of discomfort during exercise. Many cases can be significantly improved by repeated large-volume lavage with several litres of warm saline via a wide-bore tube such as a nasogastric tube. Initially, $PGF_{2\alpha}$ can be used to induce luteolysis of the corpus luteum if present, which should allow the cervix to relax sufficiently for digital exploration for the presence of any adhesions. Oestradiol or PGF_2 may also help relax the cervix. The broad-spectrum combination of antibiotics (Utrin Wash; Vetoquinol UK Ltd) and crystalline benzylpenicillin (5 megaunits) should be infused after repeated large-volume lavage and oxytocin to achieve drainage of exudate, and an endometrial biopsy is useful in assessing the degree of endometrial damage. Monitoring the uterus by a combination of rectal palpation and ultrasound provides information on the response to treatment. Even if successfully treated, the mare must be considered a susceptible mare if she is to be bred and managed accordingly.

In non-responsive cases hysterectomy can be performed following aspiration of the exudate from the uterus, although great care has to be taken to prevent contamination of the peritoneal cavity.

RETAINED FETAL MEMBRANES (RFM)

Retention of the fetal membranes (RFM) is properly regarded by veterinary surgeons as a potentially more serious affection than the same condition in cattle. This has originated from the times when draught horses predominated in the horse population and was invariably followed by serious sequelae; as a result early manual removal was the rule. Complications include acute metritis, septicaemia, laminitis and even death. With prompt and effective treatment these sequelae can be avoided. In many cases, uterine involution is delayed even if these more serious complications do not develop. The riding horses and ponies of today are less likely to suffer from these complications, but RFM should be treated as an emergency.

The average time taken for the fetal membranes to be expelled is about 1 hour, and should not exceed 2 hours, although there is debate amongst equine clinicians about the latter. RFM is one of the most common peripartum problems in the mare, with an incidence in the range of 2% to 10% (Vandeplassche et al., 1971).

Incidence and aetiology. The incidence is much higher after dystocia, which is probably due to either uterine trauma or uterine inertia. In equine dystocias treated at the Ghent Veterinary School, there was an incidence of 28% after fetotomy, and 50% after caesarean operations; in the latter, the likelihood of retention was doubled if the foal was alive at the beginning of the operation compared with if it was dead (Vandeplassche et al., 1972). These authors emphasise the branching nature of the numerous chorionic microvilli that interdigitate strongly with the corresponding labyrinth of endometrial crypts. The microvilli are better developed in the uterine horns than in the body, and are considerably more branched, as well as bigger, in the non-pregnant than in the pregnant horn. This latter property of the villi, coupled with the more marked folding of the allantochorion and endometrium as well as the slower involution of the non-pregnant horn, all combine to provide an explanation of the higher incidence of retention in the non-pregnant horn. Placental pieces from other areas can be retained and it is important to examine the fetal membranes thoroughly to determine which portion has been retained.

The precise cause of retained placenta remains unclear. The most likely is uterine inertia due to hormonal imbalance. Oxytocin has an important role in postpartum uterine contractions, and low levels of this hormone in the circulation may result in abnormal myometrial activity. This in turn leads to placental retention.

Clinical signs. The most obvious sign of RFM is the presence of a variable portion of tissue protruding from the vulva; less commonly nothing is visible. Either this means that no parts of the fetal membranes have been expelled or, more likely, portions remain attached.

Treatment. Initially, the protruding membranes should be tied in a knot to prevent them touching the hocks. As uterine contractility plays an important role in the dehiscence of the fetal membranes, administration of oxytocin is recommended as a first and most successful method of treatment in up to 90% of cases. It is a good rule not to wait longer than 6 hours after delivery of the foal; the time interval should be shorter in heavy breeds. This method of treatment avoids manipulation within the uterus, with the risk of introducing microorganisms. Oxytocin can be given via the intramuscular route (20–40 IU), which can be repeated after 1 hour if the membranes have not been expelled. Alternatively, use slow intravenous infusion of 50 IU oxytocin in 1 litre of physiologic saline over 1 hour. Symptoms of colic often follow injections of oxytocin and commonly precede natural expulsion so that pain-relieving drugs and sedation may be required.

Only if this treatment fails and the membranes are almost detached but retained within the uterus should one attempt gentle manual removal. This interference should be carried out with scrupulous regard to asepsis, and no undue force should be applied, for even moderate traction on the afterbirth may cause the uterus to become inverted and prolapsed (see prolapse of the equine uterus in Chapter 19). In most cases of retention some separation of the allantochorion has occurred and consequently a variable amount of the afterbirth hangs down from the vulva. The mare is effectively restrained and measures should be taken to protect the operator from being kicked. The tail is bandaged and held to one side by the attendant while the obstetrician thoroughly washes the perineum and rear of the mare. With the hand and arm protected by a clean plastic sleeve, the extruded mass, or failing that the freed part lying within the vagina, is grasped and twisted into a rope. The gloved hand anointed with lubricant is gently introduced along the ‘rope’ to the area of circumferential attachment in the uterus. As the ‘rope’ is gently pulled and twisted, the tips of the fingers are pressed between the endometrium and the chorion. The villi are easily detached, and as the allantochorion is gradually freed it is taken up by further twisting of the detached mass. The allantochorionic membrane is gently separated from the endometrium by moving one of the hands between them. The tightest attachment is usually at the tip of the horn. The process of separation usually goes quite smoothly, and the complete sac of allantochorion can be gradually detached from the pregnant horn. There is a tendency for attachment to be firmer in the non-pregnant horn, and occasionally retention is confined to this horn. If it is found impossible to detach the apical portions of the allantochorionic sac without tearing the membranes it is better to desist and to try again in 4–6 hours, by which time a successful outcome will be likely. Unwanted side-effects of this manual removal may be serious haemorrhage, invagination of one of the horns and a higher chance of retention of microvilli in the endometrium. Vandeplassche and his colleagues (1971 and 1972) refer particularly to the residue of microvilli that is present in the endometrium even after a normal expulsion of the afterbirth and is vastly increased when manual removal is effected in a case of retention. During a difficult manual removal only the central branches of the chorionic villi are removed while practically all the microvilli are broken off and retained; rupture of endometrial and subendometrial capillaries may also occur. The consequences of difficult removal are increased puerperal exudate, containing much tissue debris; endometritis and laminitis; uterine spasm and delayed involution of the uterus. It is for these reasons that Vandeplassche and his colleagues (1971 and 1972) prefer to treat severe equine retention by means of intravenous drip administration of oxytocin rather than by persistence with manual removal.

A third method described in the literature, and which may be successful under some circumstances, is the placement of some 10 litres of warm, sterile saline inside the chorioallantoic membrane. Stretching of the uterine wall stimulates uterine contractions, via endogenous oxytocin release, and may assist in the separation of the microvilli from their endometrial crypts. This treatment should be used in combination with exogenous oxytocin administration. After removal, it is always important to examine the membranes for completeness confirming that all the allantochorion has been removed. If necessary, the uterus should be flushed and siphoned to remove any fluid exudate remaining in the uterus by using a stomach tube and funnel. Aftercare includes (depending on the severity of the case) regular general clinical examination, particularly the uterus (for involution and contents) and, if indicated, flushing and siphoning the uterus once or twice daily for a few days in combination with further injections of oxytocin. The rationale for uterine lavage is to remove both debris and bacteria from the uterus. Warm, sterile physiologic saline should be used in 2–4 litre flushes (until the recovered fluid is clear). Vandeplassche and colleagues (1972) deprecate the use of any antiseptic solution to rinse the uterus after the expulsion of the afterbirth, because this depresses phagocytosis. Special attention is paid for signs of laminitis, and non-steroidal anti-inflammatory drugs are given when laminitis is a suspected complication. Tetanus antitoxin is recommended and, if indicated, treatment with antibiotics. If there is a risk of the mare developing a toxic metritis, she should be treated with systemic and intrauterine antibiotics. The dominant infective organism is often *Streptococcus zooepidemicus* initially, but infection with Gram-negative bacteria such as *Escherischia coli* frequently develops. The antibiotics chosen should have broad-spectrum activity and should be effective against endotoxin-producing organisms. Cyclo-oxygenase inhibitors such as flunixin meglumine should be given to either treat or minimise the risk of development of endotoxaemia.

Provided treatment is begun at the correct time and no secondary complications develop, the prognosis for a case of retained placenta is good.

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27 Infertility in the sow and gilt

Pig producers expect high levels of fertility, and any shortfalls represent a serious economic loss (Glossop and Foulkes, 1986). The efficiency of a pig operation may be described in terms that take into account a financial component, e.g. the number of pigs sold per sow place per year, or the number of kilograms of pig meat sold per square metre of pig unit (Douglas and Mackinnon, 1992). Consideration of reproductive efficiency, however, requires an evaluation of fertility level, which may be expressed in various ways (definitions taken from PIC, 1990–1):

1. *Farrowing rate* – the number of sows that farrow to a given number of services, normally expressed as a percentage.
2. *Farrowing index* – the number of farrowings per sow per year.
3. *Conception rate* (or non-return rate) – the number of sows that conceive to service expressed as a percentage of those served. The conception rate is usually estimated as the non-return rate to oestrus (28 days after service) or is identified by pregnancy diagnosis at 30 days or more, after service. This term does not necessarily equate to the farrowing rate, as pregnancy can end at any time, but it can provide an earlier warning of a problem.
4. *Non-productive or empty days* – the number of days in which a sow is not pregnant. There are, of course, days during which it is not possible for a sow to be pregnant (e.g. in lactation, and during the weaning to oestrus interval), which should be taken into account.
5. *Piglets born per sow per year* – this figure divides into two components: total numbers born, and numbers born live.

All fertility parameters interrelate, and Figure 27.1 illustrates the relationship between them (Douglas and Mackinnon, 1992). Each producer must establish targets for reproductive performance. In order to do this in a realistic way, it is first necessary to consider the physiological potential of the sow. The reproductive cycle of the sow comprises:

Gestation	= 115 days
Lactation	= 21–8 days
Interval from weaning to oestrus	= 5 days
Total no. days per cycle	= 141–8 days.

From these calculations the maximum potential farrowing index is 2.5–2.6 (Glossop, 1992), although this assumes a farrowing rate of 100%, which is unrealistic. The calculation does, however, highlight factors that will influence the farrowing index; these include gestation length (which is, of course, fixed), lactation length and weaning to oestrus interval along with conception rate and farrowing rate.

Analysis of reproductive data from any of the bureau recording schemes demonstrates the shortfall between the physiological potential and the reality. Table 27.1 details fertility data for herds recording in the UK (PIC, 1998). It is worth comparing overall herd performance with that achieved by the top-performing herds. Clearly, some herds are getting close to the potential farrowing index of 2.5–2.6. The aim is to raise *overall* herd performance in line with this.

Any discrepancy between the targets and the reality represents an economic loss resulting from suboptimal fertility. Targets set for a particular unit must take into account all management factors that influence fertility. Realistic performance targets for most herds are set out in Table 27.2. The reproductive performance of a herd relies upon the exercise of tight control over such factors. The purpose of this chapter is to examine

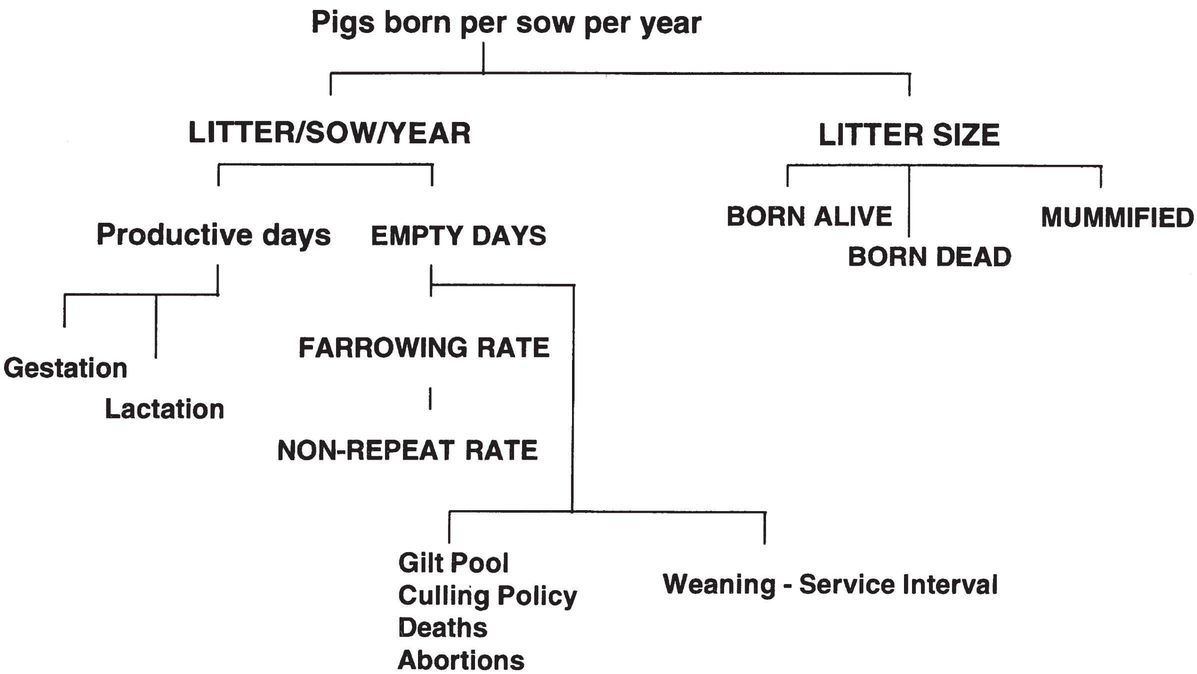


Fig. 27.1 Flow chart showing reproductive factors influencing fertility (Douglas and Mackinnon, 1992).

Table 27.1 UK herd performance (averages weighted by sow herd size) (PIC, 1998)

	All farms	*Bottom 33%*	*Top 33%*	*Top 10%*
No. farms	110	36	37	12
No. sows	23 611	6192	8067	2370
Farrowing rate	80%	74%	85%	88%
Farrowing index	2.29	2.18	2.39	2.43
Live births per litter	10.74	10.43	11.04	11.22
Live births per sow per year	24.64	22.74	26.34	27.27

Table 27.2 Targets for reproductive efficiency (Douglas and Mackinnon, 1992)

Litters/sow/year	>2.3
Farrowing rate	>85%
Non-repeat rate	>90%
Litter size (total)	>11.5
Born live	>11.0
Born dead	<0.5
Mummified	<0.5

these factors and to gain an understanding of how they may be optimised.

FACTORS THAT AFFECT FERTILITY

In Wrathall's (1977) classification of factors that affect fertility the importance of management and stockmanship is emphasised; it is vital to recognise this when attempting to solve a fertility problem. Any investigation of herd infertility should take into account management factors before making a detailed study of other issues. The quality of stockmanship will be reflected in such basic procedures as oestrus detection, supervision of service, general hygiene and record-keeping. As units grow larger and as management systems change in response to consumer demands, stockmanship characteristics and requirements may have to be adjusted, but they remain, none the less, of paramount importance. English (1991) discusses this subject in great detail, pointing out that good stockmanship involves a combination of sound basic knowledge, patience, empathy, sensitivity, organisational skills and an appreciation of priorities. Management determines the nature of the sow's environment which, in turn, affects the endocrine control of reproduction. In large intensive units, irregularities of reproduction are often more apparent than in less intensive systems. The keeping of large numbers of breeding animals together favours the build-up and spread of microbial and viral diseases that may interfere with fertility.

An investigation of a fertility problem in the pig is rarely, if ever, considered on an individual sow basis. The parameters of reproductive performance demonstrate this, with fertility being expressed in terms of percentages of the herd, and averages. Sows that deviate from the norm are invariably removed from the herd rather than being treated or being allowed to continue in a subfertile way. Herd fertility investigations are therefore exactly as they sound – investigations of overall reproductive performance. This being so, it must be remembered that a herd comprises a number of individuals; in order to understand and fully appreciate a fertility problem it is valuable to consider individual sow records.

Any investigation of herd infertility must begin with an evaluation of the problem. This requires an initial understanding of the history of the problem (Douglas and Mackinnon, 1992), and examination of the records (Muirhead, 1976), which may be presented in various ways. Ideally, the unit will record fertility data along with all other performance data on an in-house or bureau computer system, and Figure 27.2 shows examples of records maintained in this way. Where this is not the case, time must first be spent handling the raw data that may be in the form of pocket record books for the service house and the farrowing house. This is an extremely laborious process but the information is vital to any investigation.

Parameters of relevance to the investigation include:

- herd size
- age profile and replacement rate
- return rate/conception rate
- distribution of return intervals
- weaning-to-oestrus interval
- farrowing rate
- total numbers born
- total numbers born live
- lactation length
- number of non-productive (empty) days.

It is of particular importance to evaluate the data with reference to:

- reproductive performance of the herd before the problem, and the time over which it changed
- reproductive performance of other herds
- physiological potential reproductive performance.

Examination of records should provide a definition of the nature and extent of the problem. This exercise is followed by a clinical appraisal of the stock, post-mortem examination and laboratory diagnosis as appropriate (Douglas and Mackinnon, 1992). At the outset it should be recognised that the route of a fertility problem is often multifactorial.

The problem will probably fall into one or more of the following categories:

- anoestrus
- conception failure
- pregnancy failure.

ANOESTRUS

Anoestrus is defined as 'the absence of oestrous behaviour (standing to a boar or to a riding test) but excludes the normal interval (dioestrus) between two successive oestrous periods' (Meredith, 1984). By definition, delayed oestrus is included in this category (Douglas and Mackinnon, 1992).

EFFICIENCY REPORT

Farm Number

Run on 28 Sep

Dated 16-May in Week No.19 - Second Quarter

Pigtales™

	PERIOD 1 From 15-Nov To 16-May (ACTUAL)	PERIOD 2 From 17-May To 16-May (ACTUAL)	 (TARGET)
SERVICES Total	302	581	554
Repeat %	8	8	10
Matings/Service	2.0	2.0	2.0
Matings/Boar/Week	2.1	1.9	1.6
FARROWINGS Total	240	469	471
Livebirths/Litter	2686/11.2	5299/11.3	5652/12.0
Mummified % Total	1.2	1.4	0.0
Stillbirths % Total	9.5	9.0	2.5
Index (Interval)	2.25(162)	2.25(162)	2.25(162)
Born/Sow/Year	25.2	25.4	27.0
Born/Female/Year	23.5	23.7	25.3
LOSSES Total	395	747	678
Losses % of Liveborn	14.7	14.1	12.0
WEANINGS Normal (& Late)	237(0)	470(0)	471
Pigs Weaned/Litter	2265/ 9.6	4561/ 9.7	4974/10.6
Late Foster % Weaned	0.0	0.0	0.0
Sub-Standard % Weaned	0.0	0.0	0.0
Weaned/Sow/Year	21.5	21.8	23.8
Weaned/Female/Year	20.1	20.4	22.4
STOCK Sows	214	209	210
Boars	11	12	13
Sow:Boar Ratio	19.5	17.4	16.0
Gilts	15	15	14
Sow:Gilt Ratio	14.3	13.9	15.0

(a)

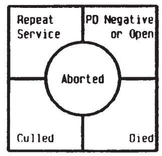
Fig. 27.2 Herd fertility records

FARROWING CONTROL CHART

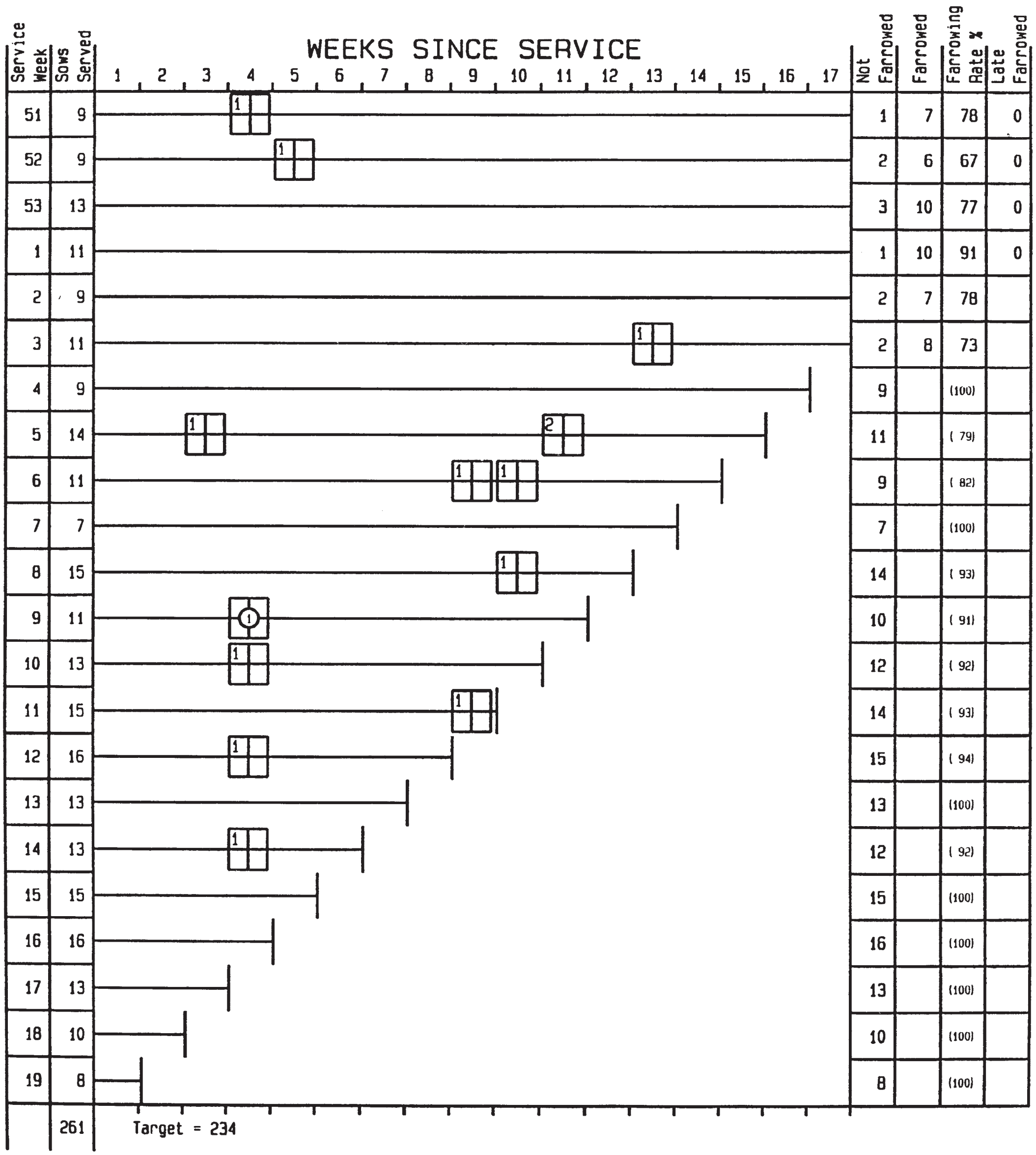
Farm Number

Run on 28 Sep

Dated 16-May-93 in Week No.19 - Second Quarter



Pigtales™



Service Week	Sows Served	Not Farrowed	Farrowed	Farrowing Rate %	Late Farrowed
51	9	1	7	78	0
52	9	2	6	67	0
53	13	3	10	77	0
1	11	1	10	91	0
2	9	2	7	78	
3	11	2	8	73	
4	9	9		(100)	
5	14	11		(79)	
6	11	9		(82)	
7	7	7		(100)	
8	15	14		(93)	
9	11	10		(91)	
10	13	12		(92)	
11	15	14		(93)	
12	16	15		(94)	
13	13	13		(100)	
14	13	12		(92)	
15	15	15		(100)	
16	16	16		(100)	
17	13	13		(100)	
18	10	10		(100)	
19	8	8		(100)	
	261				

(b) Farrowing Rate (from week 30 to week 2) = 85% -- Target = 85%

Fig. 27.2 *continued*

PIGTALES SOW CARD

SOW **951**

Sire: LAST FARROWED 06-05 BORN/YR 32.5

Dam: SOW'S AGE 2 Yrs 10 Mths WEAN/YR 26.9

LITTER NUMBER	1	2	3	4	5	AVGE	6
BOAR(S) USED	1xMIX	1xMIX	1x	1x	1x		1x
FARROWING INTERVAL·		146	143	147	140	144	
SERVICES/MATINGS	1/3	1/3	1/3	1/2	1/3	1.0	1/3
PROBLEMS							
STILLBIRTHS	1	0	0	0	0	0.2	
MUMMIFIED	0	0	1	1	0	0.4	
LIVEBIRTHS	7	12	15	15	15	12.8	
FOSTERED ON	1	0	0	1	0	0.4	
FOSTERED OFF	-1	0	-2	-3	0	-1.2	
PRE-WEANING DEATHS	-1	0	-2	-2	-3	-1.6	
WEANED	9	12	11	10	11	10.6	0
SUB-STANDARD	0	0	0	0	0	0.0	0
AV.BIRTH WEIGHT	1.1	1.3	1.1	1.5	0.0	1.3	
AV.WEANING WEIGHT	5.3	6.7	7.0	5.3	5.4	5.9	
AGE AT WEANING	23	24	28	21	28	24.9	

Boars Used (Breed)............ LEN( ) ZAK( ) WILL( )
Service date.................. 15-06 (Wk 24)
3 Week date................... 06-07 PD ______
Due to Farrow................. 08-10
Date Farrowed................. ________
(c) Date Weaned................... ________

Fig. 27.2 *continued*

Anoestrus is inevitable at certain stages in a sow's life (e.g. before puberty, and during pregnancy and lactation), and this should be taken into account in any investigation. It must be recognised, however, that even 'normal' periods of anoestrus represent non-productive days and should be kept to a minimum in the non-pregnant sow (Meredith, 1984). The average weaning to oestrus interval is 4–6 days, and any delay in this results in loss of production due to an increase in empty days. A confounding factor is that fertility appears to be lower in sows with an extended weaning-to-oestrus interval. The net result of anoestrus is economic loss, and every effort should be made to minimise the interval. Anoestrus is one of the commonest reproductive disorders of sows and gilts (Meredith, 1979). In some studies its prevalence is higher during the summer months (Hurtgen et al., 1980), although this may be influenced by other factors such as parity, geographical location and boar presence (Bassett et al., 1996).

The term 'suboestrus' refers to a condition in which cyclic animals show no obvious external signs of oestrus. Suboestrus is characterised by the presence of corpora lutea. Meredith (1977) demonstrated by rectal palpation that the cervix is relatively small and firm in anoestrus and softer in suboestrus under the influence of this luteal tissue.

Firstly, it is important to establish that the problem is truly one of anoestrus and not simply inadequate oestrus detection. Accurate oestrus detection involves time and effort, and strategic use of boar presence, in conjunction with good record-keeping. The boar is 98% accurate at oestrus detection (Almond and Dial, 1987), and also makes a positive contribution to the stimulation of this event. Sows should be exposed to the boar for at least 20 minutes each day from weaning and be observed at this time for signs of oestrus. Early oestrus (day 0–day 4) may be missed if detection is not started on the day of weaning, and this can result in an apparently delayed oestrus 21 days later. Oestrus detection can be an even greater challenge in gilts. Observations in Australian pig units have indicated that there is considerable room for enhancement in reproductive efficiency by improving oestrus detection in this particular group of breeding females. Hemsworth (1988) suggests that the detection rate of oestrus in gilts can be as low as 50–60%.

Investigation of anoestrus

Having established from the records, and from observation on the actual unit, that anoestrus really is a problem, it is necessary to investigate the situation further.

Ovarian function tests

The ovaries of sows that demonstrate no physical signs of oestrus may be truly inactive (anoestrous), active with oestrus inapparent (suboestrous) or pregnant (Meredith, 1983). Tests for ovarian function will differentiate between these conditions. Ovarian activity has been identified in apparently anoestrous gilts by identifying changes in plasma progesterone concentration with time (Einarsson et al., 1978; Christensen, 1981). It is, therefore, important to investigate ovarian activity before attempting to treat an anoestrous female. Progesterone may be measured in plasma or whole sow's blood by enzyme-linked immunoassay (ELISA) as described by Glossop and Foulkes (1986). Weekly blood progesterone assays will differentiate between truly inactive, pregnant and normally cyclical ovaries (Glossop and Foulkes, 1990).

An incidence of sows that have normal cyclical ovaries, but are apparently suboestrous, may result from inadequate oestrus detection, perhaps due to lack of emphasis on this important stage of the breeding cycle, or to suboptimal conditions for observing signs of oestrus. Ideally, the producer should be encouraged to observe for oestrus in the presence of the boar (Almond and Dial, 1987) at least once (and preferably twice) each day from the day of weaning. Oestrus may be inhibited by stress, unsuitable environment, psychological inhibitions, exogenous hormones or lameness (Meredith, 1979). The presence of the boar is a key issue in the stimulation of oestrus in both sows and gilts, and its accurate identification (Glossop, 1992).

Factors that influence the appearance of oestrus in sows and gilts are summarised in Figure 27.3 (Meredith, 1979). Walton (1986) considers exposure to mature boars to be the single most important feature of the post-weaning environment to ensure rapid return to oestrus and ovulation.

Post-mortem examination

Ovarian function may be assessed by post-mortem examination of the reproductive tracts of sows culled from the herd under investigation. This type of study can reveal a range of abnormalities:

1. Acyclic ovaries: inactive, with some small follicles (<5 mm diameter), absence of corpora lutea
2. Cystic ovaries:
   - Multiple large cysts (usually <14 mm in diameter), generally containing some luteal tissue that produces progesterone – these may regress, but some persist and can inhibit oestrus (Figure 27.4)
   - Multiple small cysts – these often produce oestrogens, which results in sows having markedly irregular cycle lengths and exhibiting intense signs of oestrus (nymphomania)
   - Single cysts – these rarely affect sow fertility and tend to be incidental findings at post-mortem.

Realtime ultrasound

This technique can be used to observe changes in the size and shape of the ovaries by a non-invasive method in the live animal (Weitze et al., 1990a). Such investigations are time-consuming and require expensive equipment and a considerable level of expertise. They do provide, however, a valuable tool for diagnosis of infertility and research into ovarian activity.

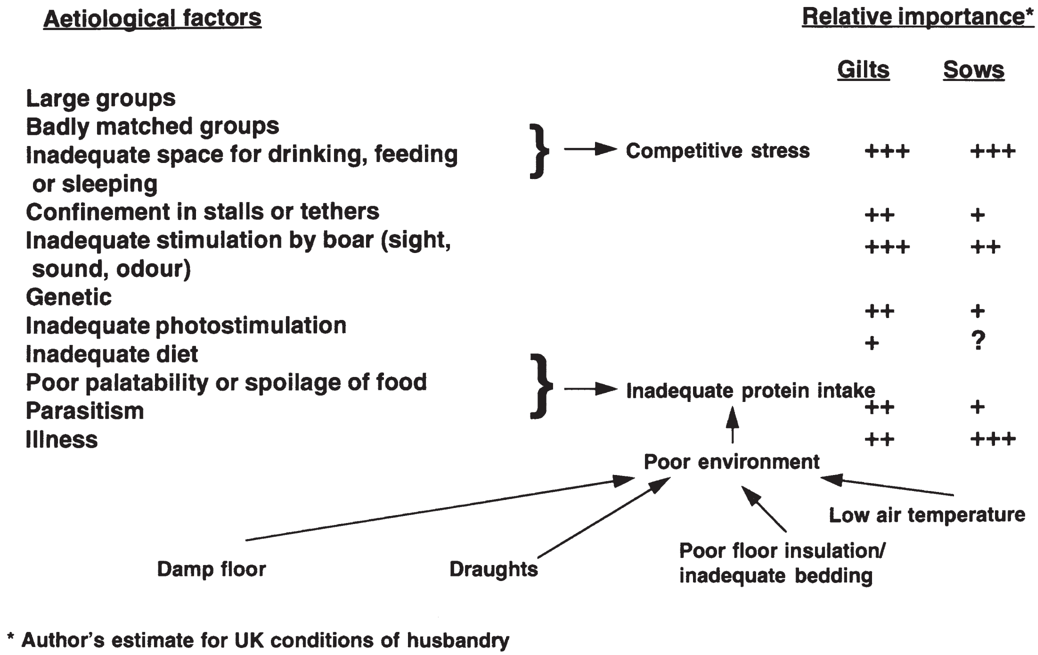


Fig. 27.3 Factors that influence the appearance of oestrus in sows and gilts (from Meredith, 1979).

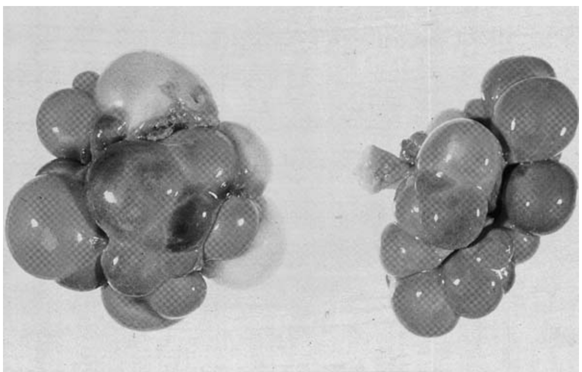


Fig. 27.4 Cystic ovaries.

Treatment of anoestrus

Anoestrus is of economic significance and must be treated promptly by identifying and remedying all contributing factors. In serious cases where ovaries are truly inactive, it may be worth considering hormone therapy as a means of restoring cyclical activity. Various hormone preparations are available but a combination of equine chorionic gonadotrophin (eCG) and human chorionic gonadotrophin (hCG) is still probably the preparation of choice (Meredith, 1979). Injection of a preparation containing 200 IU hCG and 400 IU of eCG (PG600, Intervet) into 363 anoestrous sows resulted in 87.6% showing signs of oestrus within 3–8 days (Schilling and Cerne, 1972).

CONCEPTION FAILURE

This problem is recognised by an increased number of regular returns to service (i.e. returns at 18–24 days after service). The conception rate (or non-return rate) in breeding herds should be at least 90% (see Table 27.2) and an incidence of returns higher than 10% should be regarded as abnormal and unacceptable. Conception failure suggests that viable ova did not come into contact with viable spermatozoa at the appropriate time. The result of this will be either total conception failure (i.e. regular return to service) or partial conception failure (i.e. reduction in litter size). Assuming that ovulation has taken place, conception failure must be due to one or more of the following factors.

Timing of service

Lynch and O'Grady (1984) said that 'A single mating at the appropriate stage of oestrus should be sufficient to get a high proportion of ova fertilised at the optimum time to ensure a high embryonic survival rate and large litter size at birth'. The problem comes in identifying this moment correctly. Existing service regimens seek to compensate for inaccuracies in timing. The single most important factor in achieving an acceptable conception rate is timing.

The sow ovulates, on average, 36–44 hours after the onset of standing oestrus (Hunter, 1988; Weitze et al., 1990). Ova have a finite life span following ovulation and must come into contact with the spermatozoa at an early stage after ovulation, and in any event within 8 hours. Spermatozoa can survive for approximately 24 hours inside the sow's reproductive tract (Soede et al., 1996; Waberski, 1996). A service regimen must take all these factors into account and aim to ensure that the uterus contains viable spermatozoa in advance of, and during, the time when ovulation may occur (Glossop, 1991). Inappropriate timing of service results in conception failure. An optimal service management regimen should ensure that each sow is served on the day of onset of standing oestrus and at least once more, 18–24 hours later.

Quality of service

One cannot assume that service has been performed properly without careful supervision to ensure that the boar has actually achieved intromission (Douglas and Mackinnon, 1992). Service pen design, particularly with reference to the floor surface, is of direct relevance to the quality of service. Young boars need to be trained to natural service, and should be well supervised each time they mount a sow or gilt (Hollier, 1979).

Semen quality

This aspect of fertility is often overlooked, although semen quality can be affected by a wide range of factors such as age, environmental temperature, frequency of use and disease (see Chapter 30 on male fertility for a more detailed discussion on this subject). Where semen quality is affected in terms of ejaculate volume, sperm count, sperm motility or morphology, it is possible that conception rates may be affected. Where boars are used for natural service, particularly in a cross-service regimen, it may be that subfertile or infertile individuals remain unidentified. Consideration of boar fertility should also take into account physical breeding soundness, paying particular attention to feet and leg conformation.

Having established an abnormally high incidence of regular returns to service, it is necessary to consider the pattern of the problem. For example, is the problem prevalent within a particular group of individuals differentiated by parity, lactation length, weaning-to-oestrus interval, day of service or service regimen? All of these factors can be related to conception rates and should be considered with care by categorising each return and attempting to establish a pattern.

The next essential step in the investigation is to visit the unit on a busy service day to observe routine procedures. The following questions should be asked:

- Are service pens adequate in terms of size, shape, construction and floor surface?
- Are services properly supervised?
- Are boars being worked in rotation or are some boars being overworked?
- Is semen quality acceptable?
- Are the boars physically sound for breeding?
- What is the physical condition of the sows?
- Is sufficient time spent on oestrus detection?
- Are conditions adequate for oestrus detection? (For example, is there sufficient light in the service house?)
- When are sows served in relation to the onset of standing oestrus?
- What service regimen is used?
- Where artificial insemination is used, do staff understand all the procedures involved?
- Is there evidence of an infectious disease?
- Do the staff have any idea of the cause of the problem?

Despite the reference to infectious disease, regular returns to service, particularly in the absence of other reproductive signs, are more likely to be due to a management problem. It is important to remember that where oestrus detection is suboptimal, a proportion of first returns may be missed, becoming apparent at the second return. For this reason, emphasis should be placed on continued careful observation for signs of oestrus in the presence of a boar throughout pregnancy, bearing in mind that a significant proportion of returns occurs outside the 'normal range' of 18–24 days after service (Glossop and Foulkes, 1988).

PREGNANCY FAILURE

Pregnancy failure may be divided into two main sections:

- failure to establish pregnancy
- failure of an established pregnancy.

The reaction of the conceptus to an adverse factor varies markedly with age, and it is helpful to categorise the various stages accordingly. Wrathall (1975) classifies the stages as:

- pre-attachment (before days 13–14)
- embryonic (from days 14–35)
- fetal (after day 35).

Failure to establish pregnancy

Unattached conceptuses within the uterus are susceptible to damage by many factors. Where pregnancy fails around the time of maternal recognition of pregnancy (i.e. around days 12–13) sows tend to return to oestrus outside the normal range for 'regular returns'. In an experiment where blastocysts were flushed from the uterine horns of pigs on days 10–13 after service, those flushed on day 12 or 13 returned on days 26–30. Those flushed on day 10 or 11 returned at the 'normal' time (Meulen et al., 1987). A proportion of so-called 'late returns' have been recorded in herds with fertility levels within the normal range, and it has been proposed that failure of establishment of pregnancy is the cause of this (Glossop and Foulkes, 1988). In a study of 2472 return intervals, 25.6% of returns before day 31 occurred after the time when oestrus is commonly expected (i.e. day 18–24). The relatively high incidence of this phenomenon in apparently normal herds represents a financial loss that may be avoidable. The exact mechanism of action for this has not yet been clarified, but it is likely to involve a problem with the maternal recognition of pregnancy; any form of stress should be avoided from day 7 after service. Moving sows, regrouping, exposure to extremes of temperature or changes of diet may all contribute to such problems. An infectious challenge at this time may also have a deleterious effect on the establishment of pregnancy. Failure of pregnancy at this stage may be total (resulting in an irregular return to service) or partial (resulting in resorption of some embryos, and consequent reduction in litter size) (Wrathall, 1975).

Investigation of early pregnancy failure is extremely difficult from clinical signs alone. It is necessary to establish the presence of a pregnancy that subsequently fails, and strategic use of hormone assays can be of value in this situation (Glossop and Foulkes, 1990). A regimen in which plasma progesterone is measured on days 21 and 28, and oestrone sulphate on day 28 after service can provide valuable information in such cases. Identifying that conception has probably taken place (by progesterone assay) but that the pregnancy has terminated by day 28 (by a negative result for oestrone sulphate) provides useful information as to the possible course of events (Table 27.3).

Failure of an established pregnancy

Death of conceptuses during the embryonic stage tends to result in resorption if abortion does not occur. Dissolution of embryos in the absence of anaerobic bacteria appears to be an aseptic, autolytic process resulting in complete disappearance of the products or a vaginal discharge (Wrathall, 1975). Partial failure of pregnancy will result in a reduction in numbers born as being the only presenting sign. Once pregnancy has been confirmed (e.g. by the Doppler ultrasound technique on days 28–35), fetal death is more likely to result from an infectious disease. Beyond 35 days, fetal death will result in mummified fetuses at farrowing. Mummification is the most common clinical manifestation of a viral infection (e.g. Aujeszky's disease, porcine parvovirus, porcine reproductive respiratory syndrome or swine fever)

Table 27.3 Use of hormone assay to identify anoestrous, cycling or pregnant sows (Glossop and Foulkes, 1990)

	Day 21: progesterone	*Day 28: progesterone*	*Day 28: oestrone sulphate*
Anoestrous	Low	Low	Low
Cycling	Low	High	Low
Pregnant	High	High	High
Early embryonic death	High	High/low	Low

at this time, although only a proportion of the fetuses may be affected. Environmental conditions may have an effect on the maintenance of pregnancy. Social interaction with boars may enhance the maintenance of pregnancy (Wilson and Love, 1986).

INFECTIOUS CAUSES OF INFERTILITY

An infectious form of infertility can be of great economic significance to a unit. It is vital, however, to ensure that management and stockmanship are adequate before searching for an infectious agent in any investigation into infertility. A whole range of management factors (e.g. environment, stress and nutrition) may lower the natural defence mechanisms, rendering an animal population more susceptible to disease (Fiennes, 1970). In other words, all factors must be taken into account even when it is likely that an infectious agent exists. UK Veterinary Investigation Centre records demonstrate the frequencies of pathogens isolated from fetuses presented for examination; such information is of value when assessing the relative importance of individual pathogens (Table 27.4).

Wrathall (1971) classified infectious causes of infertility into three groups.

Group 1

Group 1 infections are associated with ubiquitous microorganisms that are present in the majority of pig populations.

Under normal circumstances such organisms are generally harmless but they may act as opportunist pathogens when other predisposing factors allow them to gain access to a susceptible reproductive tract. An episode of this type would tend

Table 27.4 Percentage frequency of isolation of pathogens from porcine fetopathies examined by Ministry of Agriculture Veterinary Investigation Centres (source: VIDA-II)

	1984	*1988*	*1989*	*1992*	*1993*	*1994*	*1995*	*1996*	*1997*	*1998*
Erysipelothrix rhusiopathiae	0.8	8.2	5.2	4.1	5.4	0	15.1	9.7	14.2	16.1
Leptospira spp.	NR	NR	NR	24.0	34.0	35.3	22.1	34.7	9.5	35.7
Listeria monocytogenes	0	0	0.7	0.6	0	1.0	0	0	0	NR
Streptococcus spp.	5.4	6.2	3.9	8.2	6.2	6.9	4.7	2.8	16.7	14.3
Parvovirus	70.2	57.4	68.0	40.0	21.7	23.5	25.6	23.6	28.6	14.3
Pasteurella spp.	NR	NR	NR	NR	6.2	8.8	9.3	1.4	4.8	3.6
E. coli	NR	NR	NR	NR	8.5	4.9	7.0	4.2	0	NR
Other pathogens	23.6	28.2	22.2	23.4	17.8	19.6	16.3	23.6	26.2	16.1
Total identified	500	195	153	171	129	102	86	72	42	56
Total submitted	1410	661	482	527	450	384	271	236	206	183
Percentage diagnosed	35.5	29.5	31.7	32.4	28.7	26.6	31.7	30.5	20.4	30.6

NR = Not recorded

to be sporadic in nature. Examples of this type of organism include:

- *Escherichia coli*
- *Erysipelothrix rhusiopathiae*
- *Listeria spp.*
- *Mycoplasma spp.*
- *Pasteurella spp.*
- *Salmonella spp.*
- *Klebsiella spp.*
- *Corynebacterium spp.*
- *Staphylococcus spp.*
- *Streptococcus spp.*
- *Campylobacter spp.*

Clinical signs could include conception failure, abortion, stillbirths, perinatal death and endometritis. Diagnosis and control of Group 1 infections can be difficult due to the ubiquitous nature of these organisms in normal healthy populations. Control measures must include removal of all predisposing factors, enhancement of resistance in susceptible animals and reduction of the weight of infection to exposed individuals. Hygiene in the farrowing house and at service is particularly important. The boar should not be forgotten as a potential source of infection.

Group 2

Group 2 infections result from certain common contagious microorganisms that are present on a high proportion of pig units, e.g. porcine enteroviruses, and porcine parvovirus (PPV). A strong immunity to such infectious agents is usually developed during early postnatal life. Such viruses rarely cause clinical disease in adult sows and boars but they are highly contagious and can spread rapidly through a susceptible population.

PPV is endemic in most herds and may cause reproductive failure associated with embryonic death, mummification, stillbirths and subsequent reduction in litter size. The virus has been recovered from aborted and stillborn piglets, piglets that died soon after birth, and from vaginal mucus and semen (Cartwright and Huck, 1967). Infection must occur during the first half of pregnancy in order to result in disease. Transplacental infection has been demonstrated (Cartwright et al., 1969). Gilts are particularly susceptible at their first exposure, after which a lifelong immunity will develop. Management of this disease requires exposure of all gilts to the virus before service by careful integration into the herd. A more controlled alternative is vaccination. Diagnosis is by serology, and serological testing also gives an indication of the immune status of the herd.

Porcine reproductive respiratory syndrome (PRRS) is a relatively new member of Group 2 diseases. It was first recognised in the USA in 1987 (Dial and Parsons, 1989) and has since occurred in mainland Europe and Britain (Done et al., 1992). The effect on the reproductive performance of a herd can be devastating (Christianson et al., 1992). The clinical signs of PRRS are rather variable but include some or all of the following (de Jong et al., 1991; Loula, 1991; White, 1991; Done et al., 1992; Hopper et al., 1992):

- *In the sow*:
  - inappetance (for 7–10 days), which may appear in waves in the herd
  - fever
  - listlessness
  - regular and irregular returns
  - vaginal discharge
  - anoestrus
  - abortions (not a major feature)
  - early farrowings
  - stillbirths and mummification
  - poor milking
  - secondary discharges due to cystitis or pyelonephritis
  - sudden death.
- *In the boar*.
  - lethargy
  - inappetance
  - semen quality – affected for up to 13 weeks, or occasionally on a permanent basis (personal communication).
- *In the piglets:*
  - weakness
  - puffy eyes
  - lameness
  - high pre- and postweaning mortality
  - respiratory signs.

Gross pathological lesions tend to occur in the respiratory system with a confluent consolidation of the lungs affecting all lobes. Extensive bronchopneumonia and occasionally fibrous pleuritis are also features of this disease (Loula, 1991). The most likely method of spread of the disease is by the introduction of infected pigs on to the premises, although local airborne spread between herds has been suspected (Cromwijk, 1991; van Alstine, 1991) over distances of up to 3 km (Robertson, 1992). The virus has been isolated in boar semen, and infection has followed insemination with infected semen (Christopher-Hennings et al., 1995).

The serious economic impact of this disease results from its devastating effect on herd productivity in terms of farrowing rate, number of live piglets born, pre- and postweaning mortality and performance of surviving piglets. De Jong et al. (1991) suggest that a herd may lose 10% of production. Diagnosis of PRRS is made on the basis of clinical signs, changes in herd performance, serology and histopathology (Done et al., 1992).

Treatment involves the use of antibiotics for some of the secondary effects although they cannot prevent the reproductive losses. Seropositive herds need to adapt their gilt intake programme to ensure that exposure to the virus occurs at least 4–6 weeks prior to mating.

Group 3

Group 3 infections occur relatively infrequently, but tend to result in severe reproductive loss, e.g. leptospirosis and Aujeszky's disease.

Reproductive losses from leptospirosis have been reported on a world-wide basis. The causative agents are a variety of spirochaetes belonging to the genus *Leptospira*. Serogroups of greatest importance to pig populations are *australis* (including the *bratislava* and *muenchen* serovars), *pomona* and *tarrasovi*. The last two groups have not yet been found in the UK (Ellis, 1992). Incidental infections in pigs may also result from *canicola, icterohaemorrhagiae, autumnalis, hardjo, mozdak* and *muenchen*. The epidemiology of the disease is complicated by the fact that some strains are specifically adapted to the pig, and others to dogs, horses, hedgehogs and other wildlife.

The most important route of infection is thought to be via the mucous membranes of the eye, mouth, nose (Alston and Broom, 1958; Michna and Campbell, 1969) or vagina (Chaudhary et al., 1966). A bacteraemia occurs 1–2 days after infection, may last for a week and coincides with acute clinical disease. Presenting signs include anorexia, pyrexia and listlessness (Hanson and Tripathy, 1986). Primary signs of chronic leptospirosis are abortions, stillbirths and birth of weak piglets (Bohl et al., 1954; Fennestad and Borg-Petersen, 1966). Leptospires can localise in the kidneys, multiply at this site and appear in the urine in varying degrees of intensity and for different lengths of time (Ellis, 1992). Leptospires can also localise in the uterus of pregnant sows; when this happens in the last half of gestation abortions and stillbirths often result, occurring 1–4 weeks after infection (Hanson and Tripathy, 1986). Infection with the *bratislava* serovar has also resulted in persistence of leptospires in the uterine tube and uterus of non-pregnant sows (Ellis et al., 1986a) and in the genital tract of boars (Ellis et al., 1986b).

Usually, diagnosis of leptospirosis is based on serology using the microscopic agglutination test (MAT) (Faine, 1982), although the presence of seropositive animals in the herd does not always result in clinical signs. Demonstration of leptospires in the fetus provides a definitive diagnosis of leptospiral abortion (Ellis, 1992).

Control of leptospirosis depends upon the combined use of antibiotic therapy and management. Systemic streptomycin at 25 mg/kg body weight (Dobson, 1974) or oral tetracyclines at levels of 800 g per tonne of feed (Stalheim, 1967) have been used to eliminate carriers, although this type of strategy is not always successful. The main management factor involves prevention of contact between pig populations and other domestic stock or wildlife, which can be difficult to achieve, particularly on outdoor units. Vaccination is an option in some parts of the world, although vaccines are not available in many countries in Western Europe (Ellis, 1992).

The causative agent of Aujeszky's disease (or pseudorabies) is a herpesvirus. Aujeszky's infection usually gains access to the pig by inhalation or ingestion of the virus (Wrathall, 1975). It may also be transmitted by coitus although there is some argument as to whether true venereal transmission occurs. Aujeszky's disease is characterised by nervous and respiratory signs associated with a rise in temperature and often death in young piglets. Infection in adults may result in stillbirths and abortion (Taylor, 1995). In adult boars and sows, the clinical signs of this disease are seldom severe and usually consist of pyrexia, depression and anorexia that lasts for up to a week. Of great significance to the breeding herd is the fact that the virus causes embryonic death, fetal mummification and stillbirths.

Brucella suis is a widespread infection of pigs in the USA but has not appeared in Great Britain. In countries where it does occur, it should always be considered as a cause of herd infertility or abortion. Pigs of both sexes are much more susceptible to infection after weaning. Once infection has been introduced into a susceptible herd (usually by pig movements), it spreads quite rapidly by ingestion or by venereal transmission (Wrathall, 1975). An infected animal suffers an initial generalised bacteraemia similar to undulant fever in humans, which may last for several weeks or months (Deyoe, 1967). Service by an infected boar results in uterine infection, although establishment and proliferation of the organism do not appear to interfere with fertilisation. Abortion is the most significant effect of venereal infection and can occur at any stage during pregnancy, although the rate is highest when infection occurs at the time of breeding. Sows usually abort only once.

There is also a higher incidence of stillborn and weakly piglets. In sexually mature boars, infection can localise in the testis, resulting in clinical orchitis (Kernkamp et al., 1946) with consequent impairment of spermatogenesis, loss of libido and infertility. Poor reproductive performance of the boars exacerbates the overall infertility. Herd diagnosis is made by means of a complement fixation test (CFT) or a serum agglutination test (SAT). *B. suis* may be isolated from aborted fetuses.

There is no treatment for swine brucellosis, nor is there any means of conferring artificial immunity. In infected commercial herds, all pigs should be slaughtered as they reach a suitable marketable weight and the unit left empty for 6 months before restocking. In the case of a valuable breeding herd, depopulation may be out of the question. In such situations all pigs are assumed infected, and a clean herd built up by isolating the piglets at birth and retaining those that pass the agglutination test at weaning age. The public health issues should be borne in mind as this is an important zoonosis.

Other viruses that interfere with gestation include swine fever (hog cholera), foot and mouth disease, classical swine influenza, transmissible gastroenteritis, Japanese B encephalitis and Japanese haemagglutinating virus. Experimental infection with attenuated classical swine fever virus has caused various effects that vary according to the stage of gestation at which sows were inoculated. In the USA, Dunne et al. (1965) associated enteroviruses from two serologically distinct groups (A and B) with an epizootic disease of pigs characterised by stillbirths (S), mummification (M), embryonic death (ED) and infertility (I) ('SMEDI viruses').

Vulval discharges

Pig producers often complain of a high incidence of vulval discharge in the herd, and it is worth considering the types and aetiology of these in some detail. Vulval discharges are the most obvious clinical sign of bacterial genital infections, although their detection varies according to a range of factors including the level of stockmanship and the type of sow accommodation. Discharges are also seen in a number of more generalised infectious forms of infertility, and their intermittent nature confounds the problem. The time of appearance of the discharge is significant to the investigation.

In most cases, uterine infections in non-pregnant sows do not appear to affect return intervals, although infection in early pregnancy can be associated with regular or irregular returns to service. In late pregnancy uterine infection can lead to abortion (Meredith, 1991).

Investigation of an outbreak of vulval discharge involves identification of the source of the discharge by speculum examination per vaginam. Discharges may originate from the vestibule, the vagina, the uterus or the bladder. Cytological examination of the discharge differentiates between those composed of urinary sediments (which are rarely responsible for disease), and those containing leucocytes and bacteria. The consistency of the discharge may vary from a thin pale yellow fluid without blood or mucus, to one with necrotic debris and mucus with or without blood (Muirhead, 1986). The latter type is closely correlated with cystitis that may be associated with *Corynebacterium suis* (Soltys, 1961). Microbiological sampling of the cervix generally yields a mixed flora of Group 1 commensal organisms.

Differential diagnosis

Some discharges are quite normal, particularly those that are watery or slightly cloudy in appearance, and occur in pro-oestrus and oestrus. After mating, seminal fluids, including gel, may be expelled, and again these do not indicate disease. A slight discharge may also be seen during pregnancy, and following parturition a lochial discharge will normally persist for up to 5 days.

Abnormal discharges can vary in quantity, consistency and colour (Table 27.5). Production of large volumes of creamy discharge (up to 500 ml) usually indicates endometritis. In the early stages, a tacky mucus can appear 15–21 days after service; these sows usually return to oestrus (Muirhead, 1986). Discharges immediately after farrowing have also been reported in association with the mastitis, metritis, agalactia syndrome (Leman et al., 1972). Muirhead (1986) did not recognise an association between post-farrowing discharges and those observed after service.

Treatment involves improved hygiene, particularly in the service house, antibiotic injection of sows at weaning and/or a programme of in-feed medication.

Table 27.5 Differential diagnosis of vulval discharge (guidelines) (Meredith, 1991)

Type of discharge	*Quantity*	*Consistency*	*Colour*	*Malodorous?*
Normal				
Pro-oestrus/oestrus	Small	Watery, slightly tacky	Clear, cloudy or white (depending on cell content)	No
Seminal (during/shortly after mating or artificial insemination)	Varied	Mainly semen components, some fluid and cells from female	Clear, cloudy or white	No
Post-mating (up to 2 days after service or artificial insemination)	Small	Thick, tenacious	White, grey or yellow	No
Pregnancy (probably from cervix)	Small	Thick, tenacious	White, grey or yellow	No
Postpartum lochia (up to 5 days postpartum)	Up to about 15 ml present at one time Decreasing by 3rd day	Usually thick	Varied	Slightly
Abnormal				
Vaginitis/cervicitis	Small	Thick, tenacious	White to yellow	Severe cases only
Endometritis	Varied	Varied	Varied	Severe cases
Endometritis (puerperal)	Often >15 ml present at one time	Usually thin, may be lumps	Varied	Usually
Abortion (bacterial)	Varied	Varied	Varied (may be blood)	Occasionally
Urolithiasis (oxalates, phosphates)	Varied	Often gritty when rubbed between fingers	Cloudy, white or yellow	No
Cystitis/pyelonephritis	Varied	Varied	Often blood-stained	Severe cases (ammoniacal)

STRUCTURAL ABNORMALITIES OF THE FEMALE REPRODUCTIVE TRACT

Anatomical defects of the female genitalia have been reported in pigs and include intersexuality, gonadal hypoplasia and other miscellaneous abnormalities (Wrathall, 1975). That such anomalies are congenital rather than acquired is shown by their relatively higher incidence in gilts than sows. This aspect of pathological reproduction in swine has been studied by Wilson et al. (1949) and Nalbandov (1952), who found its incidence in sterile swine to be 21.5%. Despite the relative importance of a developmental abnormality in an individual, it should be remembered that on a herd scale such defects are not usually of great significance.

Inherited hypoplasia of the gonads

This is an important condition in farm livestock because it can lead to a substantial reduction in fertility without being obvious clinically (Wrathall 1975). Both sexes may be affected but it is, of course, more readily apparent in the male. In pigs, gonad hypoplasia has not been studied extensively although it has been described in the boar (Holst, 1949).

Intersexuality

Such abnormalities are not uncommon with a prevalence of up to 0.5% (Wrathall, 1975). It appears to be a hereditary condition determined by recessive genes. Most porcine intersexes are male pseudohermaphrodites; they have testes that may be subanal or intra-abdominal. The mammalian embryo has dual potentiality in the sense that initially it possesses two sets of tubular duct systems – the Wolffian ducts and the Müllerian ducts. If the embryo is male the Wolffian ducts are stimulated to develop, whereas the Müllerian ducts regress and are usually vestigial by the time of birth (Hunter, 1988); in the case of the female, the reverse takes place, with the Müllerian duct system developing instead. In the intersex, a combination of both duct systems is present, the animal possessing a mixed set of tubular organs (Figure 27.5), both Wolffian and Müllerian ducts developing side by side. Externally, intersexes resemble the female and micturate through the vulva, although a phallus may be present. The animal may be considered a gilt until puberty, when it starts to demonstrate male behaviour.

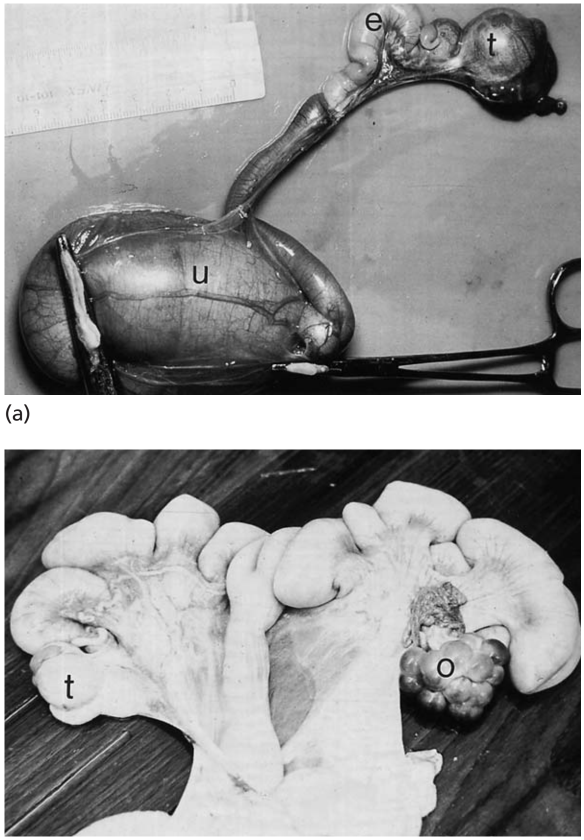


Fig. 27.5 Intersexuality. (a) Distended uterine horn (u), epididymis (e) and testis (t). (b) Genitalia of a porcine hermaphrodite showing testicle (t) and ovary (o) (courtesy of R. G. A. Douglas and J. D. Mackinnon).

Bilateral uterine tubal lesions

Structural sterility resulting from bilateral tubal lesions (e.g. hydrosalpinx, pyosalpinx and ovarobursal adhesions) has been shown to occur in up to 33.3% of sows and gilts that failed to breed (Warnick et al., 1949). Apart from the uterine tubes, other parts of the tubular genital tract may

Fig. 27.6 Complete pregnant genital tract of a gilt showing uterus unicornis. Five piglets were present.

show aplasia or duplication, but only when the whole tubular system is aplastic, or when the vagina, cervix or uterine body is imperforate, will sterility result. The condition of uterus unicornis (Figure 27.6) will lead only to lowered fecundity.

Absence of one or both ovaries

The absence of one or both ovaries and a generalised underdevelopment of the whole reproductive tract (infantilism) occurs occasionally. Other lesions include double vagina, septae or 'strings' in the vagina and hymenal residues; Teige (1957) suggested that this type of defect may cause problems at service. Meredith (1982) described incidents of pain and/or haemorrhage at mating associated with hymenal strictures and urethral intromission.

SEASONAL INFERTILITY

Reduction in fertility in pigs in the summer and early autumn has been reported in many countries and appears to manifest as a range of problems including delayed puberty in gilts, delayed post-weaning oestrus in sows, regular and irregular returns to oestrus (Wrathall, 1987), delayed return to oestrus (Love, 1978, 1981), reduction in the farrowing rate (Bray et al., 1994a,b), embryonic death, ovarian cysts and silent oestrus (Williamson et al., 1980). Autumn abortion syndrome may also be connected to seasonal infertility (Wrathall, 1987).

It has been suggested that heat stress is particularly damaging during the first 8–14 days post-mating. Improved management of sows to avoid stressful and overheated conditions during the hot summer months can reduce the problem (Hennessy and Williamson, 1984; Hancock, 1988). More information is needed on the description and causes of this significant loss of production, particularly with the increasing trend to outdoor pig production in various parts of the world.

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28 Infertility in the bitch and queen

There have been considerable advances in our knowledge of reproduction in the bitch and queen, and over the last few years there has been increased awareness of infertility in these species. In the dog in particular, this has been the result of breeding animals for working and show purposes, and following the substantial developments in the use of dogs as assistance animals for disabled people. Nevertheless, much attention has been paid to the control of reproduction, principally as a result of the considerable problem world-wide of pet overpopulation (Olson and Moulton, 1993). However, there is no doubt that reproductive failure is common, and this may be attributed to the high degree of inbreeding (Wildt et al., 1983).

The extent of infertility in the bitch and queen is unknown. In the bitch, reduced fertility may not be fully appreciated because matings are relatively infrequent, when compared with other domestic species, and because the majority of breeding animals are housed singly or in small groups. The situation is further complicated because 'fertility' usually represents an owner's personal expectation of the reproductive performance of the bitch or queen; this expectation may differ between a commercial breeding establishment and the owner of an individual pet. In addition, there are wide breed variations, particularly in litter size, which make it difficult to compare animals of different breeds.

It is with increasing frequency that both individual breeders and the managers of breeding colonies approach the veterinarian for help with breeding problems. This may be because of the value of an individual animal which fails to reproduce, or because of a concern for the decline in the productivity of a breeding colony. In the former case, continuation of a breeding line is the ultimate aim, whilst for the latter, greater productivity allows lower numbers of breeding animals to be maintained. The breeding of large numbers of dogs and cats is common for pharmaceutical and biological laboratories, and also for the production of working and assistance animals such as guide dogs for visually impaired people. For the latter, colony management requires not only a high output but a consistent production throughout the year.

The normal expectation of fertility in the bitch is a conception rate in the region of 70–80% (Hancock and Rowlands, 1949; Strasser and Schumacher, 1968; Andersen, 1970; England, 1992), whilst the queen may rear between one and three litters per year (Stabenfeldt and Shille, 1977; Cline et al., 1981; Concannon, 1991). There are considerable variations from this, depending upon age and breed. For the bitch, the peak in reproductive efficiency occurs at approximately 3 years of age, with a significant decline in the number of pups born in bitches aged 7 years and above (Blythe and England, 1993). Blythe and England (1993) also demonstrated a variation in the prolificacy between different breeds of dog. Similarly in the cat, an age-related reduction in the number of litters per year and average litter size has also been noted (Robinson and Cox, 1970; Schmidt, 1986), which is most marked after 6 years of age (Lawler and Bebiak, 1986). The decreased litter size in older bitches may be associated with an increased frequency of stillbirths. However, it should be remembered that there are marked breed variations for normal litter size in both the dog and cat (Lyngset and Lyngset, 1970; Robinson and Cox, 1970; Robinson, 1973); these should be considered when an animal is presented because of alleged subfertility.

The investigation of infertility in the bitch and queen is complicated by the fact that failure to conceive does not result in an immediate return to oestrus as occurs in polyoestrous species.

However, the early diagnosis of pregnancy is now possible using real-time ultrasonography (Yeager and Concannon, 1990; England and Yeager, 1993), the detection of acute phase proteins in serum (Eckersall et al., 1993) and the measurement of plasma concentrations of relaxin (Concannon et al., 1996). These methods allow improved investigations into why an individual animal fails to produce live offspring.

As with other species, infertility in the bitch and queen may be categorised according to whether the cause is structural (including congenital, acquired and neoplastic diseases), functional (including endocrinological abnormalities), infectious or managemental. The influence of the male should always be investigated; collection and evaluation of a semen sample provide a basic assessment of the male's fertility (see Chapter 29). In addition, attention should also be given to the mating routine since owners, who are unfamiliar with normal mating behaviour, may inadvertently be hindering conception. Examples of this are the belief that ovulation always occurs a set number of days after the onset of pro-oestrus in the bitch, and that only a single mating is necessary to induce ovulation in the queen.

THE BITCH

Structural abnormalities of the reproductive tract

Congenital abnormalities

Agenesis of an ovary is rare and does not cause infertility unless both ovaries are affected. In some cases there may also be agenesis of the ipsilateral uterine tube and/or uterine horn, although the latter may occur with a normal ovary. Ovarian dysplasia has also been reported in a bitch with an abnormal number of chromosomes (Johnston et al., 1985), and evaluation of the karyotype may be useful when investigating the cause in these cases.

Bitches with uterine tube and/or uterine horn agenesis and normal ovaries usually exhibit typical oestrous behaviour, but either fail to become pregnant (bilateral lesion) or have low numbers of offspring (unilateral lesion). Diagnosis usually relies upon direct inspection of the reproductive tract via laparotomy or laparoscopy; the use of radiography following the injection of radiopaque contrast media into the uterus (Lagerstedt, 1993) is not as useful in the bitch as for other species, particularly for demonstrating lesions of the uterine tube, which in this species is very small and rarely fills with contrast medium.

Other congenital anomalies of the tubular genital tract include segmental aplasia of the Müllerian duct system. The aetiology of this condition remains uncertain; however, the inadvertent administration of exogenous hormones during pregnancy may result in the partial or complete absence of a connection between the Müllerian ducts and the urogenital sinus (Christiansen, 1984). Complete aplasia of the vagina results in infertility (Wadsworth et al., 1978; Hawe and Loeb, 1984) and allows the accumulation of uterine fluid, producing similar signs to those of pyometra. In these cases the only treatment is ovariohysterectomy.

Strictures of the caudal reproductive tract are common in bitches. These may produce clinical signs associated with vulval pruritis or chronic vaginitis (Holt and Sayle, 1981; Soderberg, 1986); however, most commonly they are first recognised during a prebreeding examination or when there is pain associated with intromission. Circumferential strictures are most commonly found at the junction between the vestibule and the vagina; these may be stretched under general anaesthesia during pro-oestrus to allow mating during oestrus. Larger transverse fibrous bands may also be present; these require an episiotomy and extensive dissection to restore the vaginal lumen. Such congenital strictures may be considered to have five aetiologies. The first is the result of hypoplasia of the genital canal which causes vestibulo-vaginal hypoplasia and a vaginal stricture of some length. The second aetiology is the result of poor or inadequate fusion of the Müllerian ducts to the urogenital sinus causing annular fibrous strictures at this site. The third relates to tissue vestiges remaining at the vestibulo-vaginal junction resulting in hymenal remnants or a complete hymen. Fourthly, incomplete fusion of the two Müllerian ducts may result in a double vagina or a vertical fibrous division within the vagina, and the final aetiology is imperfect joining of the genital folds and genital swellings resulting in vestibulo-vulval hypoplasia.

Congenital abnormalities of the external genitalia are rare. Vulval hypoplasia associated with perivulval dermatitis has been described (Christiansen, 1984); the relationship between this condition and early neutering has not been fully established. Masculinised female pups that have an abnormally shaped vulva may be produced following androgen or progestogen administration during pregnancy (see abnormalities of phenotypic sex below).

Intersex. Intersex animals have ambiguous genitalia. In the bitch this is usually recognised because of an abnormal phenotypic sex appearance; externally the animal appears female but when it reaches puberty the clitoris enlarges and male-like behaviour may develop. Intersex animals may be classified as those with abnormalities of chromosomal, gonadal or phenotypic sex. These conditions have recently been reviewed (Meyers-Wallen and Patterson, 1989; Meyers-Wallen, 1993).

Abnormalities of chromosomal number include phenotypic females (X0 or XXX) which have underdeveloped genitalia, and chimeras and mosaics that arise from two cell populations with different chromosome constituents. In chimeras and mosaics, there may be both ovarian and testicular tissue (true hermaphrodite); the phenotype of the animal depends upon the amount of functional testicular tissue (Meyers-Wallen and Patterson, 1989).

Animals with abnormalities of gonadal sex are those in which chromosomal and gonadal sex are dissimilar. Such individuals are called sex-reversed. XX sex reversal is inherited as an autosomal recessive trait in the American cocker spaniel, and appears to be familial in other breeds (Meyers-Wallen and Patterson, 1988). Affected animals may conform to one of three categories: (1) true hermaphrodites with one ovotestis, bilateral uterine tubes and normal external female genitalia, (2) true hermaphrodites with ovotestes and/or epididymides and masculinised external male genitalia, and (3) XX males (Meyers-Wallen and Patterson, 1989). Animals with abnormalities of phenotypic sex are those in which chromosomal and gonadal sex are the same; however, the internal or external genitalia are ambiguous. Animals may be either female or male pseudohermaphrodites. Female pseudohermaphrodites generally occur as the result of androgen or progestogen administration during pregnancy; they have masculinisation of the external or internal genitalia but with two ovaries. The clinical appearance may vary from simple clitoral enlargement to almost male-like external genitalia. Progestogens administered during pregnancy have been most frequently implicated since these agents are used by some veterinarians to prevent alleged luteal deficiency. Male pseudohermaphrodites have testes, but the internal or external genitalia are feminised. This may be the result of failure of Müllerian duct regression or the failure of androgen-dependent masculinisation. In many cases, the exact aetiology remains unknown. However, removal of the reproductive tract including gonads is usually necessary. Following gonadectomy an enlarged clitoris may reduce in size although clitoridectomy may be necessary subsequently (see Chapter 4).

Acquired abnormalities

Acquired atrophy of the genitalia has been seen with neoplasia of the hypothalamus or pituitary (Arthur et al., 1989); this is termed Fröhlich's syndrome.

Other acquired abnormalities of the reproductive tract include endometrial hyperplasia and pyometra (which are discussed later) and vaginal hyperplasia (Figure 28.1). The latter condition, which is often wrongly called vaginal prolapse, may cause infertility by preventing mating. The aetiology is not clear; however, in some bitches the vaginal mucosa cranial to the urethral orifice becomes hyperplastic during pro-oestrus and oestrus and may protrude from the vulva and prevent mating. In some cases this is a simple tongue-shaped piece of tissue, whilst in other cases it may involve the entire circumference of the vagina, resulting in the protrusion of a cylindrical-shaped mass through the vulval lips. The hyperplasia appears to be an accentuated response to normal circulating oestrogen concentrations, which regresses at the beginning of metoestrus (dioestrus) but returns at the subsequent oestrus. In many cases, conservative

Fig. 28.1 Bitch with hyperplasia of the vagina during oestrus. The entire circumference of the vaginal mucosa is hyperplastic and protrudes from the vulva. Top is the dorsal commissure.

therapy using emollient creams and topical antimicrobial agents is sufficient. Recurrence may be prevented by performing an ovariohysterectomy during the subsequent anoestrus. However, if the bitch is required for breeding a submucosal resection may be performed during early oestrus. Breeding from these bitches should, however, be questioned since a familial tendency has been reported (Jones and Joshua, 1982).

True vaginal prolapse is very rare but has been reported during oestrus (Schutte, 1967a), and recently chronic prolapse during pregnancy requiring hysteropexy was described (Memon et al., 1993).

Neoplasia

Ovarian tumours are uncommon in the bitch, accounting for approximately 1% of all neoplasms (Cotchin, 1961; Hayes and Harvey, 1979). There is an increased incidence of ovarian neoplasia in older dogs (Jergins and Shaw, 1987); the mean age of occurrence is 8 years (Withrow and Susaneck, 1986). Ovarian tumours may be germ cell, epithelial or sex cord stromal in origin. The most important are granulosa cell tumours, which may become very large and produce clinical signs related to a mass effect or ascites. These tumours do not frequently metastasise and are usually endocrinologically inactive; however, they may secrete progesterone and produce cystic endometrial hyperplasia and pyometra, or oestrogen and produce signs of persistent oestrus or possibly bone marrow suppression. Rarely, alopecia is a presenting clinical sign. A less common tumour is the papillary cystadenocarcinoma, which may occur bilaterally (Neilsen, 1963). These tumours commonly metastasise to the peritoneal lymphatics producing obstruction and ascites. This neoplasm has been found to be a consequence of prolonged administration of stilboestrol.

The diagnosis of ovarian tumours is usually made on the basis of clinical signs, abdominal palpation, radiography and ultrasonography (Goodwin et al., 1990). Ovariectomy or ovariohysterectomy may be curative if performed early.

Uterine tumours are uncommon (Brodey and Roszel, 1967). The most frequently reported such lesions have been fibroleiomyomata. These are discrete and non-malignant, but haemorrhage may occur, resulting in a sanguineous vulval discharge. Uterine tumours may be diagnosed using realtime B-mode ultrasound (Figure 28.2).

Tumours of the cervix are rare, but benign tumours of the vagina and vestibule are more common and include fibromata, fibroleiomata and lipomata (Withrow and Susaneck, 1986) (Figure 28.3). These often originate from the ventral vaginal floor cranial to the urethral orifice and may cause a local vaginitis and haemorrhage. Usually they can be removed via an episiotomy; concurrent ovariectomy/ovariohysterectomy reduces the risk of recurrence.

Fig. 28.2 Ultrasound image of the uterus of a bitch demonstrating a hypoechoic uterine tumour (T). The uterus (U) lies dorsal to the bladder (B).

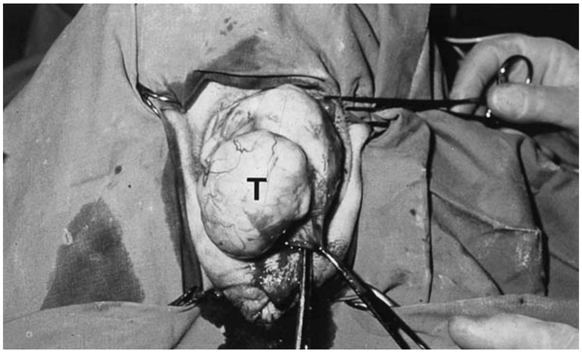

Fig. 28.3 Surgical removal of a large vaginal tumour (T) via an episiotomy.

The transmissible venereal tumour (TVT) (Figure 28.4) affects the vagina and external genitalia of the bitch and the penis of the dog (see Chapter 30). Transmission of the tumour occurs at coitus when infected cells 'seed' the genital mucosa of the recipient (Cohen, 1974). Autotransmission to the nasal and oral mucosa may occur by licking of the tumour. The lesions, which are often friable and multilobulated and may be single or multiple, generally reach their maximum size after 5–7 weeks and then regress spontaneously within 6 months (Moulton, 1961). The use of surgical debulking and various chemotherapeutic regimes has been described, including cyclophosphamide and vincristine, and radiation therapy (Calvert et al., 1982; Thrall, 1982). Such tumours are more common in tropical countries, and in the UK are generally only seen in imported animals (Booth, 1994).

Fig. 28.4 Transmissible venereal tumour within the vestibule of a bitch.

Functional abnormalities of the reproductive tract

Delayed puberty and prolonged anoestrus

The age of puberty in the bitch ranges between 5 and 24 months (Andersen and Wooten, 1959; Rogers et al., 1970; Concannon, 1991), although this is influenced by the breed, body weight and environmental conditions (Christiansen, 1984; Feldman and Nelson, 1987a; Concannon, 1991). Bitches that do not reach puberty by 2 years of age are therefore considered to have delayed puberty. Since it is not uncommon for there to be few signs associated with the first oestrus, bitches that are thought to have delayed puberty may simply have had an unobserved oestrus. Failure to identify oestrus should also be considered in bitches that have prolonged interoestrus intervals. A high peripheral plasma progesterone concentration demonstrates that ovulation has occurred within the last 60 days.

The normal interoestrus interval is between 26 and 36 weeks (Christie and Bell, 1971), and this is variable both within and between breeds (Linde-Forsberg and Wallen, 1992) and cannot be used to predict the next oestrus in an individual bitch (Bouchard et al., 1991). Therefore, it is difficult to define prolonged anoestrus, except that it is an interoestrus interval greater than that which was anticipated for a particular individual. The Basenji dog frequently exhibits its pubertal oestrus at approximately 300 days of age, and thereafter cycles annually (Concannon, 1993).

To investigate both delayed puberty and prolonged anoestrus it is necessary to ensure that an oestrus has not been missed and that the animal's body weight and nutritional plane are normal. Debilitating disease may result in a failure to cycle, as may the use of certain drugs including progestogens, androgens and anabolic steroids. Progesterone-producing ovarian cysts that prevent a return to oestrus have been described in the bitch (Burke, 1986), although these are very rare.

There has been considerable interest in the role of hypothyroidism as a cause of acyclicity in the bitch (Manning, 1979; Johnston, 1989). The mechanism of this is not fully understood, although the administration of thyrotrophin-releasing hormone (TRH) causes the release of prolactin (Reimers et al., 1978); therefore, factors that affect TRH are likely to affect both thyroid function and prolactin secretion (Concannon, 1986). It is rare for only reproductive signs to be present in dogs with hypothyroidism. Recently, hypothyroidism was shown not to be related to poor reproductive performance in greyhounds (Beale et al., 1992).

Induction of oestrus. It may be possible to induce cyclicity in bitches provided that there is no underlying disease. A variety of agents may be used for this purpose including oestrogens, luteinising hormone (LH) and follicle-stimulating hormone (FSH), gonadotrophin-releasing hormone (GnRH) agonists and prolactin inhibitors (England, 1994). Unlike in other domestic species, it is not possible to induce oestrus in the bitch by shortening the luteal phase using prostaglandins since the luteal phase is followed by a variable, but prolonged, period of anoestrus.

Prolactin is the principal luteotrophic hormone in the bitch. Administration of prolactin inhibitors (cabergoline, bromocriptine, metergoline) during the luteal phase rapidly removes the support for the corpora lutea, and results in a dramatic decline in plasma progesterone concentration (Onclin and Verstegen, 1999). Cessation of therapy at this time results in the bitch entering anoestrus. Prolactin also appears to play a role in the regulation of interoestrous intervals possibly by affecting gonadotrophin secretion and/or ovarian responsiveness to gonadotrophins (Concannon, 1993); if prolactin inhibitors are administered continually during anoestrus, there is a rapid return to oestrus (Okkens et al., 1985; van Haaften et al., 1989; Verstegen et al., 1999). There is also a rapid return to oestrus when anoestrus is prolonged (Arbeiter et al., 1988; Jöchle et al., 1989; Handaja Kusuma and Tainturier, 1993). Administration of cabergoline daily during anoestrus usually results in a return to oestrus within 30 days. If treatment is stopped once pro-oestrus has begun, there is usually a high pregnancy rate similar to natural oestrus. A similar regimen may be used for bitches in the luteal phase, although the return to oestrus is slower and pregnancy rates are lower, presumably because a certain period of time is required to allow endometrial sloughing before the next follicular phase. Currently, the use of prolactin inhibitors appears to be the most reliable and successful method of inducing oestrus in the bitch.

Exogenous oestrogens produce an increased responsiveness to basal concentrations of LH, which results in follicular growth and the production of endogenous oestrogen. Oestrogens have been used in a variety of regimens for oestrus induction, most using low doses for 7–10 days with or without subsequent gonadotrophin administration. Moses and Shille (1988) gave diethylstilboestrol (5 mg daily orally) until 2 days after the signs of pro-oestrus developed. If no response was elicited by day 7 the dose was increased to 10 mg daily for a maximum of a further 7 days. Subsequently, intramuscular injections of LH (5 mg) and FSH (10 mg) were given on days 5 (LH) and days 9 and 11 (FSH). All seven bitches in the study exhibited oestrous behaviour, were mated and whelped normally. However, subsequent studies by the same worker (Shille et al., 1989) were unsuccessful. Recently, diethylstilboestrol alone has been found to be very effective (Bouchard et al., 1993a). However, overall the pregnancy rates following oestrogen-induced oestrus are poor. One further concern is the risk of oestrogen toxicity, which includes dose-related bone marrow suppression, coat changes, mammary and vulval enlargement and potentiation of the stimulatory effects of progesterone on the uterus producing cystic endometrial hyperplasia and possibly pyometra.

Many protocols have used exogenous gonadotrophins for the induction of oestrus. Equine chorionic gonadotrophin (eCG) and human chorionic gonadotrophin (hCG) were first used by Scorgie (1939). Other workers have subsequently used these preparations at different dosages and different regimens with varying success (Wright, 1972, 1980; Jones et al., 1973; Allen, 1982; Nakao et al., 1985). Arnold et al. (1989) showed that high doses and long treatment times could induce hyperoestrogenism, prevent implantation and induce bone marrow suppression and death. These workers found that low doses (20 IU/kg of eCG for 5 days), with a single administration of 500 IU of hCG on the fifth day, produced more physiological changes in plasma hormones; however, pregnancy rates are usually low when compared with the use of prolactin inhibitors.

Exogenous pulsatile administration of GnRH may be used in an attempt to mimic natural profiles and induce physiological concentrations of FSH and LH. The pulsatile administration of GnRH to anoestrous bitches every 90 minutes for 6–12 days induced a fertile oestrus with pregnancy in three of eight bitches (Vanderlip et al., 1987). Pulsatile infusions are necessary because the constant infusion of GnRH produces initial stimulation followed by down-regulation of GnRH receptors. However, such techniques are not practical in the clinical situation. Concannon (1989) achieved some success in inducing oestrus using a GnRH superagonist administered via a subcutaneous osmotic pump. Concannon et al. (1993) recently showed that oestrus could be synchronised in a group of bitches by initially preventing oestrus using progesterone, and subsequently inducing oestrus using a GnRH agonist.

Silent oestrus

Normal cyclical endocrine changes may occur in some bitches without obvious external signs of pro-oestrus or oestrus. This may occur in up to 25% of greyhound bitches at the first cycle after puberty (Gannon, 1976). It is possible that oestrus events are not observed by the owner because there is only slight vulval swelling and minimal serosangineous discharge, or because the bitch is particularly fastidious. On some occasions overt pseudopregnancy occurs in the absence of a preceding observed oestrus. However, ovulation can be confirmed by the measurement of peripheral plasma progesterone concentration. If suspected, oestrus can be detected by the weekly examination of exfoliative vaginal cytology.

Split oestrus (false oestrus)

Occasionally, at their first oestrus, bitches develop vulval swelling and a serosangineous vulval discharge of a short duration. However, ovulation does not occur; follicles presumably regress and the signs of pro-oestrus disappear. A normal oestrus follows several weeks later. The recognition of the split oestrus syndrome is important to ensure that mating is achieved at the correct time in relation to ovulation.

Unpredictable ovulation time

Most bitches ovulate between 10 and 14 days after the onset of pro-oestrus (see Chapter 1). However, ovulation in normal bitches may occur as early as day 5, or as late as day 30, after the first signs of proestrus (Figure 28.5). In addition to this large variation, bitches are not necessarily consistent between cycles (England et al., 1989a). Infertility may therefore result because of attempted matings at inappropriate times in relation to ovulation (see 'Management factors affecting fertility', p. 655).

Prolonged pro-oestrus/oestrus

The normal interval between the onset of proestrus and ovulation varies from 5 to 30 days (England et al., 1989a; England, unpublished observations). However, most bitches ovulate by day 14 after the onset of pro-oestrus, and those that ovulate later than this are often considered to have prolonged oestrus. These animals do not require treatment but require careful assessment of the optimal mating time. Cases in which pro-oestrus or oestrus persists longer than 30 days (Wright, 1990) may require treatment. The induction of ovulation may be attempted by the administration of hCG (20 IU/kg). It can be difficult to predict the correct time to administer hCG, since its early use may result in either failure of response or possibly luteinisation without ovulation. Commonly, hCG is administered once more than 80% of exfoliated vaginal epithelial cells are anuclear.

Oestrogen-secreting follicular cysts are very rare in the bitch, but these may produce persistent oestrus. Similar clinical signs may be seen with oestrogen-secreting ovarian tumours where high concentrations of oestrogen may lead to bone marrow suppression, resulting in anaemia and thrombocytopenia. In such cases, treatment is by

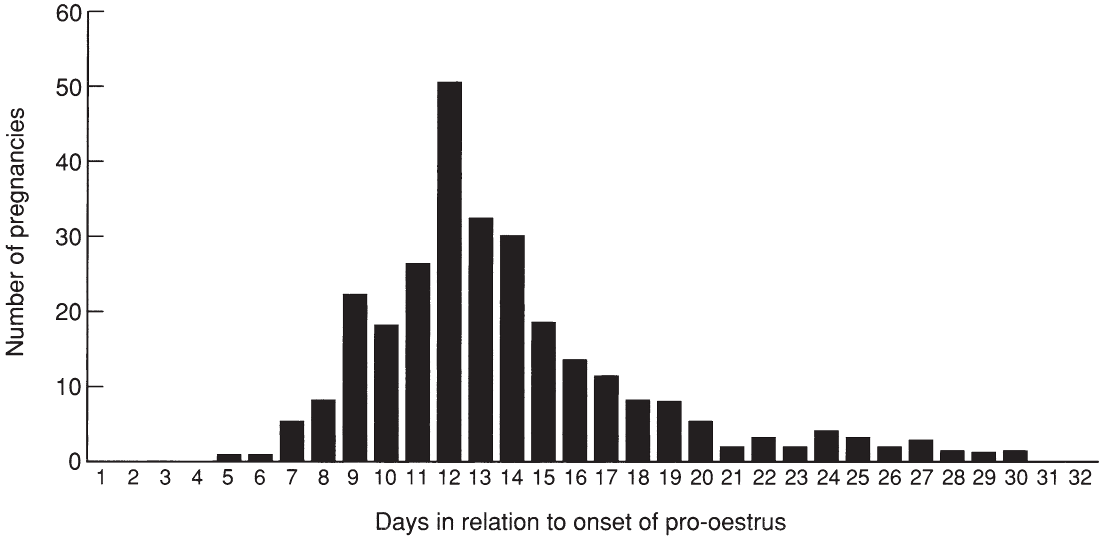

Fig. 28.5 The relationship between the calculated day of ovulation and the number of days from the onset of pro-oestrus in 278 bitches (England, unpublished observations).

unilateral ovariectomy, although consideration should be given to the fact that the bitch may be normal or have a split oestrus syndrome. The administration of lithium carbonate may be useful in cases of oestrogen-induced bone marrow suppression (Hall, 1992).

Ovulation failure

Until recently, the diagnosis of ovulation failure was most commonly made on the basis of a shortened interoestrus interval (Johnston, 1988). However, following the introduction of routine monitoring of plasma progesterone concentrations, bitches that fail to ovulate have been detected (Wright, 1990; Arbeiter, 1993). The incidence of ovulation failure has not been established; however, attempts at treatment may be made by the administration of hCG (Johnston, 1991). In some instances there may be a delay in ovulation, that is, a slow but prolonged increase in plasma progesterone prior to ovulation. These cases are also often treated with hCG, but efficacy remains to be proven.

Ovarian cysts

Cystic follicles (Figure 28.6) and corpora lutea are very rare in the bitch; most ovarian cystic structures are of parabursal origin (Figure 28.7) and have no clinical significance.

Fig. 28.6 An ovary of a labrador bitch demonstrating multiple follicular cysts.

Oestrogen-secreting follicular cysts may produce persistent oestrus with vulval discharge, flank alopecia and hyperkeratosis (Fayrer-Hosken et al., 1992); unilateral ovariectomy or ovariohysterectomy is necessary for the control of the clinical signs (Vaden, 1978; Burke, 1986). Attempts

Fig. 28.7 The reproductive tract of a bitch demonstrating multiple parabursal cysts adjacent to the ovary and proximal uterine horn.

to cause luteinisation of the cysts using hCG are usually disappointing (Arthur et al., 1989).

Luteal cysts have been identified in post-mortem studies (Dow, 1960); however, their significance is unknown. Burke (1986) suggested that they may secrete progesterone and produce prolonged anoestrus (sic) and cystic endometrial hyperplasia.

Andersen (1970) found that follicular and luteal cysts were most common in older bitches. Similar findings have been noted in the ovaries of aged bitches, which have a mucohaemorrhagic vulval discharge, a condition referred to as metrorragie (Lesbouyries and Lagneau, 1950). These bitches are often attractive to male dogs but will not allow coitus; a study of exfoliative vaginal cells demonstrates neutrophils, erythrocytes and parabasal epithelial cells. This condition is often persistent and unresponsive to medical therapy such that ovariohysterectomy is usually the treatment of choice.

Premature ovarian failure

Premature ovarian failure has been suggested as a rare but permanent cause of anoestrus in previously normal bitches (Feldman and Nelson, 1987b). For an accurate diagnosis of these cases, investigation of the karyotype and measurement of plasma concentrations of LH, FSH and thyroid hormone should be undertaken (Johnston, 1989). In valuable breeding animals, oestrus induction regimens may be contemplated; however, there is no information on the efficacy of these treatments in animals with premature ovarian failure.

Habitual abortion

There is little, other than anecdotal, evidence to suggest that habitual abortion is a clinical problem in the bitch. However, cases of abortion and resorption have been documented using real-time B-mode ultrasound (England, 1992; Muller and Arbeiter, 1993), although England (1992) suggested that there was no increased incidence in those bitches that had previously had reproductive disease. Cases of abortion are probably related to an abnormal uterine environment (cystic endometrial hyperplasia), fetal defects and/or the result of infectious agents. However, progesterone deficiency due to poor luteal function is implicated by some workers (Feldman and Nelson, 1987a; Purswell, 1991). There is no doubt that the corpora lutea remain the principal source of progesterone production throughout gestation, and that pregnancy may be terminated by ovariectomy (Andersen and Simpson, 1973) or the induction of luteolysis (Onclin et al., 1993) at any stage. However, the minimum concentration of progesterone required to support the pregnancy is only 2 ng/ml (Concannon and Hansel, 1977). Measurement of progesterone concentration at the time of an abortion often reveals that concentrations are low; however, this is likely to be the result of the abortion rather than the cause. Insufficient luteal function has been demonstrated following oestrus induction regimens (Barta et al., 1982) and in one case of oophoritis

(Nickel et al., 1991). In the author's experience, plasma progesterone concentrations of bitches with habitual abortion are not dissimilar to those of normal pregnant bitches. Progesterone or progestogen supplementation during pregnancy may produce masculinised female pups (Curtis and Grant, 1964) and cryptorchid male pups, and may possibly impair or delay parturition resulting in fetal death (see Chapter 6). Progestogen therapy should be restricted to those cases in which a true luteal insufficiency has been diagnosed.

Infectious agents

There are three categories of organism that may exert an influence upon fertility: firstly, those agents that are known to have a specific pathogenic effect upon the reproductive tract; secondly, those organisms that are present in the normal environment and under certain circumstances can become opportunistic pathogens; and, thirdly, agents that cause systemic disease and exert their effect upon reproduction indirectly.

Normal vaginal bacterial flora

There is a widespread belief among breeders and veterinarians that infertility, vaginitis and fading puppy syndrome are caused by bacteria that inhabit the reproductive tract of the dog and bitch. This arose from the work of Stafseth et al. (1937) and Hare and Fry (1938), who concluded that streptococci, especially β-haemolytic types G and L, were responsible for infertility, abortion, anoestrus and weak pups. With the advent of virus isolation techniques, several specific viruses have been identified, and it seems likely that the earlier work over-emphasised the importance of the streptococci. These bacteria are now considered to be part of the normal commensal flora and probably invade subsequent to viral damage, or are contaminants.

Many aerobic and anaerobic bacteria normally inhabit the vestibule and vagina of the healthy bitch (Olson and Mathur, 1978), and the bacterial flora is normally mixed. The aerobic bacteria isolated from normal bitches include *Escherichia coli*, staphylococci and streptococci (Olson and Mathur, 1978; Allen and Dagnall, 1982) whilst the anaerobic bacteria include *Bacteroides spp.* and *Peptostreptococcus spp.* (Baba et al., 1983). Mycoplasmas have been isolated from between 30 and 88% of normal bitches (Bruchim et al., 1978; Doig, 1981; Baba et al., 1983). Greater numbers of bacteria are found within the vestibule compared with the vagina; the uterus is normally sterile (Olson et al., 1986). The stage of the oestrus cycle may influence the bacterial flora, because there is a significant increase in vaginal bacterial numbers when oestrogen concentrations are elevated (van Duijkeren, 1992). Several authors have examined the vaginal bacterial flora of normal bitches and compared them with those of infertile bitches. These studies were recently reviewed by van Duijkeren (1992), who showed that the bacterial species cultured from infertile bitches did not differ significantly from healthy bitches. Similarly, Hirsh and Wiger (1977) found that the organisms recovered from bitches with vaginal discharge were qualitatively the same as the normal bacterial flora, although the bacterial numbers were higher.

Therefore, the results of microbiological examination of the reproductive tract of the bitch must be treated with caution because the simple isolation of bacteria from the vagina does not constitute a diagnosis of reproductive disease.

Opportunist pathogens

The bacterial species found in bitches with reproductive disease do not differ significantly from those found in healthy bitches. However, disease may result if the uterine or vaginal defence mechanisms are depressed, thereby allowing overgrowth of the normal commensals (Olson et al., 1986). Many of the normal vaginal inhabitants may become pathogens if a breakdown in local immunity occurs (van Duijkeren, 1992).

Bacteria may enter the uterus during oestrus when the cervix is relaxed, and could then cause infertility either directly by interfering with the zygote or by producing spermicidal factors (Jones and Joshua, 1982). Bacteria might persist within the uterus and be associated with the development of pyometra during the progesterone-dominant phase of the cycle.

If vaginal microbiological sampling reveals bacteria present in a pure growth or in very large numbers, then they may be considered significant, although pure growths of bacteria may also be isolated from normal dogs (Bjurstrom and Linde-Forsberg, 1992). Those bacteria most commonly thought to be significant by some workers, but not this author, include *Pseudomonas spp.*, *Proteus spp.* and some streptococci. Repeated culture after 1 week should be performed to confirm the diagnosis before attempting treatment. Appropriate antimicrobial therapy, based upon sensitivity tests, should only be administered after investigation of possible predisposing causes such as anatomical, neoplastic or mechanical abnormalities of the vagina. Parenteral and topical administration has been advocated.

Mycoplasmas and ureaplasmas have been implicated in causing reproductive disease in the bitch (Lein, 1986), although they are also frequently isolated in clinically normal animals with no evidence of reproductive tract disease. Mycoplasma colonisation of the vagina has recently been demonstrated following prolonged treatment of bitches with oral ampicillin and potentiated sulfonamides (Strom and Linde-Forsberg, 1993), which suggests that the widespread use of antimicrobial agents in healthy bitches should be avoided.

Specific infections

Brucella canis. *B. canis* is a Gram-positive bacterium that can produce abortion and infertility. It is the only bacterium known to be a specific cause of infertility in the bitch. Brucella infertility was first reported in the USA (Moore and Bennet, 1967; Carmichael and Kenney, 1968) but has subsequently been found in several countries. Barton (1977) found that between 1.5 and 6.6% of dogs in the USA had antibodies diagnostic of infection; however, *B. canis* is not present in the UK, although Taylor et al. (1975) reported brucella abortion in one bitch. *B. canis* can be transmitted in several ways, including contact with aborted fetal or placental tissue, contact with the vaginal discharge of infected bitches, venereal transmission and congenital infection. The most common method of infection is venereal (Moore and Gupta, 1970). Abortion occurs most commonly between days 45 and 55 of pregnancy; however, there may be early fetal resorption, or the birth of stillborn or more rarely weak pups.

The isolation of the bacterium from blood or aborted tissue is diagnostic of the disease; however, there may be prolonged periods when the bitch is not bacteraemic, so that a negative blood culture does not rule out infection. Fortunately diagnosis, using the plate agglutination test for screening and tube agglutination for confirmation, is not difficult, titres of 1:200 or greater being diagnostic of infection. Treatment of the condition with a combination of streptomycin and tetracycline is often effective in clinical cases; however, antimicrobial treatment does not remove the organism from tissues (Johnston et al., 1982). Since a carrier state can occur and these animals may be potential sources of infection, they are best neutered to remove them from the breeding programme.

Toxoplasma gondii. *T. gondii* infection causes abortion, premature birth, stillbirth and neonatal death (Cole et al., 1954; Siim et al., 1963). Surviving infected pups may carry the infection. The public health consequences of toxoplasma infection should be considered whenever it is diagnosed.

Canine herpesvirus. Canine herpesvirus in adult dogs generally produces a few mild signs limited to the respiratory or genital tract. However, the virus may cause genital lesions in the bitch that may be associated with infertility, abortion and stillbirths (Hashimoto and Hirai, 1986). It appears that infection of the pregnant bitch results in the production of placental lesions and the infection of the fetuses (Hashimoto et al., 1979). The infected placentae are macroscopically underdeveloped, and possess small greyish white foci characterised by focal degeneration, necrosis and the presence of eosinophilic intranuclear inclusion bodies. Experimental data suggest that infection during early pregnancy may result in fetal death and subsequent mummification, whilst infection during midpregnancy results in abortion, and infection during late pregnancy results in premature birth (Hashimoto et al., 1979). The virus has also been recovered from vesicular lesions on the genitalia of bitches (Post and King, 1971). Variable-sized vesicles are commonly observed in the vestibule (Hashimoto et al., 1983), and frequently these lesions are evident at the onset of pro-oestrus, suggesting that venereal transmission is probably important in adult dogs. Recrudescent canine herpes, with virus shedding from the vesicular lesions, may be stimulated by the stress of pregnancy and parturition. Pups may become infected at birth, during passage through the vagina, and subsequently die with characteristic widespread histological necrotising lesions (Carmichael, 1970). Pups that survive the illness may show persistent neurological disorders (Percy et al., 1970). Pups are only at risk whilst in utero and during the first 3 weeks of life; attempts to produce the generalised disease in older pups have failed (Wright and Cornwell, 1970a). In the pups, the disease is rapidly fatal and treatment is often unrewarding; symptomatic therapy is all that is available since specific antiviral agents are not efficacious (Wright and Cornwell, 1970b). Canine herpesvirus infection is becoming increasingly recognised within Europe. As a result of this a new vaccine has recently been licensed for use in breeding animals, and is commercially available in France.

Canine adenovirus. It is well established that infection with canine adenovirus during pregnancy can result in the birth of dead or weak pups that die within a few days of parturition (Spalding et al., 1964). In most cases, however, the virus is ingested and causes neonatal mortality (Cornwell, 1984). Carrier bitches may therefore act as a source of infection for pups.

Canine distemper virus. Experimental exposure of pregnant bitches to canine distemper virus was found to produce either clinical illness in the bitch with subsequent abortion, or subclinical infection of the bitch and the birth of clinically affected pups (Krakowka et al., 1977). This provides evidence for transplacental transmission, although the frequency of this under natural conditions is unknown.

Canine parvovirus. Canine parvovirus has been implicated by some breeders as a cause of infertility in their kennels. However, Meunier et al. (1981) found that the conception rate, incidence of stillbirths, average litter size or average number of pups weaned per litter did not change after the introduction of canine parvovirus to a kennel of 2000 brood bitches. Canine parvovirus may cause an acute generalised infection in pups less than 2 weeks of age, which can occur as a consequence of uterine infection or as a result of exposure to the virus soon after birth (Guy, 1986).

Cystic endometrial hyperplasia and pyometra

Aetiology. Although the exact aetiology of cystic endometrial hyperplasia and pyometra is uncertain, this syndrome is probably best categorised as an infectious cause of infertility even though the role of the endocrine environment is significant. It has been suggested that cystic endometrial hyperplasia, which precedes pyometra as a clinical disease, may result in infertility due to conception failure and embryonic resorption. The condition may be recognised by ultrasound examination of the uterus during the luteal phase. The detection of multiple small, focal, fluid-filled cystic regions within the endometrium is diagnostic; endometrial biopsy is not necessary. In many cases cystic endometrial hyperplasia leads ultimately to pyometra, the incidence of which is high. In fact, pyometra is recognised as one of the common causes of illness and death in this species.

There has been considerable debate over the predisposing factors and the exact aetiology of pyometra. Most observers are of the opinion that the spontaneous disease is of middle-aged or old bitches. Dow (1958, 1959a) reported that the mean age of clinical cases was 8.2 years, with only 12% of cases under 6 years of age. Several workers have suggested that the condition is more common in nulliparous bitches (Dow, 1958, 1959a; Frost, 1963) whilst others have suggested that it is more common in bitches with abnormal oestrous cycles and pseudopregnancy (Dow, 1959b; Whitney, 1967). Fidler et al. (1966), however, found no relationship to parity or oestrous characteristics; this opinion is now widely accepted. Pyometra is a disease of the luteal phase, with most bitches showing clinical signs between 5 and 80 days after the end of oestrus (Figure 28.8).

Early attempts to produce the condition by introducing bacteria into the uterus were unsuccessful (Benesch and Pommer, 1930; Teunissen, 1952); however, the latter worker managed to induce endometritis when bacteria were intro-

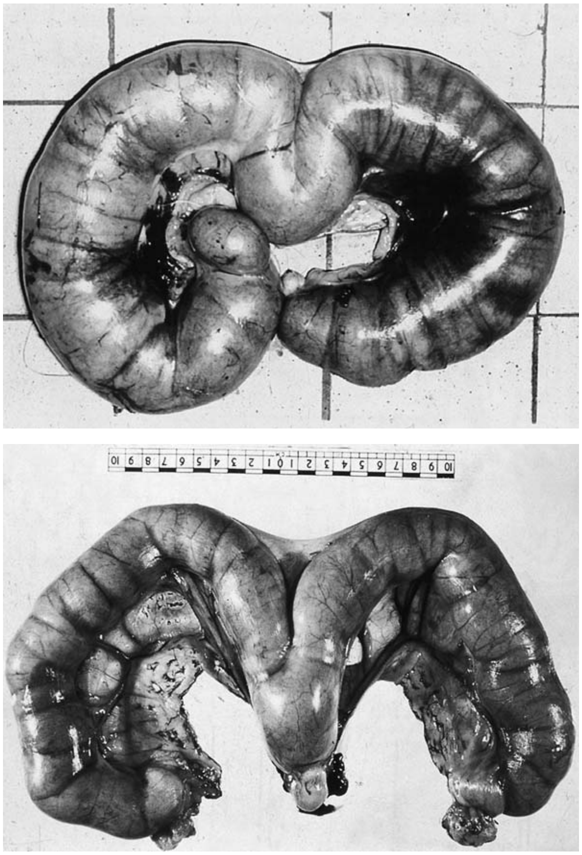

Fig. 28.8 The reproductive tracts from two bitches with pyometra. Note the different degrees of distension.

duced into the oestrous uterus during laparotomy when the uterine horn was also ligated. Although successful attempts to produce the disease following the administration of oestrogens have been reported (Bloom, 1944; von Schulze, 1955), it was the work of Teunissen (1952) that indicated the importance of progesterone in the aetiology of the condition, and also demonstrated the potentiation of the effects of progesterone by oestrogen. Teunissen's general conclusions were that progesterone was the main hormone concerned with inducing uterine glandular hyperplasia which preceded pyometra. Continuing glandular hyperplasia occurs under the influence of progesterone and regresses at the end of the luteal phase. However, during the animal's life there is progressive hyperplasia, which ultimately results in the development of pathological lesions termed cystic endometrial hyperplasia. The mucosal epithelial cells are characteristically tortuous with a hypertrophic clear cytoplasm (Hardy and Osborne, 1974). It is not known whether all cases of spontaneous pyometra are preceded by cystic endometrial hyperplasia, but this seems likely.

Much attention has been paid to the work of Dow (1959b), who was able to produce pyometra experimentally in young ovariectomised bitches by the administration of cycles of oestrogen and progesterone. Cystic endometrial hyperplasia was induced following three such cycles of treatment but there were no inflammatory changes in the endometrium. If, however, in the fifth or sixth cycle the dose of progesterone was increased, typical acute endometritis became superimposed upon the cystic glandular hyperplasia. It is worth noting that the dose rates of the hormones used were very high. Successful induction of pyometra was achieved by Teunissen (1952) without the need for cycles of oestrogen and progesterone; this difference may have been due to the age of the bitches used in the study since Dow's bitches were between 9 and 18 months of age whereas Teunissen's bitches were up to 5 years old.

It was suggested that pyometra was the result of excessive and/or prolonged stimulation of the uterus by progesterone from 'retained' or 'cystic' corpora lutea (Hardy and Osborne, 1974). However, although corpora lutea are always present within the ovaries of bitches with clinical pyometra (the result of the long luteal phase), there is no evidence of excessive progesterone production (Christie et al., 1972). Progesterone concentrations in bitches with pyometra are similar to those in healthy bitches at the same stage of the luteal phase (Hadley, 1975a; Chaffaux and Thibier, 1978; De Coster et al., 1979) and the functioning capacity of the corpora lutea has been shown to be normal (Colombo et al., 1982).

Hadley (1975b) inadvertently produced a cystic endometrial hyperplasia in bitches that were subjected to repeated uterine biopsy during the early luteal phase of the cycle. These animals were much younger than those that normally develop the lesion and had not been subjected to any hormone therapy.

Dhaliwal et al. (1997, 1999) found that various concentrations of circulating steroid hormones, particularly oestrogen and progesterone, whether endogenous or exogenous, influence the concentration and distribution of steroid receptors within the uterus of bitches. Such changes may be involved in the pathogenesis of pyometra.

Organisms isolated from the uterine fluid in cases of pyometra are those found as part of the normal vaginal and vulval microflora. It is generally agreed that the predominant bacterium isolated is *Escherichia coli* (Dow, 1960; Grindlay et al., 1973). Sandholm et al. (1975) found that the progesterone-sensitised endometrium and myometrium had an affinity for *E. coli*. These workers postulated that urinary tract infection was associated with pyometra, the uterus becoming infected during early metoestrus when receptors for *E. coli* develop within the endometrium, thus enhancing the colonisation of the uterus with bacteria. It is likely that the long luteal phase of the bitch is an important contributory factor in the development of the condition since it has been shown that in the cow, progesterone increases the susceptibility of the genital tract to infection (Rowson et al., 1953). Brodey (1968) hypothesised that anogenital bacteria entered the uterus during oestrus and were able to proliferate during metoestrus. This suggestion offers a plausible explanation of the aetiology, since the condition is more likely to develop when there is cystic endometrial hyperplasia; however, it has also been suggested that bacteria may enter the uterus either haematogenously or via lymphatic spread (Teunissen, 1952). A further factor that must be considered in the aetiology of pyometra is the use of exogenous reproductive hormones. Anderson et al. (1965) reported the occurrence of pyometra following the use of medroxyprogesterone acetate for the prevention of oestrus. Similar findings have been observed with several other progestogens. The experimental use of oestrogens in the bitch does not usually result in the development of pyometra. However, oestrogens enhance the stimulatory effects of progesterone on the uterus. For this reason, when oestrogens are administered post-mating to prevent conception, pyometra may be induced (Durr, 1975; Nelson and Feldman, 1986); this probably relates to the increasing concentrations of progesterone seen during oestrus.

Clinical signs. There are a wide range of clinical signs associated with pyometra in the bitch. When a complete history is available it is usually found that the bitch was in oestrus a few weeks prior to the illness. In some cases, when there is a vulval discharge, the owner may consider this to be a continuation of oestrus (Table 28.1).

If presented early, the general history is that the animal has been lethargic with a reduced appetite. Increased thirst and vomiting are variable findings. Some bitches may be presented later when there is a vulval discharge, which in some cases is associated with improvement in the general health. In other cases, the bitch remains unwell and there is no discharge of pus. The bitch's abdomen may become distended and she may be thought to be pregnant although systemic illness is common. These cases generally end fatally, often within 14–21 days from the onset of clinical signs; the cervix remains closed throughout. Death may be due to toxaemia alone or it may be associated with peritonitis due to rupture of the uterus. Occasionally, the cervix relaxes and there is an outpouring of pus just before death.

In a further category, there may be intermittent opening of the cervix, with relative good health following the discharge of pus, and malaise during the intervening periods. Such cases generally succumb from toxaemia in the course of a month or two.

Some cases of open-cervix pyometra may persist for years with a more or less continuous vulval discharge. Body temperature may be normal or slightly elevated in cases of open-cervix pyometra, whilst there is commonly an elevated body temperature in cases of closed-cervix pyometra. In toxaemic patients the temperature may be subnormal.

The character of the vulval discharge may vary considerably. Most often it is of thin consistency and light chocolate brown in colour, and has a characteristic odour. In other cases it is yellow in colour, often blood-tinged, and varying from a watery to a creamy consistency. The vulva is generally enlarged and there may be discoloration or scalding of the perivulval tissues and perineum.

An increased thirst is commonly observed in advanced cases, which is due to reduced permeability for water in the distal convoluted tubule of the kidney (Asheim, 1964). Renal dysfunction is probably caused by the formation of immune complexes (Sandholm et al., 1975).

Table 28.1 Differential diagnosis of vulval discharge in the bitch (adapted from Allen and Renton, 1982)

Nature of the discharge	*Condition*	*History*	*Condition of the vulva*	*Cytological findings**	*Comments*
Clear or straw-coloured	Oestrus	Expected in 'heat'	Swollen or slightly soft	LIEC, AEC, RBC, no WBC	Attractive to male
Mucoid	Metoestrus	Recent oestrus	Large but soft	PBC, SIEC, VSIEC, WBC	No malaise
Mucoid	Normal pregnancy	Pregnant/recent oestrus	Large but soft	PBC, SIEC, WBC	No malaise, does not threaten pregnancy
Purulent	Juvenile vaginitis	Before first 'heat'	Normal	PBC, SIEC, WBC	May respond to antibiotics but recurs. Recovery after puberty
Purulent	Vaginitis	Variable but often excessive licking, attractive to male	Depends on the stage of the cycle	Depends on the stage of the cycle	Specific causes include: certain bacterial or viral infections, chemical irritation (urine), mechanical irritation (foreign body), neoplasia and anatomical abnormalities
Purulent/ haemorrhagic	Pyometra	Oestrus 2–8 weeks previously	Slightly swollen	WBC, SIEC, LIEC, RBC, bacteria, cell debris	Diagnosis using ultrasonography or radiography. Often malaise
Purulent/ haemorrhagic	Metritis	Recent parturition	Large	Multinucleated cells, LIEC, uterine cells	Severe malaise
Haemorrhagic	Pro-oestrus	Expected in 'heat'	Swollen	SIEC, LIEC, RBC, WBC	Attractive to male
Haemorrhagic	Oestrus	Expected in 'heat'	Swollen or slightly soft	LIEC, AEC, RBC, no WBC	Attractive to male
Haemorrhagic	Follicular cysts	Persistent discharge	Swollen	LIEC, RBC, ± WBC	No malaise, attractive to male, may develop bone marrow suppression
Haemorrhagic	Vaginal ulceration	Recent trauma or mating	Depends on the stage of the cycle	RBC, depends on the stage of the cycle	Rare, may start up to 2 weeks after mating
Haemorrhagic	Placental separation	Pregnant	Normal or slightly swollen	RBC, mucus	Ultrasound, radiography, etc., will confirm pregnancy
Haemorrhagic	Subinvolution of placental sites	Persistent discharge after whelping	Normal or slightly swollen	RBC, large polynucleated vacuolated cells	No malaise, refractory to treatment
Haemorrhagic	Transmissible venereal tumour	Not all countries of the world	Depends on the stage of the cycle	RBC, tumour cells?	Identification of tumour on vulva or in vagina confirms diagnosis
Haemorrhagic	Cystitis	Frequent urination	Depends on the stage of the cycle	RBC, mucus	Small volumes of urine, dysuria
Haemorrhagic	Urinary tract neoplasia	Dysuria	Depends on the stage of the cycle	RBC, tumour cells?	Endoscopy may show origin of haemorrhage, positive contrast cystourethrography may be diagnostic
Haemorrhagic/ brown-coloured	Abortion	Pregnant	Slightly enlarged	RBC, mucus	Ultrasound shows uterus with similar appearance to postpartum
Green/brown-coloured	Parturition	Pregnant	Slightly swollen	RBC, SIEC, uterine cells	Panting, nest-making, milk production
Green/brown-coloured	Dystocia, placental separation	Non-productive straining	Slightly swollen	RBC, SIEC, uterine cells	Ultrasound will confirm pregnancy and fetal viability

*PBC, parabasal cells; SIEC, small intermediate epithelial cells; LIEC, large intermediate epithelial cells; AEC, anuclear epithelial cells; RBC, erythrocytes; WBC, polymorphonuclear leucocytes; VSIEC, vacuolated small intermediate epithelial cells ('metoestrus cells'). Adapted from Allen and Renton (1982).

Diagnostic features

Abdominal palpation. Before examination the animal should be given the opportunity to urinate and defecate. In cases of open-cervix pyometra the uterine horns may be detected as thickened, often irregular and slightly turgid structures from 1 to 3 cm in diameter. Their location within the abdomen is not generally altered from normal. Occasionally, some areas of the uterine horns are turgid and solid to palpate whilst others, which are distended by pus, may be indistinguishable from the surrounding bowel. Care must be taken not to confuse the colon with thickened uterine horns. In cases of closed-cervix pyometra the degree of uterine distension may be greater, and there may be visible abdominal enlargement. In large or obese patients, abdominal palpation may not be possible.

Ultrasonography. Ultrasound is particularly valuable for detecting the uterus that is filled with fluid. The uterus has an increased diameter and may be folded upon itself so that several sections of each horn may be imaged in a single plane (Figure 28.9). The diameter of the uterus may vary depending upon whether the cervix is open or closed. The uterine wall is usually relatively hypoechoic and is increased in thickness. The uterine lumen is usually grossly dilated with anechoic fluid, although small echogenic particles and mass lesions may be identified. The diagnosis is most simple when the diameter of the uterus increases above that of the small intestine. In cases where there are large volumes of uterine fluid, there is usually a far enhancement effect (Feeney and Johnston, 1986). Poffenbarger and Feeney (1986) noted that when the uterus was imaged at its proximal end the transverse image had a target configuration. Recently, Renton et al. (1993) suggested that ultrasonography could be used to monitor cases of pyometra during treatment.

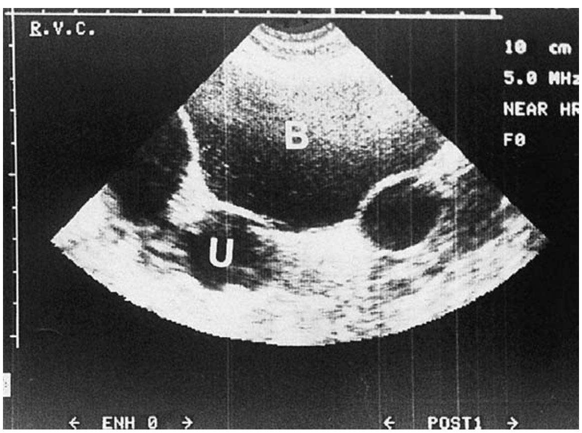

Fig. 28.9 Ultrasound image of the reproductive tract of a bitch with pyometra. The uterine horns (U) are distended with anechoic fluid and can be seen in three cross-sectional planes dorsal to the bladder (B).

Radiography. The detection of a soft tissue opacity mass lesion within the caudal abdomen, causing cranial displacement of the small intestine and dorsal displacement of the colon, has been used for some time to indicate enlargement of the uterus (Engle, 1940; Schnelle, 1940; Walker, 1965). It should be remembered, however, that these findings are not specific for pyometra, since early pregnancy has a similar radiographic appearance. Pneumoperitoneography may be a useful aid, which allows clearer radiographic differentiation of the uterus in cases of pyometra (Glenney, 1954), but this is not routinely performed.

Haematology. The total number of leucocytes is frequently elevated in cases of pyometra (Khuen et al., 1940), although the degree is much less marked in cases of open-cervix pyometra compared with closed-cervix pyometra (Morris et al., 1942). However, an elevated white cell count is not always present (Sheridan, 1979).

Rectal examination. It may be possible to palpate the distended uterus per rectum especially if slight backward pressure is applied to the abdominal wall.

Treatment. Ovariohysterectomy is the treatment of choice for pyometra. Bitches that are presented early in the course of the disease are usually a low surgical risk, and success rates up to 92% have been reported (Austad, 1952). Lower success rates may be obtained with bitches that are toxaemic. Intravenous fluid therapy is essential in all cases to ensure minimum renal toxic effects (Ewald, 1961). Attention should also be paid to plasma electrolytes and acid–base status since complications associated with septicaemia, bacteraemia and uraemia are common (Feldman and Nelson, 1987a). Whilst the ideal situation is to administer intravenous broad-spectrum antimicrobial agents and fluid therapy, it is not always possible to stabilise the patient before surgery.

If the condition is not life-threatening and the animal is particularly valuable, the question of restoration of fertility may be considered. Attempts have been made to drain the uterine fluid using a catheter placed via the cervix (Stephenson and Milks, 1934; Funkquist et al., 1983). However, this technique is difficult to perform, and surgically introduced drains have been advocated by some workers (Mara, 1971; Gourley, 1975). These are inserted transcervically via a hysterotomy and are used to flush the uterus after surgery. High success rates have been reported using this method (Mara, 1971).

In those cases where it is essential to retain reproductive function, or where surgery is not possible because of intercurrent disease, medical therapy may be considered. There have been several reports of successful medical management using oestrogens (presumably to induce cervical relaxation) (Watson, 1942; Fethers, 1943), drugs to induce uterine contraction including ergometrine (Hornby, 1943), quinine (Cowie and Muir, 1957), etamiphylline (Thomas, 1980) and several other agents (Spalding, 1923; Linde, 1966). However, since pyometra is a disease of the luteal phase and ovariectomy has been shown to produce resolution of the clinical signs (Watson, 1957), there has been considerable interest in the use of prostaglandins to cause lysis of the corpora lutea as well as for their uterine spasmogenic action (Swift et al., 1979; Sokolowski, 1980; Henderson, 1984; Wheaton and Barbee, 1993). Prostaglandins have been used successfully in the treatment of cases of open-cervix pyometra (Nelson et al., 1982; Gilbert et al., 1989), even in those cases in which progesterone concentrations were low (Renton et al., 1993).

A protocol of 0.25 mg/kg of dinoprost administered daily by subcutaneous injection has been recommended (Feldman and Nelson, 1987b), although the twice-daily administration of 0.125 mg/kg may result in fewer adverse effects. These include restlessness, pacing, hypersalivation, tachypnoea, vomiting, diarrhoea, pyrexia and abdominal pain; they may be severe and can persist for up to 60 minutes. Hospitalisation and careful observation of the patient are necessary during such treatment. Prostaglandin therapy should be combined with appropriate broad-spectrum antimicrobial agents and intravenous fluid administration. Whilst prostaglandins have been used in cases of closed-cervix pyometra (Feldman and Nelson, 1987b), this is not recommended because of the risk of uterine rupture (Jackson, 1979; Renton et al., 1993). Reported success rates have varied; one bitch of three treated by uterine drainage subsequently became pregnant (Lagerstedt et al., 1987), whilst Feldman and Nelson (1987b) found that 37 of 42 bitches subsequently whelped after treatment of open-cervix pyometra with prostaglandin. Gilbert et al. (1989) achieved a clinical cure in 33 of 40 bitches, and of these, nine eventually produced litters. The long-term complications were anoestrus, recurrence of metritis, failure to conceive and abortion.

More recently, the combined use of the prolactin inhibitor cabergoline (5.0 μg/kg/day) and cloprostenol (5.0 μg/kg every other day) for up to 10 days has been shown to be successful. In our laboratory we have treated five bitches, each of which has subsequently become pregnant.

Newer treatment opportunities including the administration of the progesterone receptor antagonist aglepristone (Breitkopf et al., 1997) may be useful in the future for treatment of this condition.

Management factors affecting fertility

The majority of bitches presented for fertility investigation are normal healthy fertile animals whose apparent infertility is related to a misunderstanding of proper breeding management (Feldman and Nelson, 1987a). In modern breeding protocols, the dog and bitch are often not allowed to display normal courtship behaviour, since they are introduced when the owner considers that the time for mating is correct. This is usually based simply upon the number of days from the onset of vulval swelling and the appearance of a serosanguineous vulval discharge.

Whilst the majority of bitches ovulate between 10 and 14 days after the onset of pro-oestrus, this event may occur as early as day 5 or as late as day 30. In addition, bitches are not necessarily consistent between cycles (England et al., 1989a). Therefore, should a bitch be mated on days 12–16 after the onset of pro-oestrus (which is common breeding practice) this may be inappropriate and result in a failure of conception.

The fertilisation period and fertile period

A surge in plasma LH concentration is the trigger for ovulation, which occurs 40–50 hours later (Phemister et al., 1973). Ovulation is spontaneous, and eggs are ovulated as primary oocytes (Evans and Cole, 1931; Doak et al., 1967). The oocytes are immature at ovulation and must reach the metaphase of the second meiotic division after extrusion of the first polar body before fertilisation (Baker, 1982); this further stage of maturation lasts 48–60 hours (Tsutsui, 1989). Eggs remain fertilisable for a further 2–3 days (Holst and Phemister, 1974; Concannon et al., 1989a); therefore the time span over which fertilisation may occur, termed the 'fertilisation period' (Jeffcoate and Lindsay, 1989), is between 4 and 7 days after the LH surge (i.e. between 2 and 5 days after ovulation) (Figure 28.10).

Dog sperm can remain viable and fertile within the uterus and uterine tubes for at least 6 days or more (Doak et al., 1967). Therefore, it is possible for matings that take place before the fertilisation period to result in conception. A second term may therefore be used, the 'fertile period', which differs from the 'fertilisation period' in that it encompasses the period of sperm survival within the female reproductive tract before ovulation and oocyte maturation. The fertile period extends from 3 days before, until 7 days after, the preovulatory LH surge, and may be even longer for dogs with exceptional semen quality. Determination of the time to mate can therefore be assessed on the basis of the time of the LH surge, or methods that may reliably indicate the 'fertilisation period' or the 'fertile period'.

Whilst it was initially thought that the LH surge occurred synchronously with the onset of standing oestrus (Concannon et al., 1975), it was subsequently shown that this may occur between 3 days before, to 9 days after, the onset of oestrus

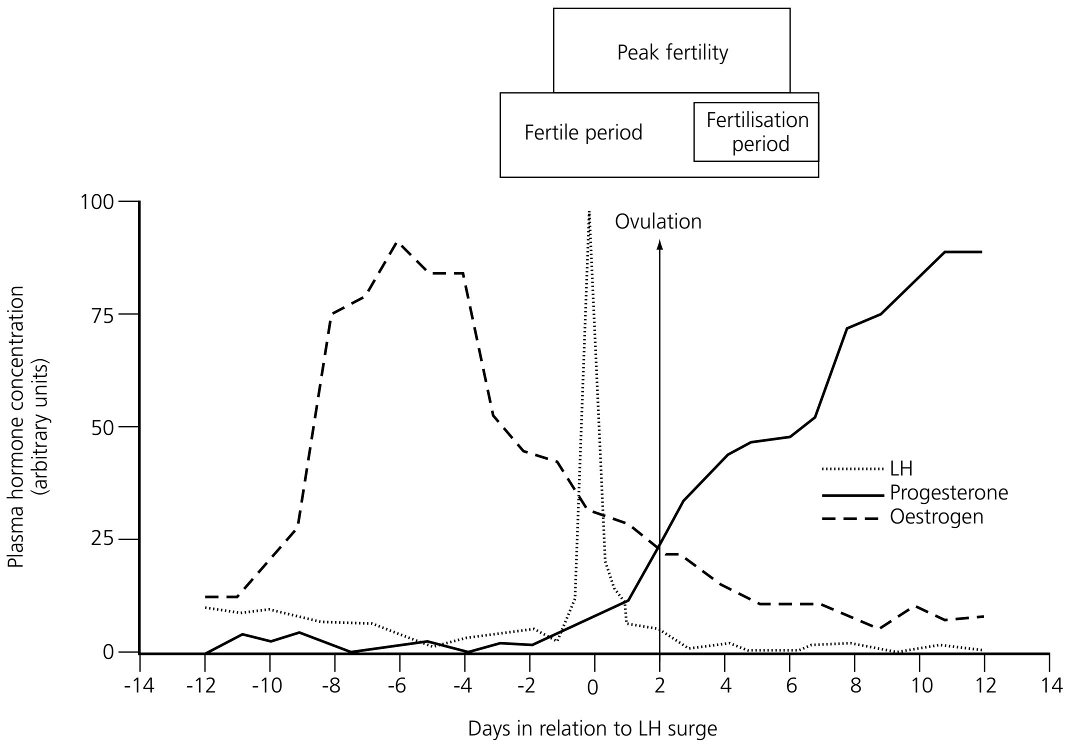

Fig. 28.10 Schematic representation of the changes in peripheral plasma hormones during pro-oestrus, oestrus and early metoestrus (dioestrus) and the relationship to the fertile period and the fertilisation period in the bitch.

(Mellin et al., 1976; Wildt et al., 1978a; Concannon and Rendano, 1983). Therefore, teasing the bitch has little value in determining the fertile period. Clinical assessment of the volume and colour of the vaginal discharge is similarly unreliable for determining the fertile period (Rowlands, 1950; Bell and Christie, 1971).

The optimal mating time

The optimal time for mating is likely to be during or immediately preceding the fertilisation period, and the period of peak fertility for natural matings ranges from 1 day before to 5 or 6 days after the LH surge (Holst and Phemister, 1974; Concannon et al., 1989a; England et al., 1989a) (Figure 28.10). Determination of the time to breed could therefore be based upon methods for estimating the time of the LH surge.

Hormone measurement. Measurement of peripheral plasma concentrations of LH is a reliable and accurate method of determining the optimum time to mate. However, there is no readily available commercial assay for canine LH, and at present measurement requires radioimmunoassay, a technique that is time-consuming and expensive. An enzyme-linked immunosorbent assay (ELISA) has recently been described for the measurement of LH concentration in fox plasma (Maurel et al., 1993), but has yet to be evaluated in the dog.

Plasma progesterone concentrations begin to increase towards the end of pro-oestrus at the time of the LH surge (Concannon et al., 1975; Hadley, 1975a). The progesterone is produced by luteinising follicles, and therefore, serial monitoring of plasma progesterone concentrations allows anticipation of ovulation. Recently, commercial test kits designed to measure the concentration of plasma progesterone by ELISA have become available. These kits have been shown to be useful for predicting the optimum mating time in the bitch (Eckersall and Harvey, 1987; England and Allen, 1989b; Dietrich and Moller, 1993; Fieni et al., 1993). Progesterone concentration may also be measured using this method on whole blood (England, 1991; Bouchard et al., 1993b) and vaginal fluid (England and Anderton, 1992).

Vaginal cytology. The examination of exfoliative vaginal cells is now commonly used to monitor the oestrous cycle. During pro-oestrus, increased plasma oestrogen concentrations cause thickening of the vaginal mucosa, which becomes a keratinised squamous epithelium. Vaginal epithelial cells may be collected either using a moistened swab or by aspiration. The relative proportions of different types of epithelial cells can be used as a marker of the endocrine environment (Plate 7). Several methods for staining of cells and various indices of cornification and keratinisation have been suggested as markers for the stage of the cycle (Schutte, 1967b; Klotzer, 1974). In general, the fertile period can be predicted by calculating the percentage of epithelial cells that appear cornified using a modified Wright–Giemsa stain (van der Holst and Best, 1976), although staining with a modified trichrome stain, allowing an assessment of the percentage of keratinised cells, has been suggested as being most useful (Schutte, 1967a). A schematic representation of the changes of exfoliative vaginal cells is given in Figures 1.31 and 28.11.

Polymorphonuclear leucocytes are absent from the vaginal smear during oestrus because the keratinised epithelium is impervious to these cells. Their reappearance during late oestrus reflects the breakdown of this epithelium (Evans and Cole, 1931). The return of polymorphonuclear leucocytes to the vaginal smear has been used by some workers as an indicator of the time of optimum fertility (Andersen, 1980).

Feldman and Nelson (1987a) suggested that breeding should be attempted throughout the period when more than 80% of epithelial cells are cornified. Whilst this is a good guide, some bitches reach peak values of only 60% cornification, whilst in others there may be two peaks of cornification (van der Holst and Best, 1976). Some bitches demonstrate poor cellular changes in the vaginal smear (Tsutsui, 1975) and typical metoestus cells may be found during pro-oestrus (Fowler et al., 1971). Allen (1985) noted that polymorphonuclear leucocytes may be found throughout the entire oestrous period; the extent of these variations has not been quantified. Recently, the aspiration of cells from the cranial vagina and the assessment of the anuclear cell index were found to increase the pregnancy rate and litter size of a group of bitches compared with a similar group mated only on the basis of the onset of proestrus

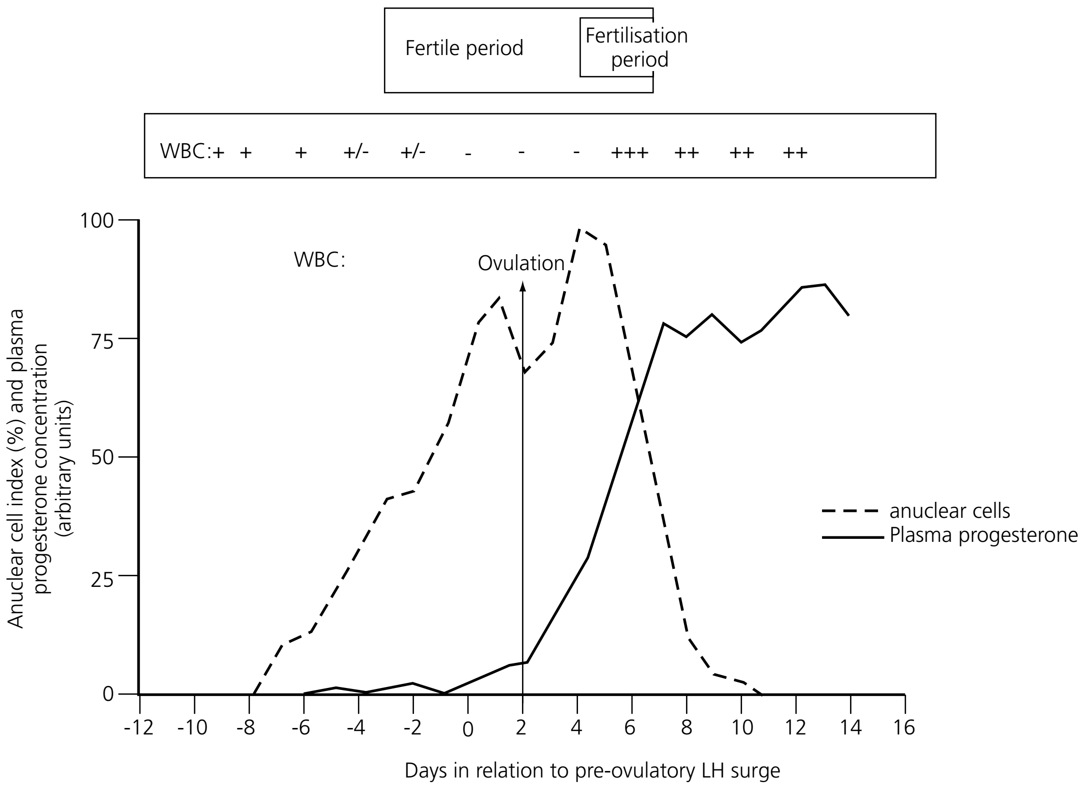

Fig. 28.11 Schematic representation of the changes in the percentage of anuclear epithelial cells in relation to ovulation and the fertile period in the bitch.

(England, 1992). The technique is particularly useful for bitches with irregular oestrous cycles and those with prolonged pro-oestrus or prolonged oestrus.

Vaginal endoscopy. Vaginoscopy is the technique of examination of the vaginal mucosa using either a rigid endoscope or a paediatric proctoscope. Vaginoscopic assessment is based upon observation of the mucosal fold contours and profiles, the mucosal colour and the characteristic colour of any fluid present (Lindsay, 1983a). Enlarged oedematous pink or pink/white mucosal folds are present during pro-oestrus and oestrus. Progressive shrinking of these folds is accompanied by pallor, effects that are probably the result of an abrupt withdrawal of the water-retaining effect of oestrogen during its preovulatory decline (Concannon, 1986). Subsequently, mucosal shrinkage is accompanied by wrinkling of the mucosal folds, which become distinctly angulated and dense cream to white in colour. These gross changes have been used to assess the fertile period (Lindsay, 1983a, b; Jeffcoate and England, 1997). Jeffcoate and Lindsay (1989) proposed a scoring scheme to allow description and recording of the vaginoscopic changes. These workers also suggested that vaginoscopy could be used to indicate the end of the fertilisation period.

Ultrasound examination. Realtime B-mode ultrasound imaging has been used in several species to monitor follicular growth and to identify the time of ovulation. Imaging of the bitch's ovaries and the detection of ovulation was achieved by Inaba et al. (1984). Subsequent work suggested that a dramatic decrease in the size or number of follicles occurred at ovulation (Wallace et al., 1989), although no details of ovarian morphology were published. England and Allen (1989a) suggested that ovulation was difficult to detect since follicles did not collapse and because corpora lutea had central fluid-filled cavities unoccupied by luteal tissue (Figure 28.12). The

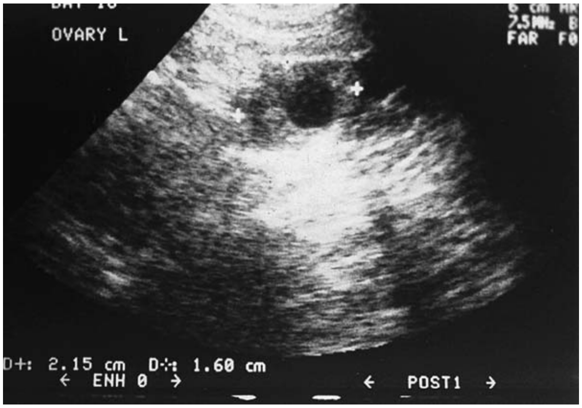

Fig. 28.12 Ultrasound image of an ovary of a bitch during metoestrus (dioestrus). A single large cavitated anechoic corpus luteum can be seen within the ovary (crosses).

central anechoic appearance of the corpora lutea was confirmed in a combined ultrasonographic and histological study (England and Allen, 1989b).

Ovulation could be detected by a decrease in follicle number and a subjective decrease in follicle size (Wallace et al., 1992), whilst England and Yeager (1993) found that ovulation was characterised by a decrease in the number of fluid-filled follicles and their replacement by similar-sized hypoechoic structures; these structures declined in number after ovulation and were replaced by fluid-filled corpora lutea.

Study of the cervicovaginal secretion. Variations in the electrical resistance of the vaginal secretion during the oestrous cycle have been described and Klotzer (1974) reported that the resistance decreased during the last part of oestrus in all bitches. These results were confirmed by Gunzel et al. (1986), and although the technique has been poorly investigated in the dog, it is used commercially for the detection of insemination time of the fox (Fougner, 1989).

Van der Holst and Best (1976) suggested that the amount of glucose within the vaginal discharge was a useful indicator of the optimum time of breeding. The principle of this technique is related to a change in the pH of vaginal secretion, since glucose is liberated from carboglutelin, which is then converted into lactic acid (Vogel and van der Holst, 1973). Although initial results were promising, this technique has not found clinical acceptance, presumably due to individual bitch variations.

Crystallisation of mucus collected from the anterior vagina has been described in the bitch (Figure 28.13), occurring after the peak in plasma oestrogen concentrations (England and Allen, 1989c). Assessment of the mucus, which originates from cervical glandular tissue (England,

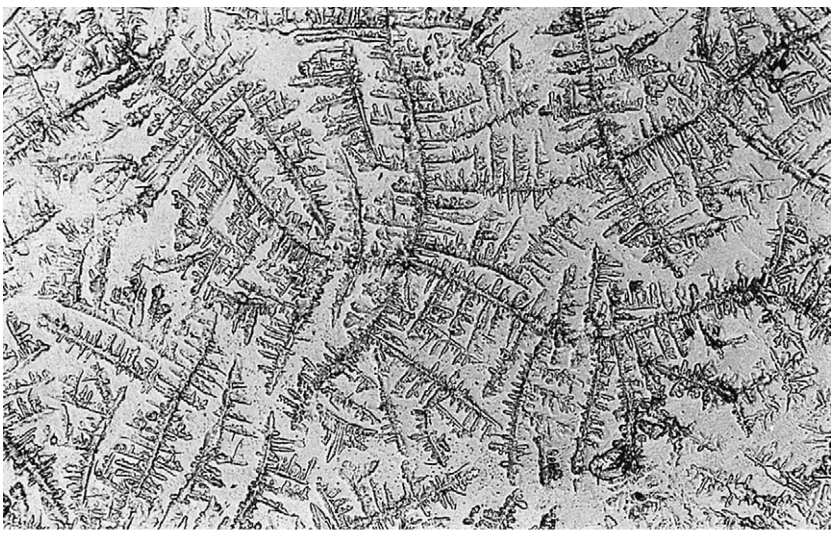

Fig. 28.13 Photomicrograph of the cervicovaginal fluid collected from a bitch during oestrus. Crystallisation of the mucus has resulted in the formation of a fern-like pattern.

1993), may be useful when combined with vaginal cytology for determining the optimal mating time (England and Allen, 1989c).

Vulval softening. During pro-oestrus the vulva becomes enlarged and turgid. There is often a distinct softening and decrease in swelling following the preovulatory LH surge (Concannon, 1986). This method is imprecise but is probably the single clinical event that has proven to be useful for assessing the optimal mating time.

THE CAT

Structural abnormalities of the reproductive tract

Congenital abnormalities

The range of congenital abnormalities of the reproductive tract of the queen is similar to that of the bitch. Ovarian agenesis is rare and results in permanent anoestrus and infertility. Small ovarian remnants containing fibrous tissue may be identified at laparotomy or laparoscopy (Schmidt, 1986). Similar to the bitch, the establishment of a karyotype may be useful in the investigation of these cases.

Ovarian hypoplasia is also rare in the queen (Herron, 1986), although phenotypically normal queens may have non-functional ovaries secondary to chromosomal abnormalities (Centerwall and Benirschke, 1975; Johnston et al., 1983).

There are a small number of reports of aplasia of the tubular genital tract, although unilateral and/or bilateral agenesis of the uterine tube and uterine horn have been identified (Herron, 1986). It is not uncommon for these lesions to be associated with absence of the ipsilateral kidney and ureter. The queen may be fertile when the abnormality is unilateral, although litter size is often reduced. In many cases, these lesions are only diagnosed at exploratory laparotomy.

Vaginal aplasia may result in the retention of uterine fluid and cause endometrial changes and infertility. The uterus may be so large that it can be detected by palpation; otherwise ultrasonography will demonstrate a tubular fluid-filled uterus present within the caudal abdomen. In rare cases simple vaginal bands or strictures may be identified and can be manually broken down, allowing normal coitus.

Disease of the caudal reproductive tract is rare. Vulval and vaginal atresia may occur separately or simultaneously. Small labia with or without stenosis of the vestibule are observed in the former, whilst stenosis of the vagina is observed in the latter (Saperstein et al., 1976).

Intersex. Several female cats with ambiguous genitalia have been described (Herron and Boehringer, 1972; Felts, 1982). In many cases, the external genitalia are underdeveloped until puberty, when the animal may demonstrate male-like behaviour. Clitoral enlargement has not been reported (Herron, 1986). An exact diagnosis may not always be reached; however, removal of the reproductive tract including gonads is necessary in most cases. Animals may be classified as having abnormalities of either chromosomal, gonadal or phenotypic sex; a full description is given for the bitch (see p. 641).

Acquired abnormalities

Acquired abnormalities of the reproductive tract of the queen are rare. Occasionally ovarobursal adhesions of unknown aetiology are seen, but these are usually unilateral and cause reduced fertility rather than infertility. The most common acquired abnormality is pyometra (see later), although hydrometra has also been reported.

Neoplasia

Ovarian tumours are uncommon in the queen. They generally reach a large size before diagnosis and are not frequently metastatic, often being granulosa theca cell tumours (Herron, 1986). These may be endocrinologically active and result in clinical signs of persistent oestrus, cystic endometrial hyperplasia and bilaterally symmetrical alopecia (Barrett and Theilen, 1977). The treatment of choice is ovariectomy. Malignant endometrial adenocarcinoma is the most common uterine neoplasm, although benign tumours have also been reported (Herron, 1986). These tumours may be associated with a persistent haemorrhagic vulval discharge and straining. In all cases, ovariohysterectomy is curative provided that metastases have not developed. Tumours of the vagina include pedunculated leiomyomata and fibromata. These are rare and generally produce clinical signs of vaginitis and straining to defecate, since they may impinge upon the colon and rectum. Local excision is usually curative, and ovariohysterectomy may reduce the risk of recurrence.

Functional abnormalities of the reproductive tract

Delayed puberty and prolonged anoestrus

The onset of puberty in the queen is influenced by both body weight and the season of birth. In most cases puberty occurs in the spring; therefore those animals born in autumn may exhibit their first oestrus at 6 months of age. However, queens born in winter or spring time may not exhibit puberty until 12 months of age (Goodrowe et al., 1989).

Queens that do not reach puberty by their second spring are considered to have delayed puberty. In the northern hemisphere, queens generally commence oestrous behaviour in January or February, an event that is dependent upon photoperiod (Herron, 1986). Prolonged anoestrus may be associated with systemic disease, poor nutrition or a high parasite burden (Mosier, 1975). If these factors are eliminated, 14 hours of daylight should abolish anoestrus in the healthy normal queen (Gruffydd-Jones, 1990). Attention should also be given to whether progestogens have been used for the control of dermatological or behavioural problems, since their use will prevent oestrus.

Induction of oestrus. When it is necessary to breed the queen it may be possible to induce cyclicity provided that there is no underlying disease and day length is suitably long. Crude extracts of FSH and LH have been used to induce oestrous behaviour and ovulation (Foster and Hisaw, 1935). More recent work has utilised pregnant mare serum gonadotrophin administered daily for 8 days with reasonable success (Colby, 1970). The queen appears to be sensitive to the effects of exogenous gonadotrophins, and high doses may result in large numbers of cystic unovulated follicles (Wildt et al., 1978a). When anoestrous queens were given a single bolus of 100 IU of eCG, followed 5–7 days later by 50 IU of hCG, ovulation and pregnancy rates were similar to those at natural cycles (Cline et al., 1980). Similarly, the administration of FSH for 5–7 days resulted in a high pregnancy rate (Wildt et al., 1978a). More recently, a decreasing dose regimen of FSH-P combined with a single dose of hCG was shown to be successful (Pope et al., 1993).

Silent oestrus

Cases of silent oestrus are usually identified in queens that are low in the hierarchy of a cat colony. The incidence of silent oestrus is unknown, and the diagnosis is made upon the absence of behavioural oestrus whilst endocrinological events are normal. Cases are best evaluated by the study of vaginal cytology to demonstrate oestrus (see later), removal of the queen to a new environment, or the induction of oestrus. Occasionally the condition occurs in queens that are lactating, although more commonly cats are anoestrous at this time.

Prolonged oestrus

Some queens appear to have a behavioural oestrus that persists for the duration of two follicular cycles, even though the endocrinological events are normal. In these cases, oestrogen concentrations return to basal values between the follicular waves, although in some cases persistently elevated oestrogen concentrations have been identified (Feldman and Nelson, 1987b). The fertility of these cycles is uncertain, although it is likely that fertility may be reduced because of inappropriate mating time and resultant failure of ovulation. In most cases, treatment is not warranted since prolonged oestrus is generally a sporadic occurrence.

Ovulatory failure

Cats are induced ovulators with eggs being fertilisable at ovulation following mating or artificial stimulation of the genitalia (Greulich, 1934). Copulation produces a rapid pituitary-mediated release of LH (Robinson and Sawyer, 1987), and usually multiple copulations are required to ensure that ovulation occurs (Concannon et al., 1980).

Failure of ovulation may occur if the queen is not mated a sufficient number of times. However, approximately 90% of queens ovulate if mated three times at 4-hour intervals for the first 3 days of oestrus (Schmidt, 1986). There is also a reduced magnitude of LH release for matings that occur later into oestrus. Therefore, breeding on the first few days of oestrous behaviour ensures the greatest success. Ovulatory failure may be diagnosed by a return to oestrus after 3 weeks (cats that ovulate but fail to become pregnant enter pseudopregnancy and have a delayed return to oestrus), and by measurement of plasma progesterone concentrations that remain basal following cessation of behavioural oestrus. Ovulation may be facilitated by ensuring that sufficient matings occur at the beginning of oestrus, or by the administration of a single dose of hCG (500 IU) on the first day of oestrus (Wildt and Seager, 1978).

Pseudopregnancy

Whilst in the bitch pseudopregnancy is not a cause of infertility, in the queen the absence of cyclical activity may be explained by this condition.

Non-fertile matings, or occasional spontaneous ovulations (Lawler et al., 1993), result in the formation of corpora lutea, which secrete progesterone. During the life span of the corpora lutea the queen does not demonstrate oestrous activity, and this results in interoestrus intervals of between 35 and 70 days, with an average of 45 days (Verhage et al., 1976; Wildt et al., 1981). Diagnosis of the condition is based upon the demonstration of elevated concentrations of plasma progesterone (Figure 28.14). Late in the breeding season, however, pseudopregnancy may be followed by anoestrus.

Ovarian cysts

In the queen, cystic structures can be associated with the ovaries. However, the majority of these are not of ovarian origin, being remnants of mesonephric and rete tubules. These cysts are endocrinologically inactive and do not produce clinical signs. True follicular cysts, associated with hyperoestrogenism, have been reported (Herron, 1986) and may be associated with exaggerated sexual behaviour and prolonged oestrus. Diagnosis may

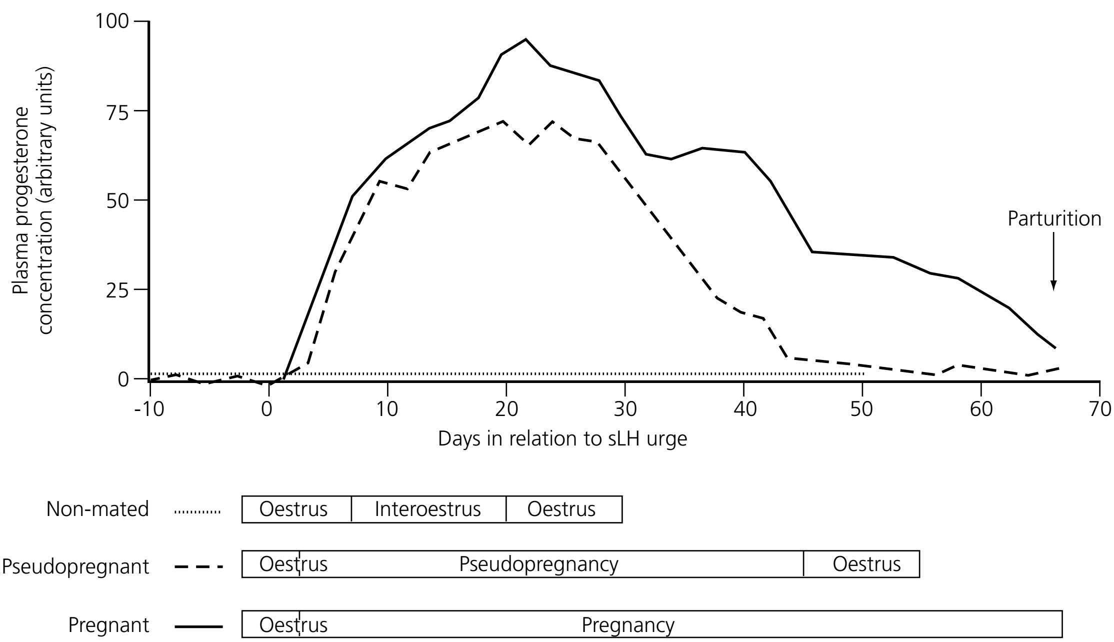

Fig. 28.14 Schematic representation of the changes in peripheral plasma progesterone concentrations in the non-mated, pseudopregnant and pregnant queen. The hormone profiles are plotted in relation to the time of the LH surge for the mated animals and the onset of oestrus in the unmated animals.

be made on the basis of clinical signs, on measurement of plasma oestrogen concentrations or by the demonstration of persistent cornification of vaginal epithelial cells. hCG may be administered in an attempt to induce ovulation; however, in most cases, either ovariectomy or the use of progestogens to suppress the clinical signs is necessary.

Premature ovarian failure

Premature ovarian failure has been suggested as a cause of permanent anoestrus in previously fertile queens, which results in a shortening of their reproductive life (Feldman and Nelson, 1987b). The condition is difficult to confirm, and diagnosis relies upon elimination of other causes of anoestrus. In valuable breeding animals oestrus induction regimens may be contemplated; however, there is no information on the efficacy of these treatments.

Habitual abortion

There is little non-anecdotal evidence to show that habitual abortion occurs in the queen; however, the condition is commonly diagnosed and treated. In many cases it is suggested that habitual abortion is the result of progesterone deficiency (Christiansen, 1984), although evidence for this is lacking. As in the bitch, the minimum plasma concentration of progesterone required to maintain pregnancy is approximately 1–2 ng/ml, with ovarian-derived progesterone being the major source throughout pregnancy (Verstegen et al., 1993a). The administration of progesterone or progestogens has been advocated to prevent habitual abortion (Christiansen, 1984); however, this therapy is empirical and suffers the risk of prolonging gestation and producing masculinised female kittens and cryptorchid male kittens. Progestogen administration should be limited to those cases in which a true luteal insufficiency has been diagnosed.

Infectious agents

There are no reported venereally transmitted infections or specific genital infections that are recognised as causes of infertility in cats. However, there are several opportunistic pathogens and specific infectious agents that may have a direct effect upon fertility.

Opportunist pathogens

The vestibule and vagina of the queen are normally inhabited by many aerobic and anaerobic bacteria. It has been suggested that they may enter the uterus at mating, and subsequently cause abortion because the progesterone-dominant uterine environment allows them to proliferate (Christiansen, 1984; Troy and Herron, 1986a). It is not clear, however, whether bacteria isolated from aborted fetuses have caused the abortion, or whether they have invaded the uterus after dilatation of the cervix at the time of the abortion. Bacteria commonly isolated include *E. coli*, staphylococci, streptococci, salmonellae and mycobacteria (Troy and Herron, 1986b). Immediately prior to an abortion, the queen may become pyrexic and lethargic. Treatment includes the administration of broad-spectrum antimicrobial agents, fluid therapy and drugs to stimulate uterine evacuation. Hysterotomy to remove fetal tissue is rarely necessary, although ovariohysterectomy may be required should a severe metritis develop.

Specific infections

Feline leukaemia virus (FeLV). FeLV has been implicated in a variety of clinical syndromes including infertility, embryonic resorption and abortion (Hardy, 1981). FeLV is believed to be the single most common cause of infertility in the queen (Jarrett, 1985). Fetal resorption is seen frequently, although abortion and the birth of permanently infected kittens also occur. The aetiology of the reproductive disease is uncertain, and whilst it is known that the virus may cross the placenta, one possibility is that secondary bacterial infections occur because of FeLV-induced immunosuppression (Jarrett, 1985). Diagnosis of FeLV may be achieved by virus isolation, immunofluorescence or an ELISA method. Owners should be discouraged from breeding from FeLV-positive queens since all offspring are born persistently infected. These kittens usually develop a FeLV-related disease soon after birth.

Vaccines are now available that provide protection against FeLV and its related diseases.

Feline herpesvirus. Feline herpesvirus I may result in abortion during the 5th or 6th week of gestation. Lesions may be found within the uterus; however, placental lesions have only been demonstrated following experimental infection (Hoover and Griesemer, 1971). In the naturally occurring disease, abortions are thought to be the result of a non-specific reaction to the infection (Troy and Herron, 1986a). Transmission of the virus occurs via the respiratory tract, with up to 80% of cats remaining as chronic carriers. The diagnosis of herpesvirus infection is based upon the clinical signs and the isolation of virus. Vaccination of queens provides good immunity and should be recommended for all breeding animals.

Feline panleucopenia virus. Feline panleucopenia virus is transmitted by direct contact with saliva, faeces and urine. Infection of pregnant queens may result in abortion, stillbirths, neonatal deaths and fetal cerebellar hypoplasia (Troy and Herron, 1986a). These effects are the result of transplacental infection leading to fetal death and resorption in early pregnancy (Gillespie and Scott, 1973), and cerebellar hypoplasia when infection occurs from the middle third of pregnancy onwards (Gaskell, 1985). Diagnosis may be made on the basis of the clinical signs, histopathological findings, virus isolation and paired serum samples that demonstrate a rising antibody titre. There is no treatment for kittens with cerebellar hypoplasia.

Feline infectious peritonitis virus. Feline infectious peritonitis virus has been implicated as a cause of infertility, stillbirths, endometritis, resorption and abortion, chronic upper respiratory tract disease and fading kitten syndrome (Scott et al., 1979; Troy and Herron, 1986b). Queens are not always ill and may suffer resorption or abortion, which is unnoticed. Abortion generally occurs during the last 2 weeks of pregnancy (Norsworthy, 1974, 1979). Diagnosis is made by serological and pathological investigation.

Toxoplasmosis. Toxoplasmosis (*T. gondii* infection) has been incriminated as a rare cause of abortion and congenital infection of cats (Troy and Herron, 1986b). Serological screening is necessary to demonstrate the role of this protozoan in cases of abortion.

Chlamydiae. There is evidence that the feline strain of *Chlamydia psittaci* causes abortion in the queen. The mode of transmission has not been elucidated, although the organism has been isolated from the genital tract of infected cats and there is circumstantial evidence associating infection with reproductive disease (Willis et al., 1984). As well as direct isolation of the organism, diagnosis is possible by demonstrating high antibody titres. It is difficult to confirm whether the isolation of this organism indicates its role in an abortion, since it may simply be an opportunistic bacterium.

Cystic endometrial hyperplasia and pyometra

Aetiology. Both natural and experimentally induced cystic endometrial hyperplasia and pyometra have been described in the queen (Dow, 1962a, b). The syndrome is more common in older animals (Lein and Concannon, 1983) and is uncommon during winter, when queens are acyclic. In naturally occurring cases, corpora lutea are present within the ovaries, suggesting that progesterone is involved in the pathogenesis, in a similar manner to the bitch (see p. 650). Interestingly, approximately half of the cases are seen in unmated queens, in which there should be no luteal phase. This may be explained by the observation of Dow (1962b) and Lawler et al. (1991) that a proportion of queens ovulate without mating. Recently, Lawler et al. (1993) showed that 35% of queens ovulated spontaneously without copulation or mechanical stimulation of the cervix. Reports of the relationship between the incidence of pyometra with parity (Colby, 1980) have been disputed (Feldman and Nelson, 1987b).

The bacteria isolated from cases of pyometra are similar to those found in the bitch, frequently being opportunistic organisms that are normal inhabitants of the genital tract of cats including *E. coli* (Joshua, 1971; Choi and Kawata, 1975; Lawler et al., 1991). Culture of vaginal swabs is therefore of limited value.

Clinical signs and diagnostic features. The severity and clinical signs of the cystic endometrial hyperplasia–pyometra complex vary considerably. In early cases there may simply be mild endometrial hyperplasia with glandular dilatation. This leads to a hostile uterine environment that may lead to fetal resorption. Queens with endometrial hyperplasia are often clinically normal, although occasionally the uterus may be enlarged and can be palpated. Ultrasound examination of these cases can reveal the presence of anechoic regions within the uterine mucosa, representing cystic glandular tissue; occasionally free uterine fluid may be detected. In breeding queens, it may be necessary to confirm the diagnosis using histological examination of a uterine biopsy. At laparotomy, a transverse wedge of uterus of full thickness to include endometrium is removed. In some cases, a presumptive macroscopic diagnosis can be made on gross examination of the uterus.

Cases of pyometra are not often difficult to diagnose, since many queens have a malodorous vulval discharge, although the queen may be particularly fastidious and regularly clean her perineum. Often oestrus will have been observed within 2 months of the onset of the discharge. The range of diagnostic methods described in the bitch (see p. 654) may be helpful to confirm the diagnosis. The use of real-time diagnostic ultrasound provides the most accurate method, although radiography, haematology and clinical examination are also rewarding. Care must be exercised in palpating the abdomen because of the risk of causing rupture of the uterus.

Treatment. As in the bitch, ovariohysterectomy is the treatment of choice for cases of pyometra. Attention should always be paid to the electrolyte and acid–base status of the animal prior to surgery; intravenous fluid therapy is always warranted. The complete reproductive tract from a queen following ovariohysterectomy is shown in Figure 28.15.

A variety of methods have been investigated for promoting uterine drainage, although cervical catheterisation has not been reported in the queen. Surgical drainage and lavage of the uterus via a laparotomy and uterotomy have been described (Gourley, 1975; Vasseur and Feldman, 1982). Following lavage, an indwelling drainage tube is placed in each uterine horn and passed through the cervix and vagina to allow postsurgical lavage.

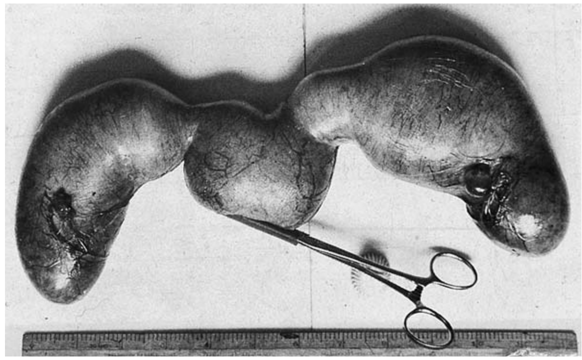

Fig. 28.15 Reproductive tract of a queen with pyometra.

Medical therapy may be useful and has been shown to have a good success rate (Feldman and Nelson, 1987b; Davidson et al., 1992). The former authors only treated queens that were less than 6 years of age and had open-cervix pyometra using 0.1 or 0.25 mg/kg of dinoprost daily for 5 days, and they found that 12 of 14 queens subsequently produced litters. The adverse effects of prostaglandin administration in the queen are similar in duration and effect to those noted in the bitch and include tachycardia, salivation, vocalisation, defecation and altered behaviour. Prostaglandin therapy should be combined with appropriate broad-spectrum antimicrobial agents and fluid therapy. Prostaglandins are known to have a direct luteolytic effect in the cat (Verstegen et al., 1993b) which, combined with their uterine spasmogenic action, explains why this regimen appears to be very successful. However, recently the luteotrophic action of prolactin and the luteolytic effects of prolactin inhibitors such as cabergoline have been demonstrated (Verstegen et al., 1993b); it is possible that these agents may be used in the treatment of pyometra as described in the bitch.

Management factors affecting fertility

In the queen, modern breeding protocols may hamper reproductive performance because of a misunderstanding of the normal reproductive physiology.

Ovulation is considered to be induced by an adequate surge of plasma LH, released following coitus. In one study, single copulations induced an

LH surge sufficient to cause ovulations in only 50% of queens (Concannon et al., 1980). When 4–12 unrestricted matings were allowed in a 4-hour period, the mean concentrations of LH were 3–6 times higher than for single matings and all queens ovulated (Concannon et al., 1980). Matings at limited, predetermined intervals on single or sequential days of oestrus can result in LH release of variable incidence, magnitude and duration (Wildt et al., 1978b, 1980; Banks and Stabenfeldt, 1982). It can be seen, therefore, that restricted mating regimens, which are common breeding practice, may result in failure of ovulation in a high proportion of queens. Multiple matings should result in ovulation, although Glover et al. (1985) suggested that repeated matings very early in oestrus may not result in adequate LH release, whilst similar matings later in oestrus are likely to be successful at inducing ovulation. Therefore, it is important that not only multiple matings but also normal courtship be allowed, to ensure that matings occur throughout oestrus and not simply at the time the breeder considers to be correct.

It is difficult to identify accurately the stages of pro-oestrus or oestrus in the queen; however, unlike in the bitch, the behavioural events are more repeatable. During the 1–2 days of pro-oestrus the queen refuses copulation but is more active and may show interest in the male; this period can only truly be identified in the presence of a male. Oestrus may last between 3 and 20 days, with an average of 8 days. During this time, the queen displays a crouching and lordotic stance that facilitates mounting by the male. This response can be elicited by firmly grasping the queen by the skin on the back of the neck. Mating should be attempted commencing at midoestrus, 3–4 days after the initial signs of pro-oestrus.

Vulval swelling during oestrus does not occur; therefore, clinical assessments of the queen are of little value for determining the optimum breeding time. A small amount of white vulvar discharge may occasionally be noticed during oestrus (Tsutsui and Stabenfeldt, 1993). The examination of exfoliative vaginal cytology is useful in the queen for determining the stage of the cycle; however, the technique does not enable the prediction of the onset of oestrus; up to one-third of queens may show signs of oestrus before the vaginal smear contains evidence of cornified cells (Shille et al., 1979). The technique is therefore most useful for verifying oestrus (Banks, 1986). The smear may be collected either using moistened cotton swab or by irrigation with an eye-dropper containing sterile saline. Staining of the epithelial cells can be achieved using a variety of stains including a modified Wright–Giemsa stain. Erythrocytes are not found within the vaginal smear, because uterine diapedesis is not a feature of oestrus in the queen. The changes in the vaginal smear are therefore limited to changes in morphology of epithelial cells, because polymorphonuclear leucocytes are also usually absent except during early metoestrus and pregnancy. The percentage of epithelial cells that are cornified changes in a similar manner to that seen in the bitch (see p. 657). During oestrus more than 80% of cells are cornified. If the queen does not ovulate, the exfoliative cells return to a state similar to that observed during anoestrus or early pro-oestrus. Early metoestrus is characterised by increasing numbers of parabasal and small intermediate epithelial cells, whilst debris, mucus and polymorphonuclear leucocytes also become evident.

Care should always be taken when collecting vaginal epithelial cells since the technique may induce ovulation.

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29 Normal reproduction in male animals

ANATOMY AND PHYSIOLOGY

The reproductive organs (Figure 29.1) of the male animal may be considered to fulfil three major functions. These are, firstly, the production of spermatozoa in the testis; secondly the maturation, storage and transport of spermatozoa within the duct system and, finally, the deposition of semen within the female genital tract via the penis. Likewise, the functions of the male hormones may be considered to be three-fold: the maintenance of spermatogenesis, the production of masculine behaviour (libido and aggression) and the development of the masculine body form.

Anatomy of the testis, spermatic cord and scrotum

The testes of all domestic male animals are located at the inguinal region within a scrotum. In the bull and the ram this structure is pendulous and has an elongated neck, but in most other domestic species the scrotum is closely applied to the inguinal region. The scrotum consists of a skin pouch overlying various fibroelastic and muscular layers, of which the most prominent is the tunica dartos (see Figure 29.2). The dartos layers are confluent between the testes, where they form the intertesticular septum. In the boar, the external spermatic fascia is also prominent. The testis itself is surrounded by two layers of peritoneum, which are formed during the descent of the testis as a single outpouching of the parietal peritoneum through the inguinal canal. The outer layer of peritoneum, the processus vaginalis (tunica vaginalis reflexa), is reflected onto the testis to form the serous outer layer of that organ, the tunica vaginalis propria. Accompanying this outpouching of peritoneum through the inguinal canal is a diverticulum of the internal abdominal oblique muscle, which inserts on to the cremasteric fascia and the vaginal tunics. This muscle, the cremaster, raises or lowers the testis in response to temperature or noxious stimuli.

The capsule, or tunica albuginea, of the testis is composed principally of fibrous tissue, but has a smooth muscle component whose function is largely unknown. Overlying the capsule is the tunica vaginalis propria. The main blood vessels of the testis are distributed over the surface of the tunica albuginea before penetrating the capsule to supply the testicular parenchyma, while the innervation of the testis is mainly confined to the periphery and little nervous tissue is found in its substance. The substance of the testis (Figure 29.3) is composed of two main tissues: seminiferous tubules and interstitial tissue. Each seminiferous tubule is a highly convoluted, unbranched tube, which opens at both ends into collecting tubules and which, in turn, open into the single epididymal duct. The seminiferous tubules are limited by a basement membrane which is partially surrounded by contractile myoid cells. Within the tubule, the seminiferous epithelium is composed of two main cell lines, somatic Sertoli cells and the sperm-producing germinal cell lines. Interstitial tissue, which consists of steroid-producing Leydig cells, blood vessels and lymphatics, exhibits much variation in its quantity and morphology between species. For example, there is prolific interstitial tissue in the boar and the stallion, but relatively little in ruminants.

The epididymis is a single, highly convoluted tube, into which the vasa efferentia drain the seminiferous tubules. Grossly, the epididymis appears as an approximately cylindrical organ, which is divided into a prominent head, situated close to the suspension of the testis from the spermatic cord, a smaller, medially situated body and a distended tail, which is continuous with the vas

Fig. 29.1 The genital organs of the bull. a, testis; b, head of epididymis; c, body of epididymis; d, tail of epididymis; e, vas deferens; f, vascular part of spermatic cord; g, ampulla of vas deferens; h, seminal vesicular gland; i, body of prostate; k, pelvic urethra surrounded by urethralis muscle; l, bulbourethral gland; m, bulbocavernosus muscle; n, crus penis; o, ischiocavernosus muscle; p, distal sigmoid flexure of penis; r, glans penis; s, retractor penis muscle; t, urinary bladder; u, pubic symphysis; v, rectum. (From Blom and Christensen (1947).)

deferens. The muscular wall of the epididymal duct moves sperm through its lumen by peristalsis, so that during passage of the epididymis, sperm, which are immature on release from the testis, undergo final maturation. The tail of the epididymis also acts as a reservoir for fully mature sperm, and becomes turgid with stored sperm in sexually active animals.

The vas deferens is a relatively thick-walled, muscular tube, which acts as both a reservoir for sperm and the means of their conduction between the epididymis and the penis. It is situated mediocaudally within the spermatic cord, in a small diverticulum of peritoneum. In addition to the vas deferens, the spermatic cord also contains the arteries, veins and nerves supplying the testis,

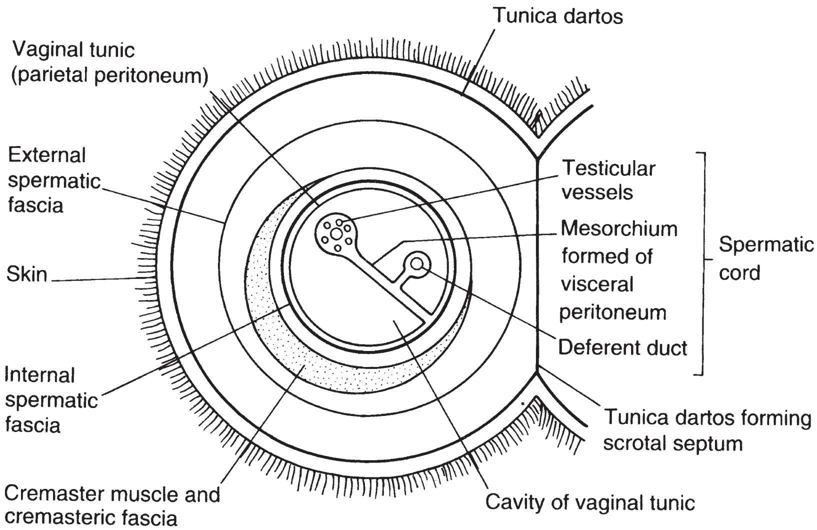

Fig. 29.2 Diagram of the anatomy of the fascial, muscular and peritoneal tissues of the neck of the scrotum. The vaginal tunic is strongly reinforced by the closely adherent and much thicker internal spermatic fascia. All of the layers are closely apposed to their adjacent structures so that the only (potential) space is the cavity of the vaginal tunic. (Redrawn from Cox (1982), with permission.)

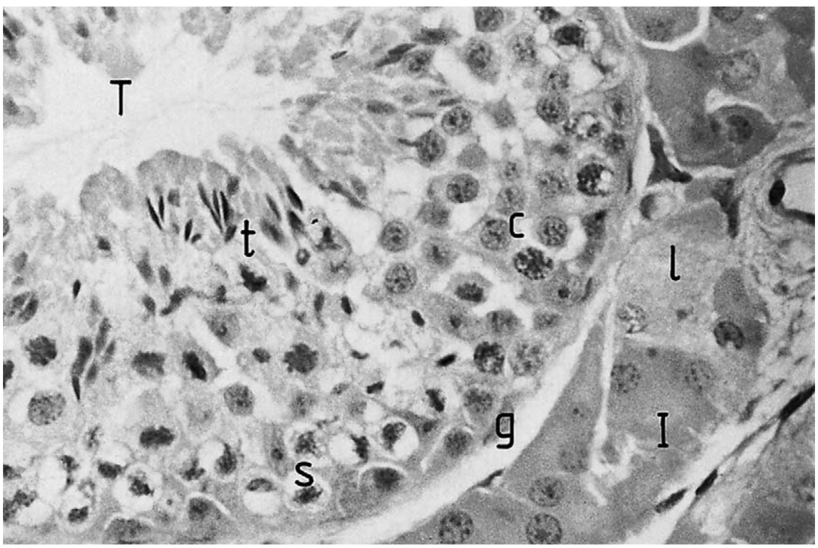

Fig. 29.3 Histology of the testis. Interstitial tissue (I) containing Leydig cells (l), blood, nervous tissue and lymphatic tissue is interspersed between seminiferous tubules (T), whose lumen is lined, in some sections, by formed spermatozoa. The periphery of the tubules is composed of spermatogonia (g) and Sertoli cells (s), with spermatocytes (c) and spermatids (t) occurring deeper in the tubules.

all of which are contained within the peritoneal vaginal tunics. Together, these structures form the spermatic cord. The spermatic sac includes the spermatic cord, the internal spermatic fascia, cremaster muscle and cremasteric fascia. The cremaster muscle is situated on the opposite side of the sac to the vas deferens, i.e. on the anterolateral surface. The vasa deferentia enter the abdomen

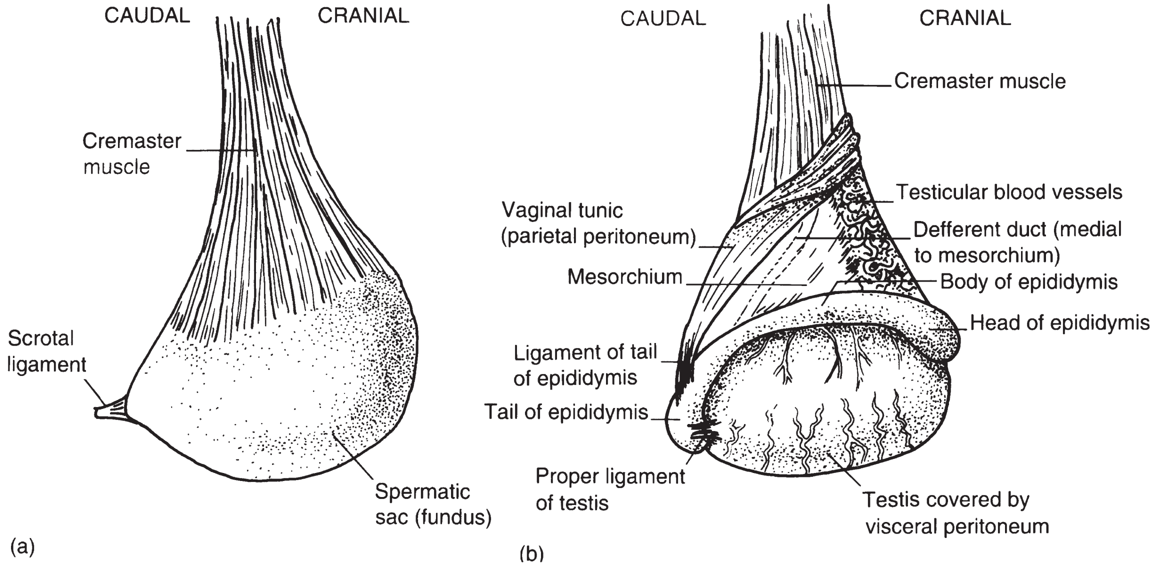

Fig. 29.4 Ligaments of the scrotum. (a) Lateral view of the right spermatic sac of the horse. (b) Lateral aspect of the contents of the right spermatic sac of the horse. The most ventral part of the spermatic sac shown in Figure 29.2 has been incised so as to enter the lumen of the vaginal tunic. (Redrawn from Cox (1982), with permission).

through the inguinal canals, whence they run in a caudal direction to join the pelvic urethra where the latter organ joins the neck of the bladder.

A number of short ligaments exist between the various structures within the scrotum, as shown in Figure 29.4. The proper ligament of the testis joins the ventral pole of the testis to the tail of epididymis, which is also joined to the vaginal tunic by the caudal ligament of the epididymis. These ligaments are derived from the gubernaculum. Finally, on the external surface of the vaginal tunic, the scrotal ligament joins the tunic to the scrotal fascia.

Blood and nervous supply to the testis

The testes are supplied with blood through the spermatic arteries, which arise from the caudal aorta, close to the renal arteries. In the domestic species, these arteries pass through the inguinal canal, enclosed in peritoneum, forming a major component of the spermatic cord. In animals with scrotal testes, the spermatic artery becomes highly convoluted from the point at which it passes through the inguinal canal, although the degree of convolution is less if the scrotum is inguinal than if pendulous. The testis is drained by an anastomosing plexus of veins (the pampiniform plexus), which arise in the tunica albuginea and return to the spermatic cord through the inguinal canal and, thence, to the inferior vena cava. Initially, many veins are identifiable in the plexus, but as the plexus ascends the spermatic cord, fewer and fewer collateral branches are apparent, until a few main veins penetrate the inguinal canal. These finally join, as a single vein, into the caudal vena cava or renal vein (Setchell, 1970). The spermatic artery surrounds, and is in very intimate contact with, the pampiniform plexus, such that artery and vein frequently share a common tunica intima. This complex vascular anatomy fulfils several functions. The length of the spermatic artery is greatly extended by its convolutions, such that the arterial pulse is almost completely eliminated by the time the artery reaches the testis (Waites and Moule, 1970), as it appears that a pulsatile arterial blood supply to the testis is incompatible with normal spermatogenesis. Secondly, spermatogenesis is more efficient at temperatures below the mammalian core body temperature. The close apposition of artery and veins allows heat exchange to occur between spermatic artery and vein, such that the temperature in the testis is several degrees lower than the core body temperature. Thirdly, it is possible that some counter-current exchange of small molecules, such as testosterone, may occur between spermatic vein and artery, although the importance of such transfers remains to be established.

The nervous supply of the testis (see Hodson, 1970) is derived from the thoracolumbar sympathetic outflow, whose visceral motor fibres innervate the smooth muscle of the testicular arterioles and of the testicular and epididymal capsules. These fibres and their accompanying visceral sensory fibres run in the spermatic cord. The scrotum has both visceral and somatic innervation, derived from nerves that pass through the inguinal canal and which arise as branches of the pudendal nerve. A further prominent feature of the innervation of the scrotum is the motor supply to the cremaster muscle and dartos. However, as might be expected from the interspecies variation in anatomy of the scrotum, there is also considerable variation in the detail of its nervous supply.

Development of the testis

Early in embryonic development, primordial germ cells migrate from the yolk sac into the mesonephros, where they induce formation of the gonadal ridge. In male embryos, the presence of testis-determining genes on the Y chromosome induces development of undifferentiated gonad into a testis. Recent evidence suggests that the critical stage in the formation of the testis is in the differentiation of the somatic component of the primitive gonad. Sertoli cells are not only crucial for the formation of the testis, but are also responsible for producing Müllerian duct inhibiting factor, which prevents development of the female genital tubular genitalia, and a meiotic inhibiting factor, which maintains meiotic arrest until puberty. Leydig cells produce testosterone, which stimulates development of the mesonephric (wolffian) ducts to form the tubular parts of the male genital tract. The only critical effector of the XY germ cell is its spermatogenesis gene, which lies on the Y chromosome, and is responsible for the onset of spermatogenesis (Burgoyne, 1988; McLaren, 1988).

As the testis develops in the mesonephros, differentiation of the definitive kidney (the metanephros) begins and, as it grows, the metanephros replaces the mesonephros as the organ of osmoregulation. The testis then migrates from its position within the abdomen towards its definitive position in the scrotum, in a process referred to as the descent of the testis. The testis traverses the abdomen (see Gier and Marion, 1970; Figure 29.5(a) and (b)) partly as a result of differential growth between the pelvis, abdomen, kidney and testis, and partly due to the tension exerted upon the testis by the gubernaculum. Before the testis itself passes through the inguinal canal, the canal is distended by a thickening of the gubernaculum and by the passage of the tail of the epididymis. Thus, the gubernaculum, tail of the epididymis and tip of the vaginal process precede the testis through the inguinal canal. Final passage of the testis is achieved by tension from the gubernaculum and pressure from the abdominal viscera (Figure 29.5(c)). The times at which the testis is first present within the scrotum are given for the main domestic species in Table 29.1.

Spermatogenesis does not commence until the time of puberty. Thus, the seminiferous tubules of the prepubertal animal consist of immature Sertoli cells and relatively undifferentiated spermatogonial stem cells. As puberty approaches, the number and complexity of Sertoli cells increase, to reach maximal numbers at the onset of spermatogenesis. Mitoses in the spermatogenic cells occur at an increasing rate as puberty approaches, with puberty itself being characterized by the onset of meiosis and sperm production. The maximum spermatogenic rate is not reached until some time after the occurrence of puberty, for both the rate of

Table 29.1 Age at which testes descend into the scrotum

Species	*Time of testicular descent*
Cat	2–5 days after birth
Dog	Between the last few days of gestation and the first few after birth
Horse	Between 9 months of gestation and a few days after birth
Cattle	3.5–4 months of gestation
Sheep	Midgestation (80 days)
Pig	After 85 days of gestation

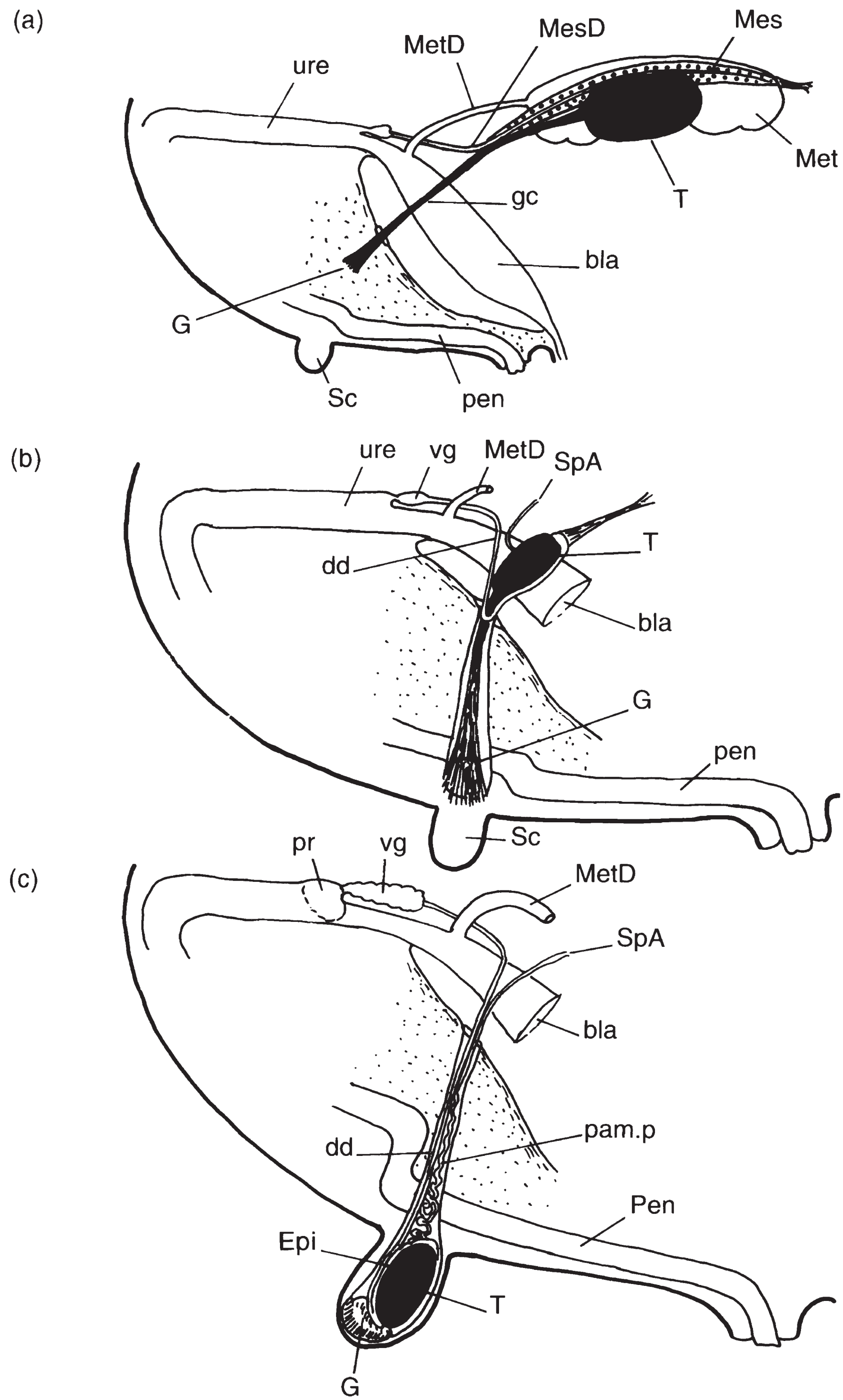

Fig. 29.5 Descent of the testis in the bull. (a) 65 day fetus, (b) 96 day fetus and (c) 140 day fetus. The changing relationships between the gubernaculum (G), testis (T) and vaginal tunics will be noted. bla, bladder; dd, ductus deferens; Epi, epididymis; gc, gubernacular cord; Mes, mesonephros; Met, metanephros; MetD, metanephric duct; pam P; pampiniform plexus; pen, shaft of penis; pr, prostate; Sc, scrotum; SpA, spermatic artery; ure, urethra; vg. vesicular gland. (Redrawn from Gier and Marion (1970), with permission.)

cell divisions and the size of the testis continue to increase into adulthood (see Hochereau-de-Reviers et al., 1987).

Physiology of the testis

Endocrinology

All aspects of male reproductive physiology are under the endocrine control of the two major gonadotrophins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The secretion of LH is pulsatile, with irregular episodes of secretion occurring every 2–4 hours. The actions of LH are primarily upon the Leydig cell, where, acting through adenylate cyclase, it promotes steroidogenesis by regulating the rate-limiting step of steroidogenesis; namely, conversion of cholesterol into the testosterone precursor, pregnenolone. Peak testosterone concentrations follow those of LH by about 40 minutes and decline back to prestimulation values over a further 40–80 minutes (D'Occhio et al., 1982a; Figure 29.6). Testosterone is required for the production of sperm in the testis and their subsequent maturation in the epididymis, for the function of the accessory sex glands and for the development of masculine secondary sexual characteristics. After aromatization into oestrogen within the brain, testosterone is also responsible for negative-feedback regulation of LH secretion and for male behaviour. Curiously, in long-term castrated animals, neither negative feedback nor libido can be restored by testosterone administration, for brain aromatase activity is eventually lost, and oestrogen itself has to be given for the restoration of these effects (D'Occhio et al., 1982b). Within the lumen of the seminiferous tubule, testosterone is converted by 5-reduction into 5-dihydrotestosterone (DHT), which is not susceptible to aromatization and is a more potent androgen than testosterone itself. Both testosterone and DHT are bound within the tubule lumen by the secretory product of the Sertoli cells, androgen-binding protein (ABP). The role of ABP therefore appears to be to maintain high androgen concentrations in the lumina of the seminiferous tubule and epididymis.

The main target of FSH is the Sertoli cell, where it also acts through adenylate cyclase-linked enzyme systems. Under the influence of FSH, Sertoli cells secrete ABP (Gunsalus et al., 1981) and aromatize testosterone into oestrogens (Setchell et al., 1983). Adeqate FSH stimulation is also required to permit Sertoli cells to support

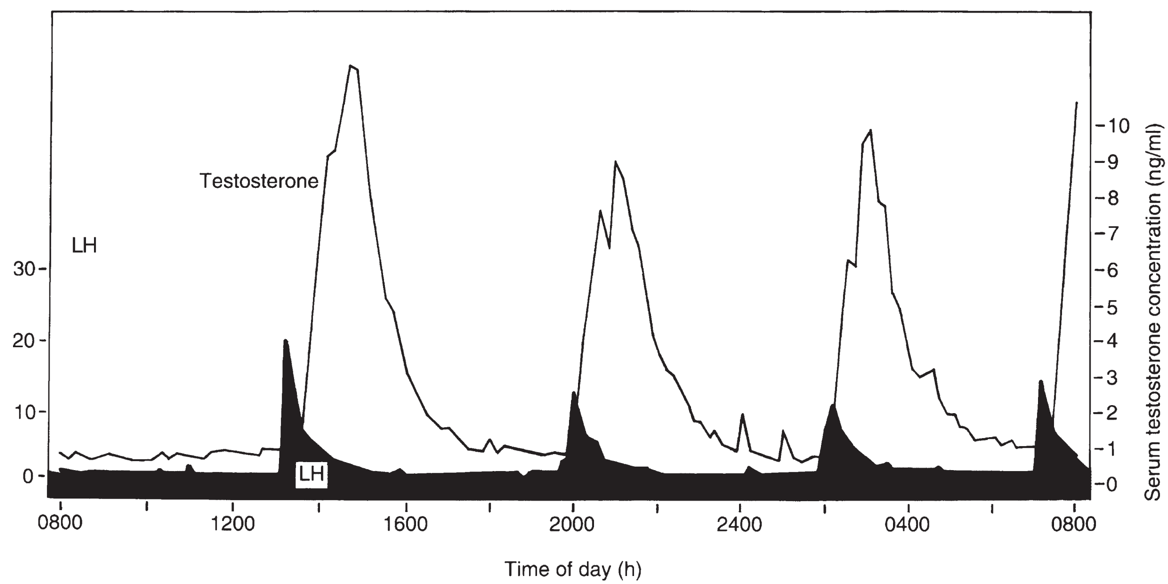

Fig. 29.6 Typical profile of the patterns of LH and testosterone secretion in the ram. (Redrawn from D'Occhio et al. (1982a).)

spermatogenesis. Some evidence suggests that the production of pyruvate and lactate, which act as energy substrates for germ cells, may be a key role of the FSH-stimulated activity of the Sertoli cell in maintaining spermatogenesis. Debate remains over the pattern of secretion of FSH; some consider it to be pulsatile, a manner analogous to LH, while others consider its secretion only to exhibit longer-term fluctuations. FSH secretion is regulated by both gonadal steroids and inhibin, the regulatory protein secreted by Sertoli cells (Baird et al., 1991).

The actions of the gonadotrophins are, however, not limited to the somatic cells of the testis, as both LH and FSH regulate aspects of germ cell activity. For example, experiments upon hypophysectomized rams indicated that the rate of division of stem spermatogonia is controlled by LH, while the rate of subsequent divisions and the ability of cells to undergo meiosis are regulated by FSH (Courot and Ortavant, 1981). Whilst these actions upon germ cells are, in part, mediated through the stimulation of activity in the somatic component of the testis by the gonadotrophins, circumstantial evidence indicates direct actions upon germ cells themselves. A schematic summary of the endocrine relationships of the testis is given in Figure 29.7 (Amann and Schanbacher, 1983).

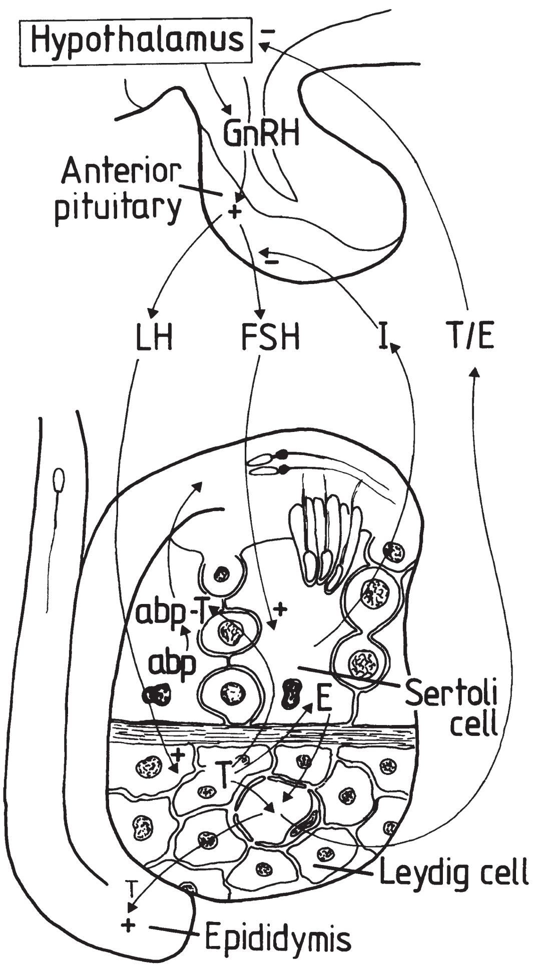

Fig. 29.7 Diagram of the endocrine relationships controlling the testis. abp, androgen-binding protein; E, oestrogen; GnRH, gonadotrophin-releasing hormone; I, inhibin; T, testosterone. (Redrawn and adapted from Amann and Schanbacher (1983).)

Spermatogenesis

Spermatogenesis is the basic process of male reproduction, resulting in the production of spermatozoa. It is carried out in the seminiferous tubule of the adult testis and comprises three main processes. Initially, the relatively undifferentiated spermatogonia undergo a period of mitotic, multiplication, divisions, followed by the meiotic reduction of the diploid to haploid genome. Finally, the postmeiotic cells undergo the morphological transformation of spermiogenesis, resulting in the release of formed spermatozoa into the lumen of the tubule.

These processes of spermatogenesis are reflected in the functional morphology of the seminiferous tubule (see Courot et al., 1970). The basement membrane of the seminiferous tubule is surrounded externally by fibroblasts and myoid cells. The blood supply is limited by the basement membrane and does not pass into the tubule itself. Within the tubule there are somatic Sertoli cells and the various stages of the seminiferous cell line, which together form the seminiferous epithelium. Sertoli cells rest upon the basement membrane, but extend through the entire thickness of the seminiferous epithelium, so that the germinal cells in all stages of spermatogenesis are in contact with the plasmalemma of Sertoli cells. Sertoli cells are irregularly cylindrical in shape, with large, variably shaped nuclei situated close to the basement membrane. They multiply during fetal and prepubertal life, with the full complement being present at the time of puberty. Until recently it was considered that Sertoli cell numbers were fixed at the time of puberty, but it is now evident that there is an annual cycle of loss and regeneration in at least some seasonally breeding species (Johnson and Thompson, 1983; Hochereau-de-Reviers et al., 1987). Sertoli cells secrete oestrogens, inhibin, a gonadotrophin-releasing hormone (GnRH)-like peptide, proteins (including ABP), lactate, pyruvate and tubule fluid. The cells are joined by specialized tight-cell-like junctions, so that the seminiferous epithelium is separated into apical and basal compartments by the blood–testis barrier formed by these junctions (see Hochereau-de-Reviers et al., 1990).

During early fetal life, primordial germ cells enter the body from the yolk sac. In the gonadal ridge these cells differentiate into gonocytes, which undergo mitosis throughout fetal and prepubertal life. Gonocytes in turn differentiate into spermatogonia, at which stage development in the semuniferous cells is arrested until the onset of puberty. In the mature animal, spermatogonia are divided into A, intermediate and B classes, with each class further subdivided according to morphology and degree of differentiation. Thus, in the ram, A_0, A_1, A_2, A_3, intermediate, B_1 and B_2 spermatogonia occur (Hochereau-de-Reviers, 1976). A-series spermatogonia are the least differentiated and form the reservoir of stem cells within the seminiferous tubule. It is likely that stem cells are regenerated by asymmetrical divisions of early A-series spermatogonia; with one daughter cell remaining as an uncommitted stem cell, the other being committed to undergo further mitotic and meiotic divisions. All spermatogonia remain in contact with the basement membrane, but, as the final meiotic division of spermatogonia gives rise to the primary spermatocytes, the cytoplasm of the Sertoli cells starts to intervene between the basement membrane and the primary spermatocytes. DNA synthesis occurs during mitotic divisions and then, to its greatest extent, during the formation of tetraploid nuclei during meiosis (for a review, see Hochereau-de-Reviers et al., 1990). RNA synthesis occurs during preleptotene and late pachytene (Kierszenbaum and Tres, 1974). The first meiotic division then proceeds through the highly sensitive zygotene and pachytene stages. The pachytene stage is particularly sensitive to noxious damage, such as by high testicular temperature and inadequate maintenance of spermatogenesis by inappropriate gonadotrophin levels. During the first meiotic division, the cells move deeper into the seminiferous epithelium, and the tight cell junctions of the Sertoli cells form beneath the spermatocytes and degenerate above them (Russell, 1977, 1978), so that the cells effectively pass through the blood–testis barrier. Thus, the progeny of the first meiotic division, the secondary spermatocytes, move from the basal to the apical compartment of the seminiferous epithelium and are thereafter separated from the general tissue fluid compartment. The second meiotic division produces spermatids, which do not divide further. The spermatids thereafter differentiate into spermatozoa (Figures 29.8 and 29.9).

At the end of meiosis, spermatids are round cells with round nuclei, which have to then undergo the very marked changes in cell function and morphology that occur during spermiogenesis. Immediately after completion of meiosis, the spermatids undergo a period of RNA synthesis, which is then followed by the beginnings of nuclear chromatin condensation (Monesi, 1971). Simultaneously, acrosomal contents are synthesized in the Golgi, whose vesicles progressively fuse to form the acrosome. As the nucleus condenses and elongates, the acrosome forms over the basal pole of the nucleus (Courtens, 1979), while at the opposite pole the flagellum starts to form from one of the centrioles. A transient microtubular structure, the manchette, appears during the formation of the flagellum in the postnuclear cytoplasm of the elongating spermatid. The function of the manchette is unknown and it disappears after the flagellum is formed (Fawcett, 1970; Zirkin, 1971). The last stage of flagellum formation is the development of the midpiece, when a helix of mitochondria condense around the proximal part of the flagellum. During formation of the acrosome and flagellum, the cytoplasm of the spermatid is deeply invaded by a process of the Sertoli cell that extends between the forming flagellum and the residual cytoplasm. It is suggested that this process is responsible for the reduction in cytoplasmic volume of the spermatid

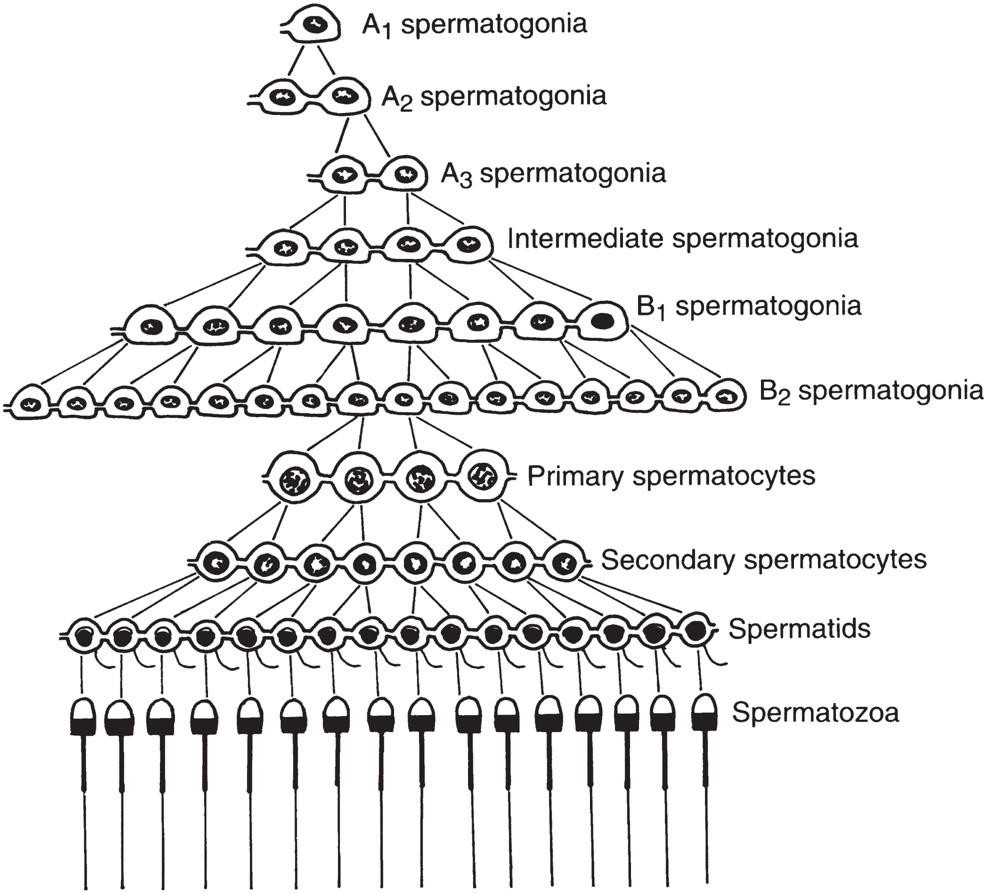

Fig. 29.8 Multiplication of cells during spermatogenesis in the bull. A_1 spermatogonia undergo a series of mitotic divisions, to produce A_2, A_3, intermediate, B_1 and B_2 spermatogonia. The final mitotic division produces primary spermatogonia, which enter meiosis, producing secondary spermatocytes after the first meiotic division and spermatids after the second. Spermatids then differentiate into spermatozoa without further cellular division.

that occurs during spermiogenesis. Finally, most remaining cytoplasm is engulfed by the Sertoli cell as the formed spermatozoon, with its remnant cytoplasmic droplet, is expelled from the crypt of the Sertoli cell into the lumen of the seminiferous tubule (see Fouquet, 1974).

The duration of spermatogenesis, i.e. the time between spermatogonial divisions and the release of the spermatozoan, is approximately 60 days in most domestic animals. Epididymal transit takes a further 8–14 days. Thus, the interval between the most sensitive stage of spermatogenesis, meiotic prophase, and ejaculation, is approximately 30 days (see Amann and Schanbacher, 1983). Hence, the interval between damage to the testis and the appearance of abnormal spermatozoa in the ejaculate is generally between 30 and 60 days, depending upon the site of damage.

The seminiferous epithelium appears as concentric layers of spermatogonia, spermatocytes and spermatids, with characteristic associations between generations of cells throughout the depth of the seminiferous epithelium. Each generation of seminiferous cells is linked by cytoplasmic bridges, so that developmental stages are synchronous within each generation, and substantial areas of seminiferous epithelium exhibit cells at a similar stage of development. Cellular associations are generally classified into type I, where two generations of primary spermatocytes and one of

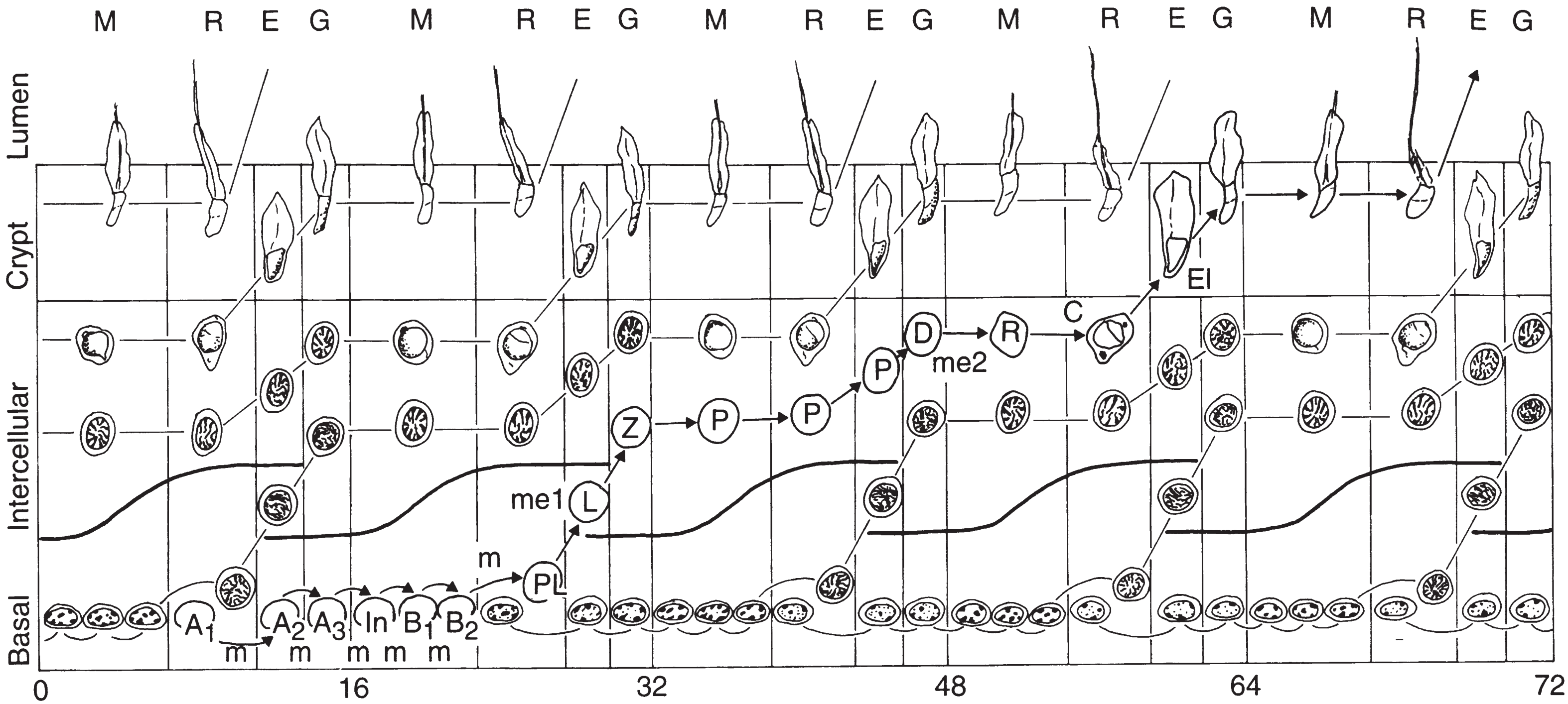

Fig. 29.9 Spermatogenesis in the bovine testis. Stem cells undergo mitotic (m) divisions producing successive generations of diploid spermatogonia (A_1, A_2, A_3, intermediate (In), B_1 and B_2), before entering the first meiotic division (me1). Preleptotene primary spermatocytes (PL) pass through the successive stages of the long first meiotic prophase (L, leptotene; Z, zygotene; P, pachytene; D, diakinesis), becoming short-lived secondary spermatocytes. These proceed through the second meiotic division (me2), producing early round spermatids (R). These differentiate into spermatozoa without further division, after nuclear condensation (C) and elongation (El). The solid horizontal line indicates the position of tight junctions between Sertoli cells: the site of the blood–testis barrier. Four major groupings of cells can be determined, based upon the morphology of the spermatids/spermatozoa; E, elongation; G, grouping, M, maturation; R, release. These recur every 16 days. (Adapted from an original drawing by Brian Setchell.)

spermatids are present, and type II, where there is only one generation of primary spermatocytes but two of spermatids (see Hochereau-de-Reviers et al., 1990). Transition between type I and type II occurs after the maturation divisions, while type II changes into type I with the release of spermatozoa and the arrival of a new generation of spermatocytes from the last spermatogonial division.

Physiology of the epididymis

Considerable changes occur to spermatozoa as they pass through the epididymis (for reviews, see Amann, 1987; Hammerstedt and Parkes, 1987). The epididymis is highly androgen-dependent; thus, if androgen levels are suppressed, epididymal function is immediately impaired. The protoplasmic remnant, which is initially sited close behind the sperm head, migrates distally to the end of the midpiece, before being finally shed in the tail of the epididymis. Sperm are immotile in the head of the epididymis, but they acquire the capacity for motility as they pass through its body. Similarly, in the head of epididymis, sperm do not have the ability to fertilize, but this is acquired during passage of the epididymal body. Less obvious, but of equal or greater importance to the morphological changes exhibited by sperm during their passage of the epididymis, are the changes in their plasma membrane, to which surface glycoproteins are added or modified by epididymal secretions and luminal cells. It is likely that these act to stabilize the acrosome while the sperm is within the female genital tract, to reduce the surface immunogenicity of the sperm and to enhance the ability of the sperm membrane to bind to the zona pellucida.

Spermatozoa take between 8 and 14 days to traverse the epididymis, according to species. In the bull, sperm take 5 days to pass through the head and body of the epididymis and a further 5–9 days to traverse the epididymal tail. The transit times of the head and body are fixed, but the tail of the epididymis has dual functions of both a site of maturation and storage, so that, in periods of high ejaculation frequency, the passage time of the tail may be reduced and relatively immature sperm ejaculated. Although sperm held in the tail of the epididymis have the capacity for motility, motility is not itself acquired until the time of ejaculation. Thus, sperm within the epididymis exhibit little motility, but are rapidly activated upon mixing with seminal plasma during ejaculation.

Structure and function of spermatozoa

Spermatozoa are divided into three main segments: the head, midpiece and tail (Figure 29.10). The head consists of little other than the condensed nucleus and the overlaying acrosome. Of the enzymes contained within the acrosome, the main two are acrosin and hyaluronidase (Morton, 1977). During the acrosome reaction, the outer acrosomal membrane fuses with the plasmalemma, under the control of intra- and extracellular calcium, whereupon exocytosis of the contents of the acrosome occurs (see Harrison and Roldan, 1990). The main functions ascribed to the acrosomal enzymes are dispersal of the cumulus oophorus and local lysis of the zona pellucida; although it has been questioned recently whether the latter function is indeed a function of the released acrosomal enzymes per se. The inner membrane of the acrosome is relatively stable and remains intact after the acrosome reaction has occurred, and some of the acrosomal enzymes are probably bound to the inner acrosomal membrane. Penetration of the zona pellucida and fusion with the oolemma are both receptor-mediated events, with specific areas of the sperm head binding to target components of the oocyte (see Wassarman, 1990).

The midpiece and tail of the sperm may be considered to form a single functional entity. The tail itself consists of a central axoneme, which, in the region of the midpiece, is sheathed in a helix of mitochondria (reviewed by Bedford and Hoskins, 1990). Sperm metabolize simple molecules, principally sugars and their derivatives (e.g. fructose, glucose, mannose and pyruvate), by both aerobic and anaerobic pathways, to provide energy for motility and the maintenance of ionic gradients across membranes (see Harrison, 1977). Forward motility of sperm results from coordinated waves of flagellar bending progressing from neck along the length of the tail. Bending of the tail occurs as the result of forces generated between adjacent peripheral doublets of the axoneme (Satir et al.,

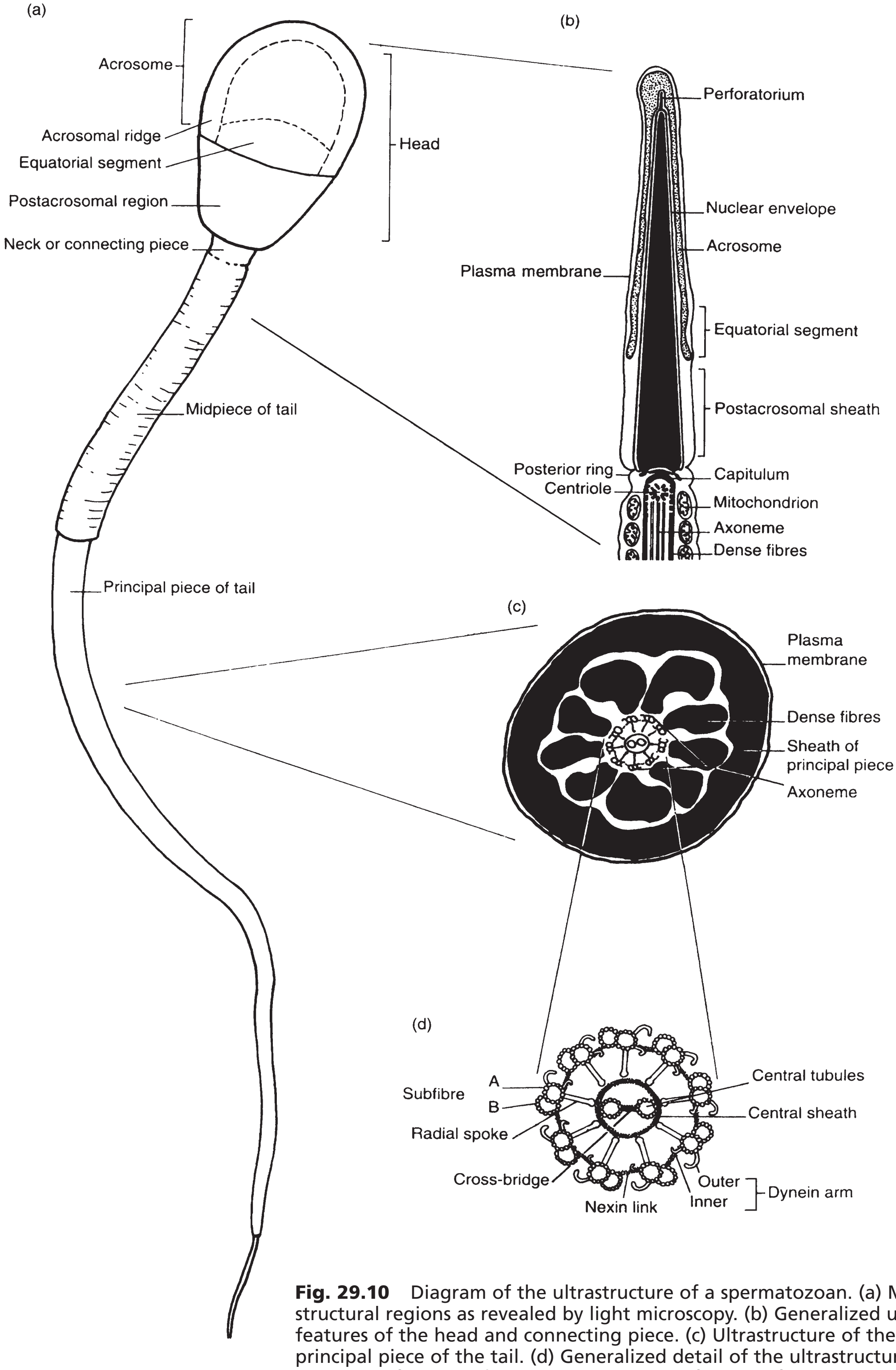

Fig. 29.10 Diagram of the ultrastructure of a spermatozoan. (a) Main structural regions as revealed by light microscopy. (b) Generalized ultrastructural features of the head and connecting piece. (c) Ultrastructure of the proximal principal piece of the tail. (d) Generalized detail of the ultrastructure of the axoneme of the tail. (Redrawn and adapted from Bedford and Hoskins (1990).)

1981). The dynein arms of the doublet, which in the resting state are bound to the adjacent doublet, unbind, elongate and then bind to a new site further along the filament. The unbinding process, which is the ATP-using step, is then repeated, resulting in a progressive bending of the flagellum. The doublets on one side of the axoneme work in opposition to each other, providing the alternating beat of the tail. After capacitation, the rate and amplitude of the flagellar beat greatly increase, and the rate of energy usage by the sperm is correspondingly elevated (Yanagimachi, 1981). The motility of the cell itself probably has little role in the movement of spermatozoa through the cervix and uterus, for this is accomplished mainly through contractions of the female genital tract (Hunter, 1980). However, passage through the uterotubal junction and within the oviduct does require sperm motility, while the enhanced, whiplash motility of the capacitated sperm is necessary for penetration of the cumulus and zona pellucida.

Accessory glands

The accessory glands include the ampullae, prostate, vesicular glands and bulbourethral (or Cowper's) glands. There is much variation between the anatomy of the accessory glands between species, which is summarized in Table 29.2.

Ampullae are present as dilations of the terminal portion of the vasa deferentia, just before they enter the pelvic urethra, where their main function is to act as reservoirs of sperm. In the bull, ram and dog, the ampullary glands that are present, contribute slightly to the seminal plasma, whereas their contribution to the ejaculate is relatively important in the stallion. The main constituent of ampullary secretion in the stallion is ergothionine (Mann et al., 1956).

Table 29.2 Accessory sex glands of the main domestic species

Species	*Ampulla*	*Prostate*	*Vesicular gland*	*Bulbourethral gland*
Cat		++		++
Dog	(+)	+++		
Horse	++	++	++	+
Cattle	(+)	++	+++	+
Sheep	(+)	++	+++	+
Pig		+	++	+++

The relative size and importance are indicated by the number of + symbols. Ampullary glands are present in species marked (+) but are not anatomically prominent.

Vesicular glands are prominent in ruminants, the stallion and the boar. They are sac-like in the stallion and boar, and are firm, lobulated structures in the ram and bull. The glands are adjacent to the neck of the bladder and lateral to the ampullae. They open into the urethra just distally to the vasa deferentia. Their secretion, which is generally watery, contributes substantially to the volume of the semen. In all species its secretion contains large quantities of citrate, while in the ruminants it also contains fructose and, in the boar, inositol (Mann et al., 1949, 1956; Mann, 1954; Marley et al., 1977).

The *prostate* is intimately related to the pelvic urethra and, in most species, is in two parts; its body surrounds the neck of the bladder and its disseminated part spreads around the pelvic urethra into which it has several openings. In the dog, the prostate is the main accessory gland and is relatively large, forming a discrete organ around the urethra. The prostatic secretion is watery and, in the dog, contains large quantities of chloride ions, but citrate, fructose and inositol are not present in high concentrations (see Huggins, 1945).

In the stallion, bull and ram, the *bulbourethral* (or Cowper's) *glands* are small, rounded structures lying between the anus and urethra. Their watery secretion is discharged prior to coitus and is considered to cleanse the urethra of urine. In the boar, the bulbourethral glands are large, cylindrical structures lying along each side of the intrapelvic urethra. In this species, their secretion is very viscid, due to its high sialomucin concentration (Boursnell et al., 1970) and combines with the secretion of the vesicular glands to produce a gelatinous phase of the seminal plasma (Boursnell and Butler, 1973).

The physiological functions of the various constituents of seminal plasma remain a matter of debate (for a review, see Brooks, 1990). There is much interspecies variation in the composition of seminal plasma, so it has been difficult to ascribe absolute functions to many of its constituents. Provision of energy, maintenance of osmotic pressure, chelation of free calcium ions and buffering are some of the suggested functions, while other possibilities include immunosuppression in the female genital tract and regulation of spermatozoan motility. Seminal plasma is also responsible for the coagulation of semen, which occurs shortly after ejaculation in many species. However, it has been argued that the wide variety in constitution of seminal plasma between species indicates that it has no critical role, an argument that is emphasized by the ability of sperm to survive in relatively simple media which bear little resemblance to seminal plasma. Paradoxically, the seminal plasma of many species appears to contain spermicidal factors, especially in the post-sperm-rich fraction of the species that produce a fractionated ejaculate.

The penis

The penis comprises three tracts of erectile tissue and the penile urethra. The urethra is surrounded by the corpus spongiosum penis (CSP), which arises at the bulb of the penis and terminates in the glans penis. The dorsum of the penis is made up of the paired corpora cavernosa penis (CCP; Figure 29.11), which arise in the two crura (roots) of the penis and terminate behind the glans. The blood supply of all three tracts is via branches of the pudendal artery, but the venous drainage of the CCP is markedly different from that of the CSP. The CCP is drained via the root of the penis, into the pudendal vein, whereas the CSP drains into the dorsal vein of the penis from its distal extremity (Ashdown and Gilanpour, 1974). Thus, both the supply and drainage of blood to the CCP are via the root of the penis, whereas the supply of the CSP is through the bulb and its drainage from its distal part. The roots of the penis are surrounded by the ischiocavernosus muscles, which, on contraction, occlude the veins draining the CCP against the ischium of the pelvis, so that the cavernous spaces of the erectile tissue in the blind-ending CCP become engorged with blood, causing stiffening and lengthening of the penis (Beckett et al., 1974). However, the detailed anatomy of the penis varies greatly between species (Figure 29.12) and, as a result, details of the functional anatomy of erection are similarly variable.

In Artiodactylae, the penis has a thick, fibrous tunica albuginea overlying the CCP and surrounding the urethra, and the individual cavernous spaces within the CCP are relatively small. The penis also has a sigmoid flexure, which is either caudal (postscrotal), as in the ruminants, or cranial (prescrotal) to the scrotum, as in the boar. In these species, relatively little blood enters the penis during erection, although the blood pressures achieved are considerable, exceeding 40 000 mmHg during ejaculation. In order that the increased pressure induced by ischiocavernosus muscle activity can be transmitted throughout the length of the penis, specialized artery-like vascular canals, the longitudinal canals, run the length of the penis. Paired canals arise in the crura of the penis, which fuse shortly afterwards to produce a single dorsal canal. Either side of the dorsal canal thereafter gives rise to a series of branches, which join together to form paired ventral canals. The dorsal canal runs for the proximal third of the penis, the ventral canals for the remaining distance, with a short distance of overlap with the dorsal canal. Lengthening of the penis is achieved partly by longitudinal expansion of the cavernous spaces between the trebeculae of the CCP, but mainly by straightening of the sigmoid flexure of the penis (Ashdown, 1970). As a result, the penis, which is normally carried high in the preputial cavity, is fully exteriorized from the narrow preputial orifice. Obliteration of the sigmoid flexure and forward movement of the penis is made possible by the very loose arrangement of the connective tissue that surrounds the penis and prepuce. In the ruminants, full erection is only briefly attained during the single ejaculatory thrust, but copulation is more prolonged in the boar. Erection is terminated by cessation of ischio-cavernosus muscular contraction, and the penis is returned to the preputial cavity by contraction of the retractor penis muscles, which restores the sigmoid flexure.

The other domestic species have a musculocavernous penis, in which the tunica albuginea is less pronounced and the cavernous spaces of the erectile tissue larger than in the artiodactyls. In the stallion (see Nickel et al., 1973; Amann, 1993),

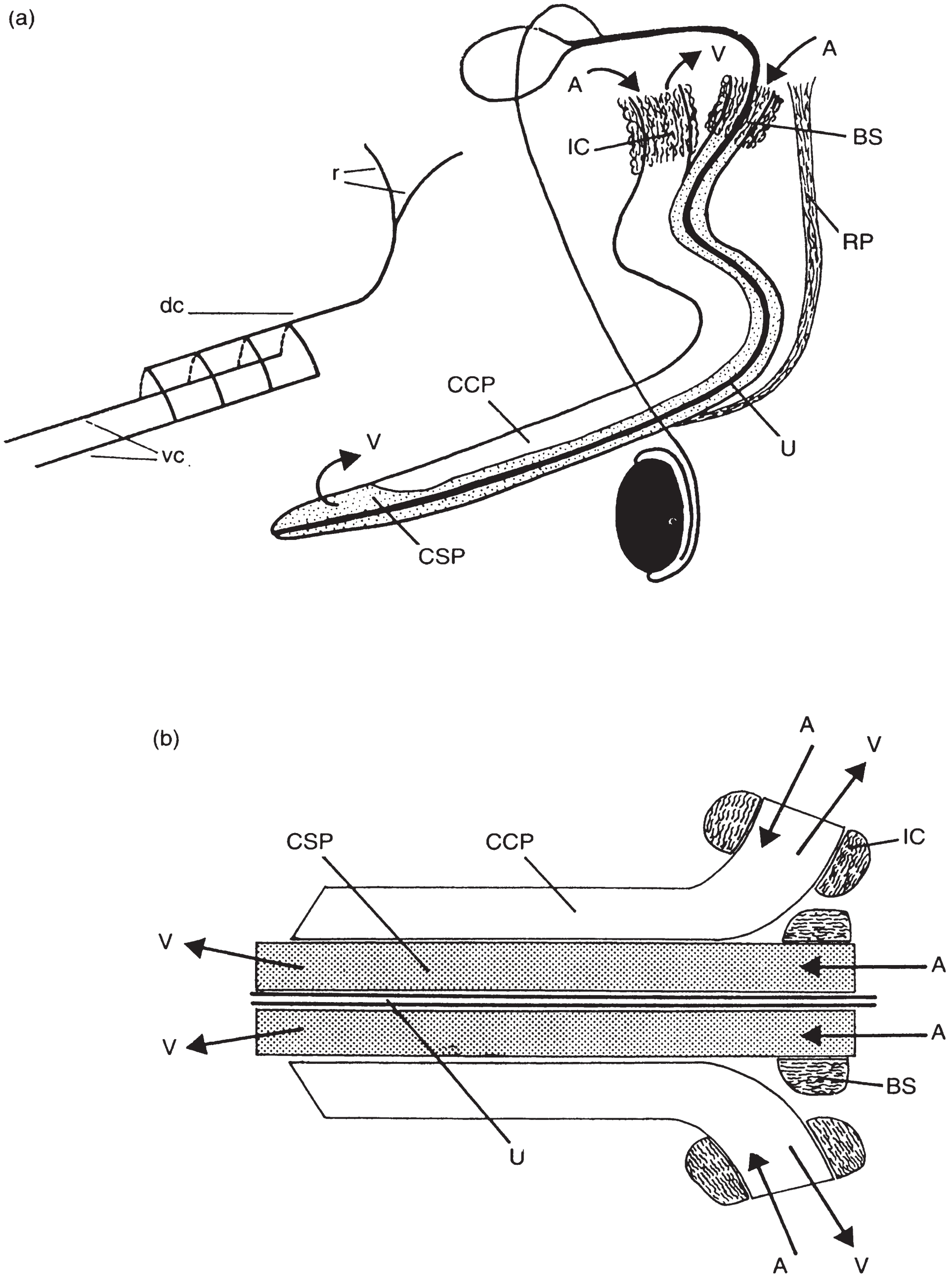

Fig. 29.11 Functional vascular anatomy of the bovine penis: (a) representational and (b) diagrammatic. Blood enters and leaves the corpus cavernosum (CCP) via the arteries (A) and veins (V) of the crura (roots) of the penis, so that contraction of the overlying ischiocavernosus (IC) muscles occludes the venous drainage and forces blood into the penis under pressure. The blood passes through the penis in longitudinal canals (inset). Two canals arise in the roots (r) of the penis and unite to form a single dorsal longitudinal canal (dc). This gives off a series of lateral branches in the region of the sigmoid flexure, which unite to form paired ventral canals (vc). The corpus spongiosum (CSP) is drained from its distal end, so contraction of the bulbospongiosus (BS) muscle only produces a transient increase in hydrostatic pressure. This is of sufficient magnitude to temporarily occlude the urethra, so that a bolus of semen can be propelled along its length. During detumescence, vascular pressure is lost and the penis is returned to the preputial cavity by the retractor penis (RP) muscle. (Redrawn and adapted from Laing et al. (1988), with permission.)

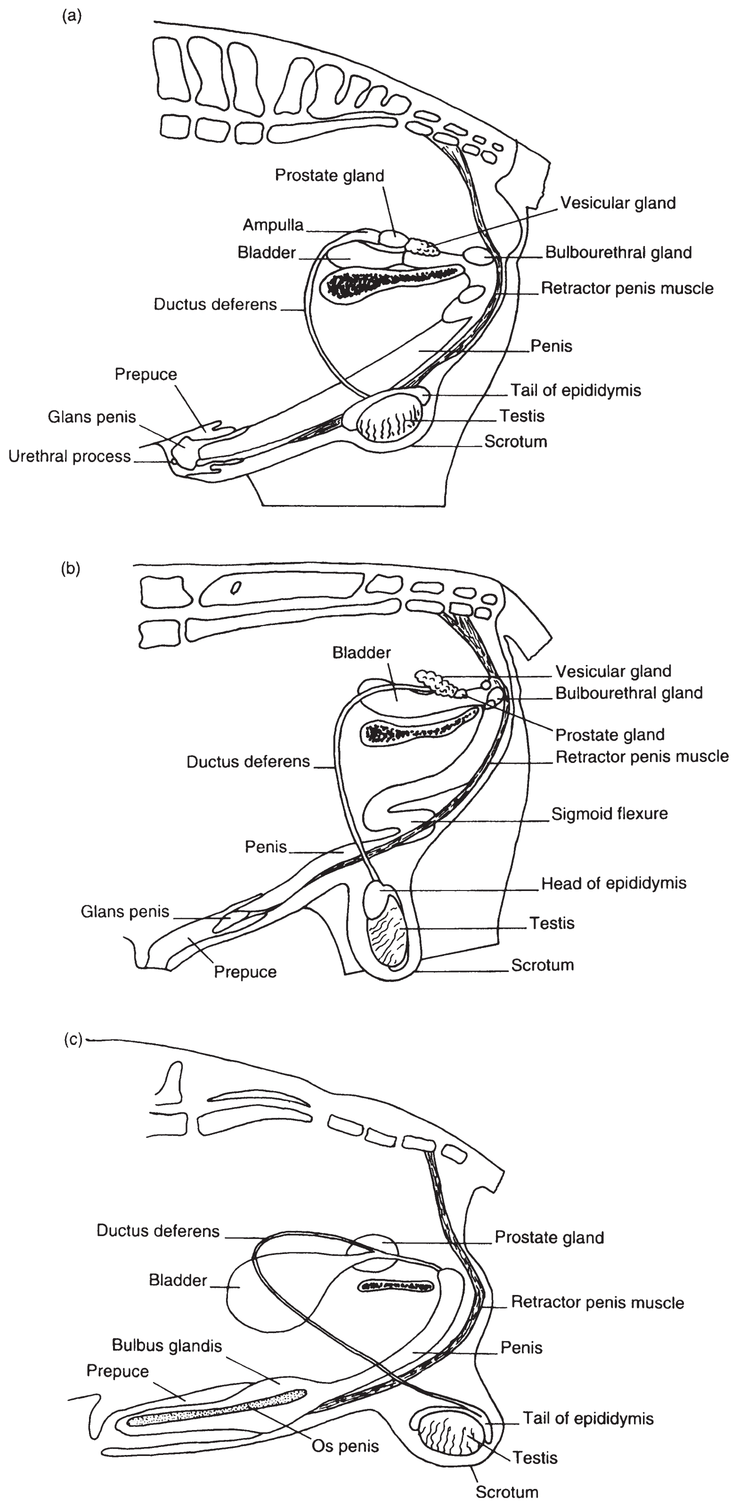

Fig. 29.12 Comparative anatomy of the penes of (a) the stallion, (b) bull and (c) dog. (Redrawn and adapted from Laing et al. (1988), with permission.)

tracts of longitudinally oriented smooth muscle fibres are associated with the trabeculae of the CCP. These are normally in a state of tonic contraction, holding the penis in the prepuce. The tone in these muscles falls during erection and micturition, leading to prolapse of the penis from the prepuce. In the stallion and dog (Evans and deLahunta, 1988), erection produces increases in both length and girth of the penis and, as there is no sigmoid flexure, the lengthening of the penis is caused entirely by vascular engorgement.

The ejaculation reflex is stimulated by sensory nerves within the glans penis that transmit to the spinal cord through the dorsal nerve of the penis, a branch of the pudendal nerve. Thereafter, erection and ejaculation are primarily coordinated as spinal reflexes in the lower lumbar and sacral segments of the spinal cord. The integrity of this nerve is essential for the ejaculation reflex to take place and, if it becomes damaged, ejaculation, though not erection, becomes impossible (Beckett et al., 1978). Pressure, tactile sensation and, in the bull, temperature are the main stimulants to ejaculation. During ejaculation, the glans of the penis of the bull and goat become coiled (Ashdown and Smith, 1969), while the vermiform appendage of the penis of the ram shows a vigorous flicking movement. It is probable that these conformational changes are to assist with deposition of semen in and around the external os of the cervix. The glans penis of the stallion and boar engage in the cervical canal, with ejaculation occurring through the cervix into the uterine lumen. Thus, the penis of the boar adopts a spiral conformation during copulation that mirrors that of the cervix of the sow, but the stallion shows only a very pronounced enlargement of the glans penis during ejaculation. The penis of the dog is unique amongst the domestic animals in having an os penis, in whose grooved ventral floor lies the urethra. During copulation, the penis of the dog is gripped by the levator vestibuli of the vagina of the bitch, whereupon engorgement of the bulbus glandis occurs. Ejaculation occurs over a prolonged period of time, with the brief production of the pre-ejaculatory and sperm-rich fractions being followed by a very protracted deposition of prostatic fluid during the copulatory tie.

Propulsion of semen along the urethra is achieved by contraction of the bulbospongiosus muscle that overlies the CSP in the bulb of the penis. Because the CSP drains from its distal end, the high pressures achieved in the CCP cannot be attained. Thus, each contraction of the bulbospongiosus muscle causes a transient wave of increased pressure in the CSP which progresses from the bulb to the glans, where it dissipates by dorsal venous drainage of the blood. Because the CCP is turgid, the increased pressure within the CSP causes a wave of occlusion of the urethra. This progressive wave of urethral occlusion, assisted by the contraction of the muscle that surrounds the extrapelvic urethra, causes conduction of boluses of semen along the urethra.

Development of the penis

Initial development of the phallus from the genital tubercle is similar in both male and female fetuses, but, in the male fetus, rapid enlargement occurs early in development. At birth, the penis is fused with the prepuce throughout its length, with small lateral veins draining the erectile tissue. During prepubertal development, the connective tissue joining penis and prepuce breaks down and the veins become occluded. The frenulum is the most substantial of the connections between the penis and prepuce, frequently containing quite large blood vessels. It is often the last part to break down and may not infrequently persist into post-pubertal life.

Libido and mating behaviour

Libido is primarily dependent upon androgenic steroid hormones, which allow mating and aggressive behaviour to occur, as well as maintaining the function of all parts of the male reproductive system. Libido seldom is expressed in animals that are castrated before puberty although, if a mature animal that has learnt to copulate is castrated, erection and copulation may persist for long periods or, occasionally, indefinitely. Despite the dependency of male behaviour upon androgen, there has been much debate over the relationship between absolute concentrations of androgen and libido (Foote et al., 1976; Wodzicka-Tomaszewska et al., 1981). Some have argued a permissive role of androgen, while others have demonstrated positive correlations between testosterone concentrations and measures of libido. Breeds of bull that are aggressive and respond quickly to the presence of an oestrous female tend to have higher testosterone concentrations than the more phlegmatic breeds, but whether this is a causal relationship remains unclear.

The males of those domestic species that are naturally herd animals spend a great deal of time detecting oestrus (see Chenoweth, 1981). Oestrous females of many species secrete pheromones to attract males, while others, notably the cow and some breeds of pig, exhibit homosexual behaviour as a signal to the male of the presence of oestrus. All males smell the perineal region of the female, and the odour of the oestrous female induces the so-called 'flehmen' reaction in the bull, ram and stallion: a characteristic raised posture of the head an an elevation of the upper lip. Females that are not in oestrus signal their objection to the advances of the male and, at the least, will respond by moving away or, perhaps, by attacking the male in the offensive manner peculiar to the species. During pro-oestrus, the interest of the male in the female is increased but, whereas she associates with the male, she will not permit mounting. Oestrous females signal receptivity by squatting, urinating, moving the tail to one side and remaining stationary (Figure 29.13). During this foreplay the male becomes progressively aroused; there are frequent erections of the penis, with emission of accessory fluid and many unsuccessful attempts to mount the female. Finally, mounting and copulation occur.

Stallion

Following intromission, the stallion performs a succession of copulatory movements of the hindquarters which, within a minute, culminate in ejaculation. During ejaculation, successive waves of urethral peristalsis can be palpated on the lower surface of the penis, while the stallion exhibits a characteristic 'flagging' movement of the tail (Figure 29.14). The stallion then dismounts.

Ruminants

Copulation in all of the domestic species of ruminants is brief. After detecting an oestrous female, mounting is followed quickly by the single ejaculatory thrust. The male then immediately dismounts, but frequent subsequent matings occur. Farm management of rams and bulls should take this high mating frequency into account. Thus, whereas individual sires can service quite large groups of spontaneously cyclic females, the

Fig. 29.13 Signs of oestrus in the mare include squatting, urination and moving the tail to one side.

Fig. 29.14 The main external sign of ejaculation in the stallion is tail flagging. Peristaltic waves can also be palpated in the penile urethra.

groups of females have to be much reduced if these have undergone oestrus synchronization. Furthermore, care has to be exercised in the use of young sires. The testis does not reach its full size and sperm-producing capacity until 1–2 years after puberty, and the epididymis does not reach its full length until the same time (reviewed by Salisbury et al., 1978). Until maturity has been reached, groups of females should be smaller than for adult animals. If overused, both the numbers of sperm produced are too few for adequate conception rates to be achieved and sperm from the tail of the epididymis are ejaculated before their functional maturation is complete. Thus, sperm numbers and sperm maturity are both adversely affected, causing severe limitation upon the chance of conception.

Boar

Copulation is relatively prolonged in pigs, lasting for between 5 and 15 minutes. Following intromission, the first phase is occupied by a series of vigorous thrusting movements of the hindquarters of the boar. During this phase, the first part of the fractionated ejaculate is produced, which comprises only accessory fluid. The second phase is quieter and accompanied by production of the sperm-rich fraction of the ejaculate. This is followed by a final, more vigorous phase, in which the third jelly-like accessory secretion is discharged. Ejaculation occurs directly into the uterus, which is distended with semen immediately after copulation, while the cervix is often sealed with a gelatinous plug. The large volume of semen produced by the boar is necessary to convey its spermatozoa through the extensive length of the uterus of the sow

Dog

The dog achieves intromission by vigorous thrusting of the hindquarters. Once intromission has been achieved, the bulbus glandis swells considerably, while the constrictor vulvae muscles of the bitch contract behind it, thus forming the 'copulatory tie'. The sperm-rich fraction of canine semen is ejaculated within as little as 80 seconds of intromission, so that conception may occur even if copulation does not proceed through to its second stage. In this second stage, the dog dismounts but remains connected and faces away from the bitch (Figure 29.15). This change of position causes the penis to become bent through an angle of 180°; the efferent veins of the penis are thereby occluded and the penis remains turgid. The function of the tie may be to prevent penile detumescence during the prolonged second stage of copulation, during which some 30 ml of sperm-free accessory fluid is pumped into the vagina and thence to the uterus, where it flushes the sperm-rich fraction through the uterus. The sexes remain locked until the vulval muscles relax and penile erection wanes.

Cat

During mating, the tom mounts the queen and grasps her neck with his teeth. As the tom adjusts his position the queen paddles her hindlegs, continuing to do so at an increasing frequency during the 10 seconds or so for which coitus lasts. The queen cries out during copulation and, as the tom dismounts, she may strike out at him, displaying the typical 'rage' reaction. This is followed by a period of frantic rolling and licking at the vulva. As soon as the postcoital reaction has ceased, the tom will attempt to mount again. Several matings

Fig. 29.15 The copulatory tie in the dog.

may therefore occur within the first 30–60 minutes. The cat is an induced ovulator (Shille et al., 1983), so the number and frequency of matings are important in ensuring that the LH surge is of sufficient magnitude to cause ovulation (Tsutsui and Stabenfeldt, 1993).

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30 Fertility and infertility in male animals

EXAMINATION FOR BREEDING SOUNDNESS

Examinations of male animals are made for two main purposes: either to ascertain whether normal fertility can be expected from the animal, or for the diagnosis of infertility. In either situation, the requirements are a history of the animal, a general examination, a detailed examination of the genital tract, observation of copulation, and collection and evaluation of semen.

History-taking is an important part of the examination of a suspected infertile male animal. Many of the causes of infertility do not manifest themselves until a considerable period of time has elapsed from the original insult, so that careful questioning of the owner, often over matters that may have been considered trivial at the time of their occurrence, may be needed to elucidate such causes. History-taking is also a useful way of assessing owners' expectations of their animals, for many cases of so-called 'infertility' result from no more than an unrealistic expectation of a sire's capabilities.

The history must establish whether or not the sire is likely to be the cause of the infertility, the duration of infertility and the circumstances of its onset. The number of females with which the sire's infertility has been manifest must be determined, as must the conditions under which mating has occurred. For example, it is not uncommon for dogs to be presented for infertility examination after failure to achieve pregnancy on no more than one or two occasions, with bitches that were scarcely in oestrus. Clearly, under such circumstances, the probability of a pathological cause of infertility is minimal. Amongst agricultural animals, the sizes of groups of females and the system under which mating was taking place must be determined. A common cause of apparent infertility in rams derives from no more than using groups of too many ewes, especially if these have undergone synchronisation of oestrus or are being used in out-of-season breeding regimens. Table 30.1 gives suggested ratios of females to males for the main agricultural species, under various mating systems. The time of year when the infertility was noticed may give helpful clues as to its cause, and may help to determine whether female factors are likely to have been of importance. Similarly, information regarding the previous achievements of the animal is of great importance in differentiating between congenital and acquired conditions, or between managemental and pathological causes. If records of the management and reproductive performance of the herd are available, they are invaluable in ascertaining the overall level of the fertility of the herd. Records may also provide useful comparative information for other contemporary sires and may help to pinpoint the onset and duration of the period of low fertility.

Observation of the normal environment of the sire is usually advisable. Seeing how the animal is handled, how it is housed, fed and cleaned, observing the area in which it is required to serve, how it is moved there and how it is handled during service, all may assist with one's assessment of the infertility of the animal.

The general examination of the sire must take into consideration its age and likely sexual experience, body condition, the possibility of intercurrent illness and the animal's temperament. Considerable importance can be attached to the body condition and general degree of maturity of young animals; on one hand, puberty can be delayed in poorly grown animals, while, on the other hand, animals that have achieved very high growth rates during rearing may have a body conformation that belies their sexual immaturity. It is

Table 30.1 Numbers of females per male of agricultural animals in various mating systems (derived from Roberts, 1986 and Levis, 1992)

Species		*Mating system*		*Overall ratio (females in herd per male)*
Bull	Spontaneously cyclic groups	Oestrus-synchronised	In hand[a]	
Immature	10–20	NA	2–4	20–60
Mature	20–40	10	4–12	80–120
Ram	Spontaneously cyclic groups	Oestrus-synchronised (in breeding season)	Oestrus-synchronised (out of season)	
Immature	20–30	NA	NA	20–30
Mature	40–80	10–20	5–10	40–80
Boar	Group synchronised by weaning[b]	In hand[a]		
Immature	1–2	1–2		20
Mature	2	1–4		20–30

[a] In hand: number of supervised double services per week
[b] Overall ratio of boars:sows. Boars would be rotated with larger groups of sows, with periods of sexual rest to give an overall service frequency of 1–4 per week

also noticeable that young bulls of some later maturing breeds, notably the Charolais and Holstein, may remain relatively subfertile for longer than their earlier-maturing counterparts (Figure 30.1; Coulter, 1980). Thus, the assessment of young sires can present some difficulty, for allowance has to be made for the maturity charac-

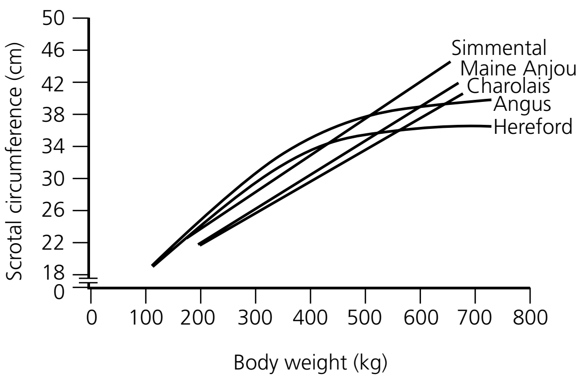


Fig. 30.1 Relationship between body weight and scrotal circumference of beef bulls. British beef breeds initially exhibit faster testicular growth than their Continental counterparts but, as the former reach mature body weights, testicular growth ceases. The later-maturing Continental sires therefore have a longer period of testicular growth, related to their greater mature body weight (redrawn from Coulter, 1980, with permission).

teristics of the breed, yet the use of young bulls in which puberty is excessively delayed is best avoided in view of the evidence that the age of puberty in a sire is highly correlated with the age of puberty in his daughters. This problem is further compounded by the pressure of time imposed by the requirements of progeny testing, particularly of dairy bulls. In a seasonally calving national herd, semen from young bulls has to be available roughly 12 months after their birth, if progeny testing is to be carried out successfully. For Friesians, this target presented little difficulty, but a significant proportion of young Holstein bulls do not produce consistently usable semen until beyond this age. By the time they reach this age, the majority of cows in the national herd will already be pregnant, so progeny testing is delayed until the following season.

When young sires are used in natural-mating regimens further difficulties may be encountered. Firstly, it must be ascertained that they have learnt to mount and successfully copulate. Similarly, it must be determined that copulation is feasible, since, where young sires are running with fully mature females, copulation may not be physically achievable. Furthermore, it is not uncommon for those young boars or rams that are run with large, mature females to be bullied by these females, to achieve no pregnancies and to lose a great deal of body condition. Secondly, young sires are most unlikely to achieve high pregnancy rates amongst large groups of females and are generally unsuitable for use with groups of oestrus-synchronised females.

Body condition is also important in adult males. Spermatogenesis tends to be limited when body condition is poor, and can also be limited by specific micronutrient deficiencies. In general, chronic and continuing deficiencies of protein and energy are likely to be of greater overall importance than micronutrient deficiencies, although the effects upon fertility can be severe when these occur simultaneously (Salisbury et al., 1978). For most agricultural species, sires should be maintained in moderate condition, although rams should start the breeding season in a high condition score, due to the considerable weight loss they experience during the season. Conversely, ruminants that are fed on very poor-quality roughage can develop such great rumen fill that normal copulation can be physically difficult to achieve. Moreover, as excess weight can lead to damage to females during mounting, it is important to determine that the sizes of sire and females are compatible.

Whereas any systemic illness can affect reproductive performance, three groups of conditions can be noted as of particular importance: namely, diseases of the locomotor system, conditions causing pain in the caudal abdomen and conditions that result in prolonged pyrexia. Specific conditions will be considered under the first two headings later in the chapter but, in principle, it is important to note that hindleg, hindfoot or back pain are incompatible with normal mating behaviour. Furthermore, not only does locomotor pain limit mating directly, but also the stress of prolonged, unresolved pain may cause corticosteroid-mediated impairment of spermatogenesis. Systemic illness causing prolonged pyrexia can result in increased temperatures within the testis, thereby causing temperature-limited impairment of spermatogenesis.

REPRODUCTIVE EXAMINATION

A complete examination of the reproductive system requires physical examination of the genital system, observation of the response of the animal to an oestrous female, observation of mating and the collection of semen. In practice, which of these procedures are actually carried out and the order in which they are undertaken depend upon the species and the nature of the owner's complaint. For example, it is frequently desirable to observe mating or to collect semen before the animal has undergone the stress of a physical examination. Thus, for a bull, collection of semen by an artificial vagina (AV) is often better undertaken before palpation of the genitalia (especially before examination of the internal genitalia per rectum), whereas collection by electroejaculation is probably best left until the rest of the examination has been completed. Also, observing mating (or collecting semen by an AV or with a judiciously placed electroejaculator) is the easiest way of observing the penis of a bull. Conversely, in the ram, it is generally best to examine the external genitalia first, as this frequently obviates the need for causing the animal stress by collecting of semen by electroejaculation.

Choosing the conditions for the observation of mating behaviour is important. Where the inherent libido of the animal is high, such as in boars and dairy bulls, it will often be willing to mount females that are not in oestrus, or even to mount other males, castrates or dummy animals. Indeed, the willingness of a cow to be haltered and tied is often a more important criterion for her use than whether or not she is in oestrus; few bulls are willing even to attempt to mount a fractious cow that is fighting against unfamiliar restraint! Rams and beef bulls, although usually of high inherent libido, commonly refuse to mount an oestrous female in the presence of a human observer, and considerable tenacity and patience are often required before mounting occurs. Animals that are stressed by recent transport are also often unwilling to mount straight away. Taken together, these many caveats mean that, although the results of observations of mating are valuable and often provide diagnostic information in infertility examinations, one should be most cautious about condemning an animal that does not perform under observation.

During examination of the genital tract, all parts of the genitalia that are accessible externally should be palpated. When examining the contents of the scrotum, the temperature, size, texture, resilience and evenness of the testes and epididymes should be determined. The testes should be freely movable within the scrotum. It is generally possible to palpate the head and tail of the epididymis, but the body is often difficult to feel, due to its medial site. The vasa deferentia should be palpated throughout the scrotal neck and (particularly in rams) the presence or absence of vasectomy scars confirmed. The spermatic cord should be palpated up to the level of the inguinal ring for the presence of abdominal contents (scrotal hernia) or abnormalities of spermatic vasculature. Measurement of scrotal circumference is useful in animals with a pendulous scrotum (Figure 30.2), while, in the stallion, measurement of the width of the testes by calipers or ultrasonography is similarly valuable. Likewise, ultrasonography of the testes of stallions and dogs to visualise fluid-filled structures within their substance is proving to be a valuable additional examination (Figure 30.3). Scrotal circumference of yearling bulls should exceed 30 cm, while mature bulls should be over 36 cm for British beef breeds and over 38 cm for most other breeds. Scrotal circumference of mature rams depends upon body weight; values over 28 cm are acceptable for smaller breeds, and over 34 cm for larger breeds. Scrotal circumference of rams is highly dependent upon season, with a 25–35% change in size occurring between the non-breeding and breeding

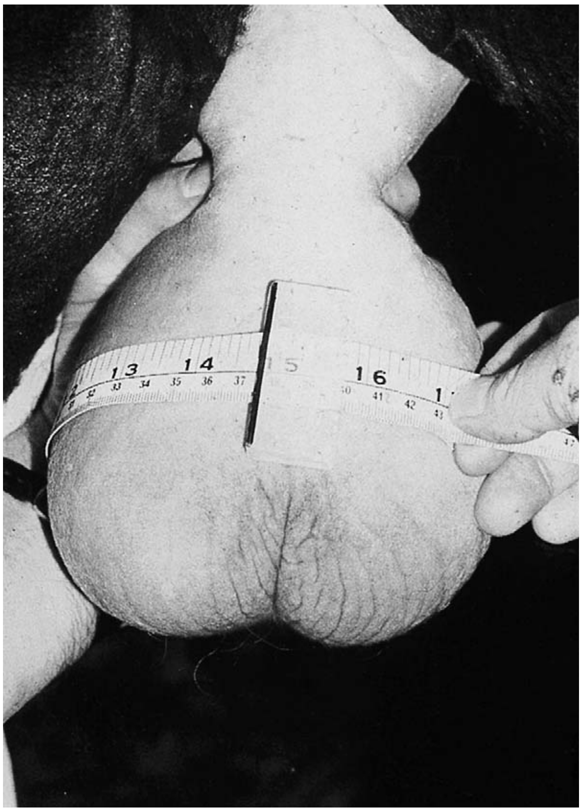


Fig. 30.2 Measurement of the scrotal circumference of the bull. The tape should be placed around the fullest part of the scrotum while the testes are held in their base by grasping the scrotal neck.

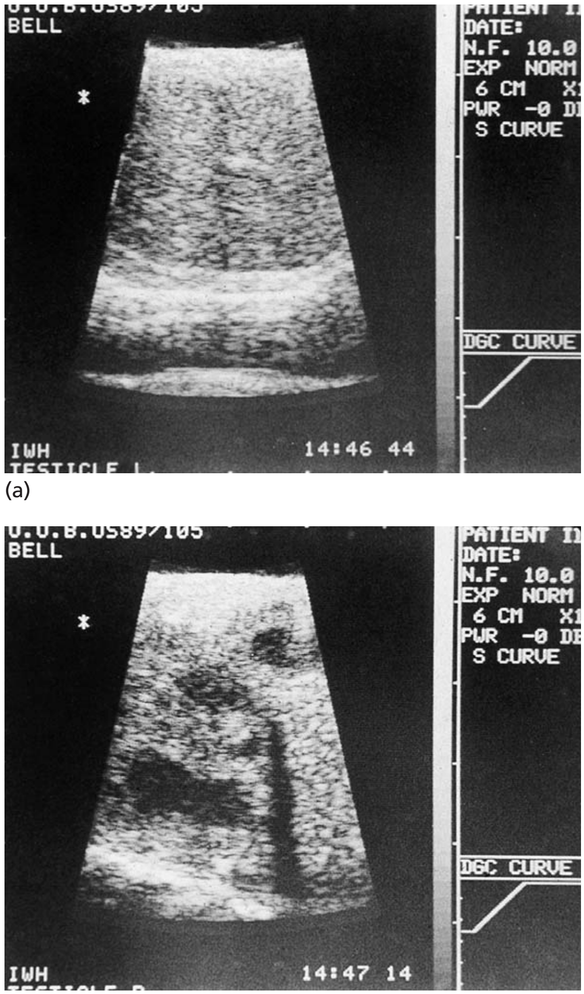


Fig. 30.3 Ultrasonograms of (a) normal canine testes and (b) a dog with epididymitis (reproduced from Barr, 1990, with permission).

seasons. The texture of the testes and the turgor of the cauda epididymes undergo parallel changes. It should also be noted that, although rams continue to produce sperm during the non-breeding season, they often fail to respond to electroejaculation during that period. Hence, much care must be exercised in interpretation of clinical findings in rams during the non-breeding season.

After palpation of the preputial part of the penis, exteriorisation of its free part where possible, palpation of the sigmoid flexures and palpation of the prepuce and preputial orifice, such of the internal genitalia as are within reach should be palpated per rectum. In the bull and stallion, all accessory glands can be palpated thus, but they are generally out of reach to a digital examination of the ram. In large boars, rectal examination is potentially feasible, but digital examination of smaller boars will only reveal the bulbourethral glands. In smaller dogs, digital examination of the prostate is possible, but radiography, which is essential when prostatic disease is suspected, is required in any case for examination of the prostate in larger dogs.

Libido testing

Assessment of libido and serving ability is widely used in the examination of bulls for breeding soundness (Chenowith, 1986, 1997). Observations of bulls' mating behaviour (Blockey, 1976a, b) suggested that serving capacity tests might be a predictor of bulls' fertility (Blockey, 1978). Moreover, libido is considered to be highly heritable in cattle, so early selection of animals for high libido is likely to result in an overall increase in this aspect of reproductive performance (Boyd and Corah, 1988). A number of tests have been devised that attempt to assess libido and serving capacity (Chenowith, 1986), utilising either females in oestrogen-induced oestrus or non-oestrous females, and scoring the number and vigour of matings or mating attempts. A number of reports have shown that high test scores are associated with good reproductive performance (e.g. Makarechian and Farid, 1985; Blockey, 1989). However, other reports have been more cautious, giving only qualified support for libido testing (Chenowith et al., 1984) or suggesting that moderate numbers of mounts were superior to high or low numbers (Coulter and Kozub, 1989). Yet other workers have failed to demonstrate a relationship between test scores and fertility (Crichton et al., 1987; Farin et al., 1989). Finally, the repeatability of individual libido scores is not high, although rankings of libido score are much more consistent (Chenowith, 1997). In consequence, the American Society of Theriogenologists advocated clinical examination, rather than libido testing, in their recent guidelines for breeding soundness examination of bulls (Hopkins and Spitzer, 1997).

Many of the factors that have been mentioned previously, such as age, sexual experience and social dominance, can affect libido and so lead to unreliability of tests. Nevertheless, when Chenowith (1986) discussed the reasons for the variability of the relationship between libido and fertility, he noted that bulls with high libido are the most efficient at detecting oestrous cows, and in multiple mating systems, bulls of high social ranking mate more cows than low-rank animals. However, even with bulls with a high intrinsic semen quality, an excessively high number of services is likely to deplete sperm numbers, resulting in a reduced chance of conception occurring to an individual service. A bull of high libido but with more mediocre semen quality would undergo such a decline in conception rates more readily. Moreover, since clinical findings, especially scrotal circumference and the presence of abnormal sperm (Coulter and Kozub, 1989), are poorly correlated with measures of libido (Chenowith et al., 1988), it is quite feasible that a bull of high libido might have inadequate semen quality to achieve an acceptable number of pregnancies. Hence, Chenowith (1986, 1997) concluded that neither clinical examination nor libido testing can be used in isolation for the evaluation of breeding soundness but, if the findings from both examinations are taken into consideration, subfertile bulls are most unlikely to avoid detection. Conversely, where range and beef bulls are examined, a combination of clinical examination and libido testing gives good results. Morris (1998), for example, suggests that a low serving capacity test score or a low scrotal circumference should preclude a bull from use, but that, if minimum criteria for both are met, the number of cows with which a bull can be run will be dictated by a combination of both scrotal circumference and service capacity test score.

COLLECTION AND ASSESSMENT OF SEMEN

Collection from the bull

Representative samples of semen can be obtained from most bulls by means of the AV (Figure 30.4), which consists of a strong outer rubber cylinder containing a latex liner. At one end of the AV, a latex extension cone carrying a graduated collecting tube is attached. The length of the AV should be adjusted so that the bull ejaculates into the extension cone, thereby producing semen that is largely devoid of particulate or bacterial contamination. The space between the rubber cone and latex liner is filled with warm water, so that the temperature in the lumen of the AV is between 45 and 48°C. If this temperature falls below about 43°C, ejaculation is unlikely. The main stimulus to ejaculation is the temperature of the AV, and its pressure upon the bull's penis is relatively less important. Clearly, however, the larger the bull's penis, the less water is required. A little inert lubricant (liquid paraffin, soft paraffin or gynaecological jelly) is placed in the lumen of the AV just prior to use.

Control of the bull and the safety of all personnel are of paramount importance during semen collection. A halter-trained, oestrous cow is the ideal object for a bull to mount, although such an animal is often difficult to provide on most farms. Where a service crate is available, the cow should be restrained in this; otherwise she should be tied to a post. The bull is led to the cow, but not allowed to mount at once. Rather, he should be allowed to see and smell the cow, but then be led away before mounting takes place. This usually causes complete or partial erection, with production of pre-ejaculatory accessory gland secretions, and is considered to cause a better ejaculation of semen when the bull is finally allowed to mount. The bull is led up to the cow with the collector standing to the right of the shoulder of the bull. Before mounting, the bull usually nuzzles the perineum of the cow, then a pumping action of the tail-head can be seen as the ischiocavernosus muscles start to pump blood into the erecting penis. As the bull mounts, full erection is achieved and the bull usually makes a single ejaculatory thrust after achieving intromission. Small, preliminary thrusts occur as the bull locates the vulva and, during these, the collector grasps the prepuce (not penis) with the left hand and deflects the penis to the right of the hindquarters of the cow, allowing it to find the entrance to the AV. The bull will then normally make the ejaculatory thrust into the AV (Figure 30.5). The entire procedure must be carried out quietly and methodically, keeping the bull under continual careful observation before, during and after collection; many bulls are at their most aggressive immediately after ejaculation.

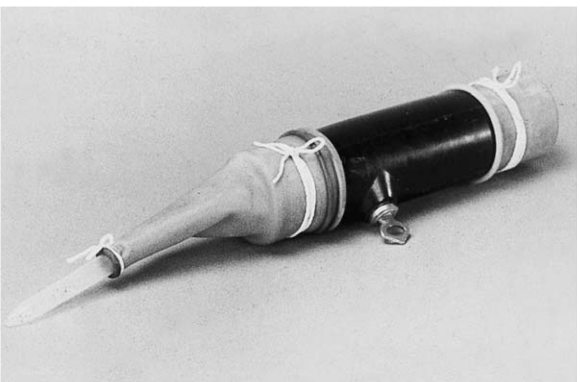


Fig. 30.4 Artificial vagina for use in the bull.

Where older bulls have been used for natural service for a number of years, they may be unwilling to ejaculate into an AV. Providing the libido of the bull is sufficient, repeated teasing or allowing the bull to mount, but deflecting his penis so he does not ejaculate, will usually provide enough stimulation for ejaculation to occur when presented with the AV. More difficulty is experienced with bulls that are unwilling to mount in the presence of humans, or which are no longer halter-trained. Some such bulls can sometimes be stimulated by allowing the cow to mount the bull, but, for many, plentiful supplies of both patience and freshly heated AVs are the only route to success.

The main alternative to collection by an AV is collection of semen by electroejaculation. Early

Fig. 30.5 Semen collection from the bull by means of an AV.

bull electroejaculators were undoubtedly unpleasant devices, but modern, variable-voltage models are much more acceptable. Electrodes, which are either in the form of a rectal probe or are worn on two fingers over a gloved hand, are placed over the ampullae via the rectum. Gently increasing voltages first elicit production of accessory gland secretions, after which semen may be dribbled through the prepuce or ejaculated from the erect penis. Electroejaculation is not as widely used in the UK as it is in North America, where it is frequently used on bulls of low libido or which are otherwise unwilling to mount. Electroejaculation is often the only feasible method of semen collection from beef or range bulls that are unused to being handled. If carefully and properly carried out, electroejaculation appears to be well tolerated by most bulls, although if performed by unskilled operators or with poor equipment, considerable distress can be caused.

Semen can also be collected by rectal massage of the internal genitalia. This technique involves the location of the vesicular glands per rectum and stroking them against the pubis, which causes accessory fluid to drip from the prepuce. The ampullae are then located and 'milked' between the finger and thumb. Success is indicated by the dripping of semen from the prepuce. It should finally be noted that, in some animals, semen can only be obtained by aspiration of the ejaculate from the vagina of a freshly served cow. Both of these last two methods have many disadvantages, and really only allow assessment of motility and morphology of sperm and do not provide accurate information over sperm numbers. Even so, the information yielded by such imprecise methods can still be diagnostic.

The stallion

Semen can be obtained from stallions by the use of an AV, a large condom or by examining the residual drips of semen left in the urethra after dismounting. Collection from the vagina of a mare is not normally possible, due to the intra-uterine site of ejaculation. The AV for a stallion is larger than that used for a bull and, for large stallions, may be exceedingly heavy. The stallion is presented with a mare in full oestrus, which is often restrained with a twitch or service hobbles. After mounting, the penis is deflected into the AV and its lower surface palpated for the presence of peristaltic ejaculatory waves (Figure 30.6). Stallions can be quite fastidious about the temperature and pressure in the AV before they will ejaculate, and some stallions object so vehemently to even the sight of an AV that collection is impossible. Care must be exercised by the collector to

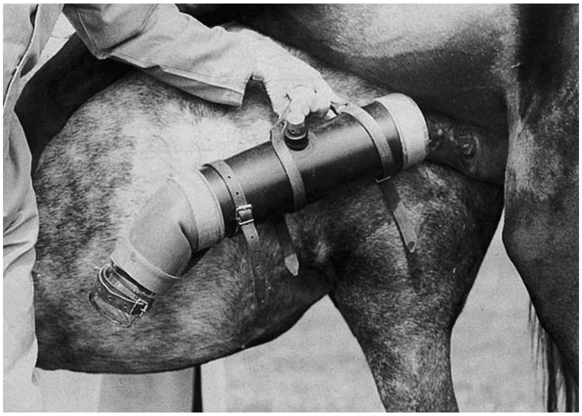


Fig. 30.6 Semen collection from the stallion. The urethra is palpated for the peristaltic waves that characterise ejaculation.

avoid getting trapped between the forelegs of the stallion and the body of the mare, as the force of the clasping movements of the legs of the stallion are quite sufficient to break an arm.

The ram

Semen can be collected from rams by electro-ejaculation, or by the use of an AV, which is essentially a smaller version of that used for the bull. Some workers consider that the spermatozoa of the ram are more susceptible to temperature shock than are those of the bull, so a warmed ejaculation cone should be provided. Most rams will not use an AV until trained to do so, and virtually all require a ewe in oestrus to mount. In consequence, most semen examinations of farm rams are undertaken by electroejaculation. A probe, containing two electrodes (Figure 30.7), is placed into the rectum of the ram and located on to the brim of the pelvis (Figure 30.8). Rhythmic stimulations of the ampullae and sacral nerve plexus should cause erection and ejaculation within a few moments. Electroejaculation is generally well tolerated but, as the electrical stimulation also causes relatively widespread muscle contraction, attempts to collect by this method should be discontinued for several minutes if the ram has not ejaculated within the first 4–6 stimulations. Where rams are taken directly from pasture, housing them on straw for 1–2 days before attempting collection produces drier faeces in the rectum and appears to cause less widespread dissemination of current. Semen collected by electroejaculation is usually of lower volume and density than that collected by an AV, and occasionally is completely immotile or completely aspermic. For these reasons, some Breed Societies' rules of sale require that infertility can only be diagnosed on semen collected by an AV. In all circumstances, further collections should be made after an immotile or aspermic sample, to ensure that the initial findings should not rightly be attributed to operator error.

Although sheep are seasonal breeders, semen can be collected from most domestic rams throughout the year. Semen quality can be maintained to a considerable extent by regular ejaculation, a phenomenon that is used to maintain

Fig. 30.7 Electroejaculator for the ram.

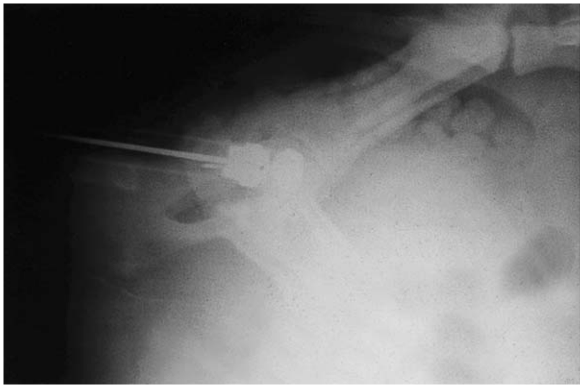


Fig. 30.8 Radiograph of the hindquarters of a ram indicating the position of the electroejaculator relative to the brim of the pelvis.

production of (frozen) semen in ovine artificial insemination (AI) centres. Farm rams exhibit a more profound decline in semen quality in the non-breeding season but, even so, epididymal reserves are sufficient for evaluation.

The boar

The main prerequisite for ejaculation in the boar seems to be the locking of the corkscrew-shaped penis in the spirally disposed cervix of the sow. This can quite easily be simulated by firmly encircling the protruded penis with the hand and fingers which are covered by a warm, lubricated, latex glove. The boar is much less sensitive to temperature than is the bull, but firm pressure is essential. The use of an AV has been advocated in the past, as an alternative to this simple method of manual collection. The AV was based upon the bovine pattern but included an air pump to vary the internal pressure. One such AV, that designed by Melrose and O'Hagen (1959), also had a tapering latex extension, intended to simulate the cervical canal of the sow. In general, the use of such AVs was less satisfactory than the manual collection method, the latter being the more widely used.

At AI centres, a dummy, which does not necessarily resemble the sow, is used for the boar to mount. As the boar mounts and makes thrusting movements, the penis is directed into the AV or gloved hand. Thrusting becomes less vigorous as ejaculation begins. The ejaculate is allowed to pass through a funnel containing cotton gauze, which retains the gel fraction but allows the fluid to pass down to an insulated bottle that is kept at 30°C. After ejaculation of the sperm-rich fraction, the boar begins to thrust again as the post-sperm gel fraction is produced.

The dog

Semen can be taken fairly readily from most dogs by digital manipulation or, less commonly, by the use of an AV. In either case, the presence of a teaser bitch, preferably in oestrus, facilitates procedures. It is generally considered that semen collected by digital manipulation is of better quality and quantity, probably due to deleterious effects of the latex of the AV upon canine sperm. Erection can be induced by applying encircling pressure with the thumb and forefinger behind the bulbus glandis. When erection is obtained, the penis can be deflected into an AV, or digital collection continued. Where an AV is used, rhythmic changes in pressure are applied until the dog attempts to tie. The AV is then repositioned to allow the penis to be directed backwards, as in the tie, while ejaculation continues. In small dogs, the AV is cumbersome to use, and it does not allow for collection of the separate fractions of the ejaculate.

In order to induce ejaculation by digital manipulation, the bulbus glandis may be rhythmically compressed (Figure 30.9), although many dogs will induce ejaculation by their own thrusting. Before production of the pre-sperm accessory fluids occurs, quite vigorous thrusting takes place, but the dog is relatively quiet during the initial phase of ejaculation. Firstly, during this quiescent phase, between 0.5 and 5.0 ml of watery, pre-ejaculatory fluid is produced, over a period of up to 50 seconds. Ejaculation of the sperm-rich fraction then follows immediately, when 0.5–2.0 ml of thick, creamy fluid is produced within a few seconds. The dog then attempts to turn and tie, whereupon the post-sperm, prostatic fluid fraction of the ejaculate is produced. This third component is again watery, comprising up to 30 ml of fluid, which is ejaculated over 3–30 minutes.

Semen examination

The purpose of semen examination is to ascertain whether the numbers of functionally normal

Fig. 30.9 Digital collection of semen from the dog.

spermatozoa present in an ejaculate are sufficient to cause pregnancy and whether the sire has an adequate capacity to produce enough spermatozoa to achieve pregnancies amongst all the females he is required to service. Details of the methodologies and interpretation of semen examination are given later in this chapter.

REPRODUCTIVE ABNORMALITIES OF MALE ANIMALS

Reproductive abnormalities causing absolute or relative infertility in male animals have classically been divided into two main classes, namely conditions causing failure of normal service (*impotentia coeundi*) and conditions causing failure of conception after normal service (*impotentia generandi*). The first group can be further divided into, firstly, conditions causing an unwillingness to mount and, secondly, conditions that prevent normal copulation from occurring, despite normal libido. Superimposed upon both groups are considerations of whether the infertility represents a pathological condition of the genital (or other) system, or whether infertility is primarily managemental in origin and could simply be alleviated by modifying aspects of the husbandry of the animal. Much of the differentiation between these major groups of conditions can be achieved by careful history-taking. A scheme of diagnosis for some of the major causes of infertility in the bull is given in Figure 30.10.

Conditions causing a lack of libido

Inability and unwillingness to copulate are relatively frequent presenting signs that accompany many disorders of the male reproductive system. The syndrome of lack of libido is, however, one of the most difficult for the clinician to unravel, for not only is it caused by genital pathology, but also it can result from intercurrent disease, management, age, maturity or season. Furthermore, many diseases that would normally be expected to show other presenting signs can, if neglected, frequently result in a sexually disinterested animal. Finally, the difficulties of achieving a diagnosis in cases of low libido are further compounded by the unwillingness of some, quite normal, sires to copulate in human company.

Maturity, age and experience

Many animals that are presented for lack of libido are either young or of advanced age. The age of the sire must be considered in relation to the normal time at which its species (and breed) exhibits puberty. The conditions under which a young male has been reared can also affect its behaviour. For example, where bull calves are reared in small groups, they continuously exhibit mounting behaviour as puberty approaches and usually learn to copulate quite quickly. However, where reared in isolation, such mounting behaviour does not occur and can seem to take an age to learn, especially in those AI stations where steers are the sole objects available for the young bull to mount. Similarly, young colts in racing yards may be violently dissuaded from exhibiting male behaviour by their grooms. When such animals then go to become stud stallions, much behavioural reinforcement has to be unlearnt before successful mating can occur.

Where immaturity is suspected as the cause of low libido, little can be achieved other than by the exercise of much patience and the provision of a plentiful supply of appropriately sized, oestrous females. Hormone therapy has been suggested, giving large doses of human chorionic gonadotrophin (hCG) (5000–10 000 IU) or gonadotrophin-releasing hormone (GnRH), in an attempt to stimulate libido through the production of elevated testosterone concentrations. Considerable caution should be exercised in the use of such hormones, however, for the high levels of testosterone they produce exert as great an effect upon aggression as on libido. Moreover, hCG, although having luteinising hormone (LH)-like properties, is not the same substance as LH, and may damage spermatogenesis through the testicular oedema that it produces. In general, the efficacy of such treatments is low. Most animals fail to respond at all, a few exhibit a short period of enhanced libido, while only in very few can success be attributed to the treatment. More seriously, the correlation between the age of onset of reproductive activity of sires and their off-

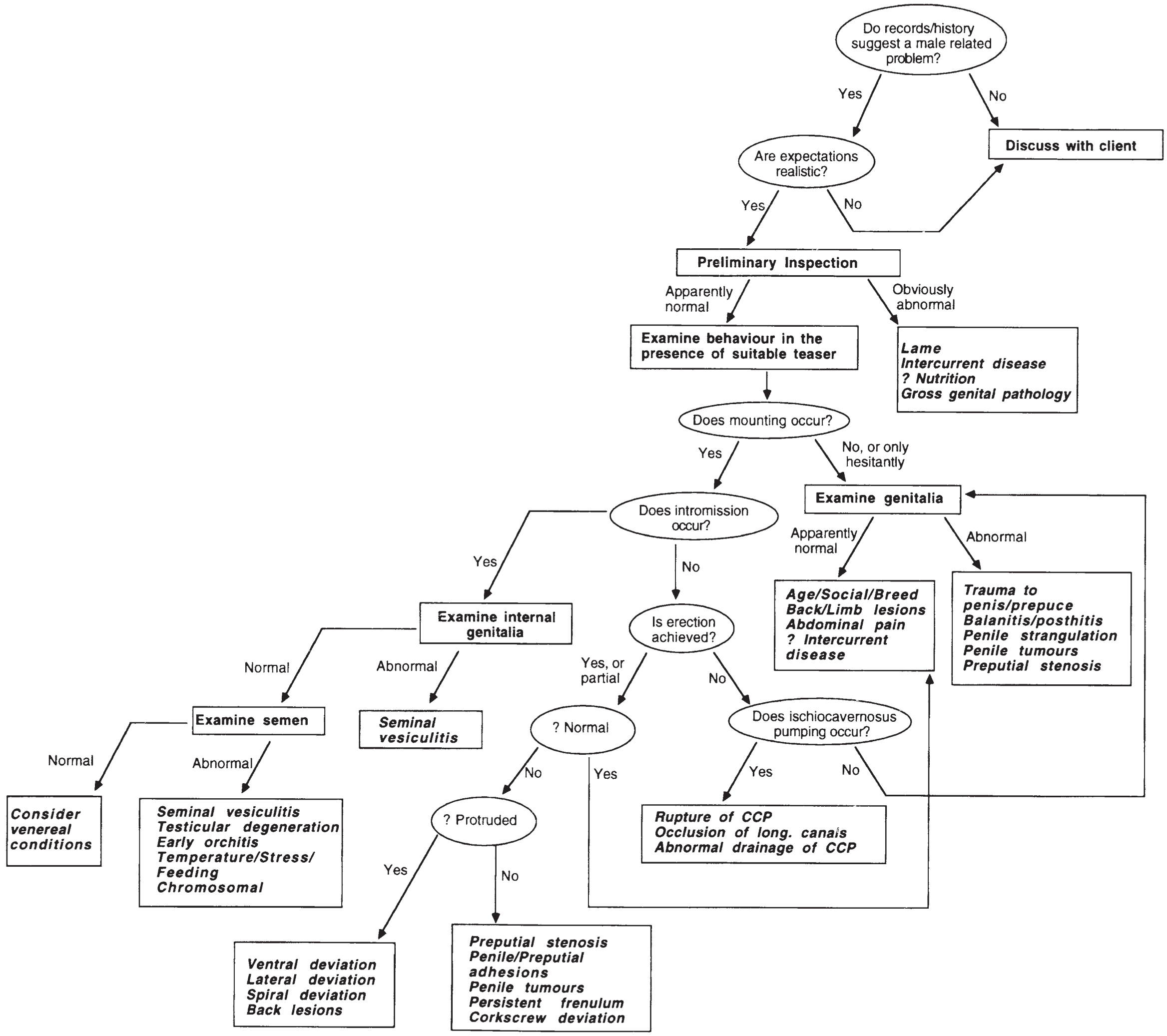


Fig. 30.10 Scheme for the diagnosis of the major conditions causing infertility in the bull (reproduced from Parkinson, 1991, with permission); CCP, corpus cavernosum penis.

spring means that it is positively undesirable to attempt to breed from animals that exhibit a gross delay in the onset of sexual activity.

Unwillingness to copulate can also result from poor service management. Slippery floors, roofs that are too low, females that are too big and stockpersons that are insensitive in their handling of their charges can all contribute to unwillingness to copulate. Similar problems pertain amongst companion animals. Tom cats frequently become conditioned to mating in one particular environment, and, if that environment should later be changed, an unwillingness to mate ensues. Dogs, which are frequently transported before mating, can also have stress-induced impairment of libido. Finally, young males of many species, notably the pig, are frequently unwilling to mate if in the sight or sound of an older, more dominant male.

Locomotor dysfunction

Most lesions affecting locomotion impair ability and willingness to copulate. Lesions of the back and hindlegs are clearly the most important of such incapacities, but, for example, in the boar, where the forelegs are used to clasp the female, painful lesions of the carpus can also preclude mating. In dogs and in aged animals of all species, lesions of the joints of the hindlimbs are important locomotor causes of impaired libido.

Amongst the large herbivores, foot lesions are probably of greatest significance. Gross pathology of the foot, such as penetrations of the sole, separations of the white line, foot rot, foul in the foot, etc., produces pain, so that the sire is unwilling to take his weight on the foot during copulation. Less obvious lesions of the foot, such as the interdigital growths of Hereford bulls, can also be important. However, it is the author's opinion that the most common locomotor-related cause of impaired libido is poor conformation of the foot. Animals with overgrown hooves, where the distribution of weight has been adversely affected, are frequently unwilling to mount or, if they do mount, are unwilling to remain mounted for long enough for successful copulation to occur. For this reason, valuable sires in AI studs receive considerable attention to the conformation of their feet. By contrast, many farm sires, especially bulls, receive little such attention until overt lameness has developed.

Similarly, any lesion of the trunk affects ability to mate. In young bulls that are overzealous in their early attempts to mate, the lumbodorsal fascia may rupture, producing the so-called condition of 'honeymoon back'. In this condition, the pain caused by the rupture of the fascia is such that the forelimb cannot be raised in preparation for mounting. This condition can be diagnosed by palpation of crepitus in the lumbodorsal region or by the presence of swollen muscle masses protruding through the fascia. It is most common in bulls that are 15–21 months old. As bulls age, progressive deposition of new bone occurs around the intervertebral joints (Figure 30.11), causing several related syndromes of incapacity. These conditions, which are most common in housed bulls receiving diets that are relatively high in calcium (Krook et al., 1969), rarely present under 7 years of age (Bane and Hansen, 1962). Firstly, progressive growth of exostoses can merely make the bull appear 'stiff', so that mounting requires increasing effort. Animals with back pain may mount, but frequently dismount again quite quickly. Affected bulls are unwilling to make the ejaculatory thrust and, if AV collection of semen is attempted, may spend a long time in the AV without thrusting (Almquist and Thomson, 1977). More seriously, spondyles of bone can fracture, usually during mounting, causing immediate, acute back pain accompanied by a complete, but usually temporary, unwillingness to mount. However, where complete bridges of bone form between several adjacent vertebrae, flexing forces

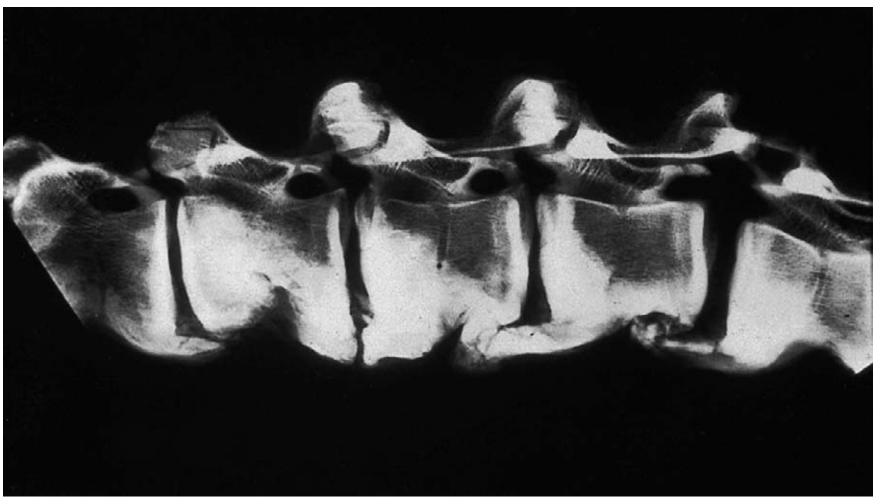


Fig. 30.11 Radiograph of the lumbar spine of an aged bull. Several of the vertebrae have substantial amounts of new bone deposition.

upon the spine can cause fractures within the spinal column, typically straight across a vertebral body. Such fractures typically occur at the moment of ejaculation, whereupon the bull becomes immediately paraplegic by spinal cord severance. The bull therefore collapses off the teaser into a dog-sitting position and exhibits complete loss of sensation of its hindlimbs.

Abnormalities of gait are a further cause of inability to mate. Details of such are beyond the scope of this chapter (for a review see Greenhough et al., 1972), other than to mention four conditions of the bull: spastic paresis, crampy syndrome, straddle gaits and the congenital ataxia of the Charolais breed. Attention is drawn to these conditions because of the importance of their recognition during examinations of bulls on behalf of prospective purchasers. However, similar conditions occur in many other domestic species.

Failure to copulate

Inability to copulate is a relatively frequent cause of infertility in domestic animals. Conditions that cause failure of copulation include failure of the penis to become turgid (i.e. failure of erection), abnormalities of erection that prevent intromission, and lesions of the penis and prepuce that prevent protrusion of the penis. Most of the conditions can be differentiated relatively easily, and a prognosis can usually be given at an early stage of investigation.

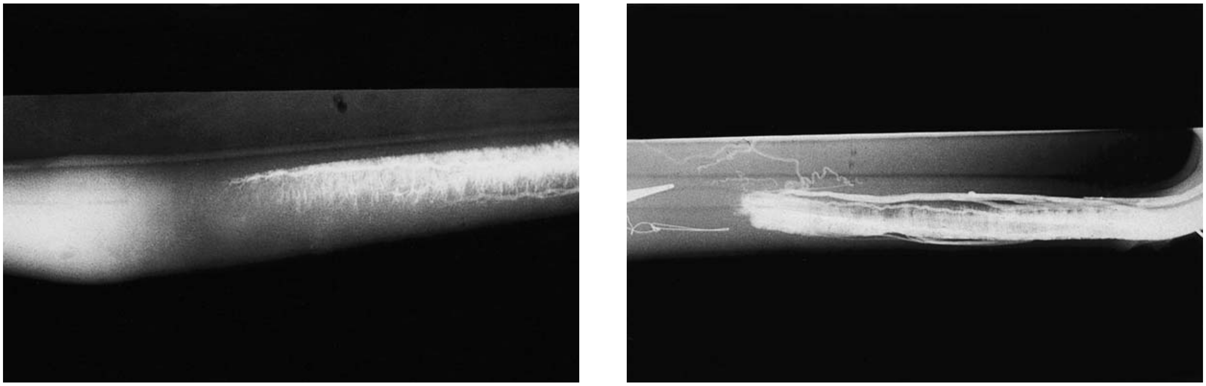
Failure of erection

Erection is achieved by the action of the ischiocavernosus muscles pumping blood into the corpus cavernosum penis (CCP). Because the CCP is, essentially, a blind-ending chamber, whose venous drainage is close to its arterial supply in the crura of the penis, the effect of the activity of the ischiocavernosus muscles is to occlude the veins and force blood into the arteries, thereby raising the hydrostatic pressure within the CCP. The hydrostatic pressures thus generated produce the lengthening and stiffening of the penis that characterises erection (Watson, 1964; Beckett et al., 1975). Thus, if any aspect of the vascular system of the CCP is perturbed, failure of erection ensues. Two main classes of abnormalities occur: those that allow blood to leak from the CCP so that it is not blind-ending, and those that prevent normal access of blood to the CCP.

Abnormal venous drainage of the CCP. This condition (Young et al., 1977; Ashdown et al., 1979a) is most commonly seen in young bulls, which are presented with normal libido, eager to mount but never achieving erection or intromission. Observation of the mating behaviour of such bulls reveals that considerable activity is present in the ischiocavernosus muscles before and during mounting, to such an extent that the tail-head appears to be ‘pumping’ up and down. However, the penis remains flaccid throughout its length. The cause of this condition is almost invariably failure of occlusion of the veins that drain the CCP during fetal life. Thus, because of the presence of veins draining the CCP, it is not a closed vessel, the high blood pressures required to produce erection cannot be achieved and the penis therefore remains flaccid.

Diagnosis of the condition can generally be made on clinical signs and history alone. However, if further confirmation of the cause of the condition is required, the presence of ectopic veins can be diagnosed by injecting radiographic contrast media into the CCP of the anaesthetised bull, then observing drainage of contrast into the dorsal veins of the penis (Figure 30.12). Because there are nearly always many small veins along both lateral edges of the CCP, surgical correction is rarely possible.

Occlusion of the longitudinal canals of the penis. In ruminants, the changes in blood pressure during erection, which are initiated by ischiocavernosus activity, are transmitted throughout the length of the CCP by the longitudinal canals. Congenital absence or acquired blockage of these canals therefore prevents erection (Ashdown et al., 1979b). Both young bulls and animals that have been successfully used as sires over a number of years can be afflicted with this condition. Young bulls present in a very similar way to animals with abnormal venous drainage of the CCP, and, in many ways, differentiation of the two conditions is rather academic, the prognosis for both being hopeless. In such young bulls, the condition is typically caused by a congenital failure of cannulation of the short segment of the single dorsal



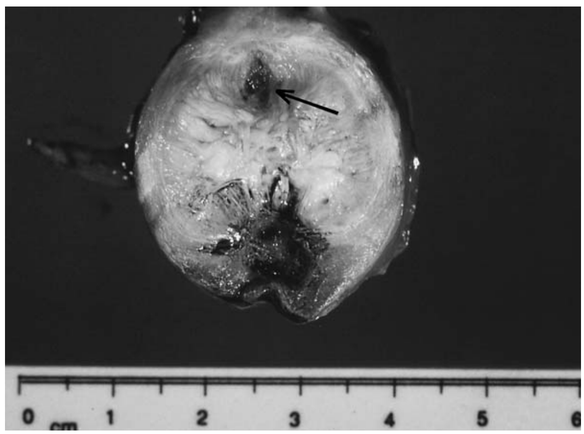
(a) (b)

Fig. 30.12 Radiographs of the penis of (a) a normal and (b) an impotent bull after the injection of contrast medium into the corpus cavernosum penis (CCP) near the root of the penis. In the normal animal, the contrast medium is contained within the CCP, but in the impotent animal contrast medium is leaking into the dorsal vein of the penis, indicating the presence of ectopic veins draining the CCP. The CCP was therefore not blind-ending and could not become turgid.

longitudinal canal (between the point of fusion of the two crural canals and the origin of the first of the lateral branches that form the ventral canal). In young bulls, the condition is diagnosed by observation of mating behaviour, when the penis remains flaccid despite considerable ischiocavernosus activity. It can be differentiated from abnormal drainage of the CCP by palpation of the base of the penis. Although the great majority of the penis is flaccid, a short length of turgid tissue is present, in the part of the penis proximal to the occlusion (Figure 30.13(a)). This can be appreciated by palpating the root of the penis just beneath the tail-head, where the penis may become so turgid that the animal may resent it being touched.

Older bulls can be afflicted with a very similar condition. Such animals generally have a history of a long period of normal service behaviour, which has latterly changed into failure of erection. Libido remains normal. The other clinical signs are similar to those exhibited by a young bull. In such older animals, the longitudinal canals are blocked by fibrinous or, more usually, atheromatous material (Figure 30.13(b)). A similar condition also occurs in rams, in which the CCP can rupture proximal to the site of obstruction, causing a peripenile haematoma in the region of the escutcheon. Similar ruptures occur infrequently in affected bulls.

(a)



(b)

Fig. 30.13 Blockage of the longitudinal canals of the penis of a bull. (a) Contrast radiography demonstrates the point at which the lumen of the dorsal longitudinal canal is blocked (arrow). (b) Transverse section of the penis, showing occlusion of the dorsal longitudinal canal (arrow) with atheroma-like material.

Rupture of the corpus cavernosum penis. This is a relatively common and potentially serious condition of bulls. It also occurs sporadically in boars and rams. The condition has many names, including rupture of the CCP, ruptured penis, fractured penis and broken penis. Rupture of the tunica albuginea occurs spontaneously if pressures within the CCP rise substantially above the pressures achieved during normal copulation (Noordsy et al., 1970; Beckett et al., 1974). Such abnormal increases in pressure can occur if the penis is suddenly subjected to shearing forces – for example, by the cow moving suddenly at the moment of ejaculation, or by the ejaculatory thrust being directed against the escutcheon of the cow rather than the vagina. Rupture (Figure 30.14) occurs most commonly either in the region of the insertion of the retractor penis muscle, or on the dorsal aspect of the distal sigmoid flexure where the trabeculae of the CCP are relatively weak. In rams, it is more common to see rupture of the CCP near to the root of the penis, above the proximal sigmoid flexure, presumably as a result of being butted from behind at the time of ejaculation.

The aetiology of the condition makes it more common in young than older bulls, probably due to the enthusiasm and inexperience of the former. Immediately after rupture, the animal may be noticeably subdued and, in most cases, will immediately refuse to make further attempts to copulate. However, some bulls continue to attempt to mate. Other clinical signs include shortness of gait and general indications of mild discomfort. Haemorrhage occurs from the site of rupture, with haematomata collecting in the surrounding tissues: cranial to the scrotum in ruptures of the distal sigmoid flexure (Figure 30.15(a)), behind the scrotum with proximal ruptures. These haematomata can become very large, especially if mating behaviour is not immediately inhibited. Distal ruptures are also characterised by preputial oedema, which is often sufficiently severe to cause eversion of the preputial mucosa (Figure 30.15(b)). Occasionally, the penis itself may be prolapsed (Figure 30.15(c)). In light-coloured bulls, the rupture of the CCP may be of sufficiently explosive nature to cause blood staining of the overlying skin. Urination is not affected.

The haematoma is initially soft and fluctuant, but later, as the clot becomes organised, it becomes firm and hard. It is not possible to determine the extent of the haematoma during the initial phase, when the prepuce is oedematous, so assessment must take place after the oedema has subsided. If untreated, a substantial proportion of haematomata become infected and produce

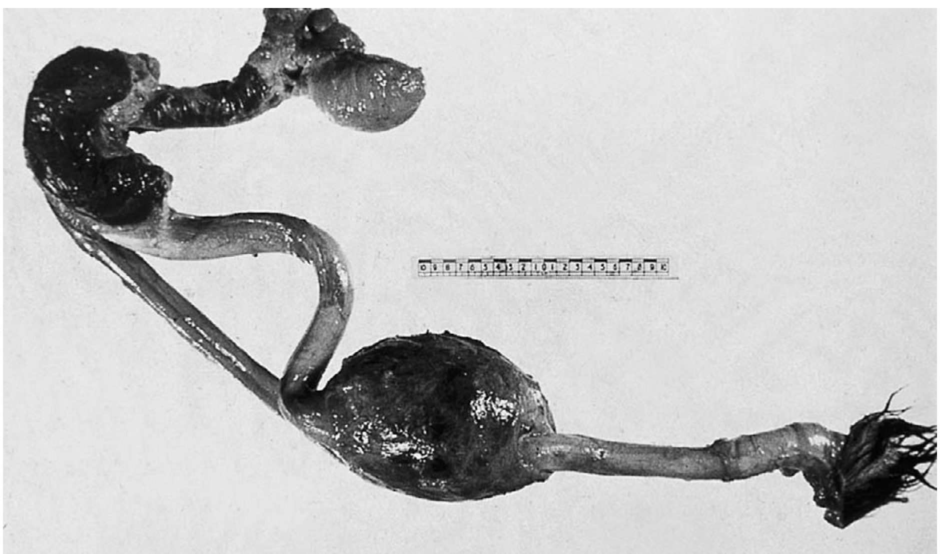
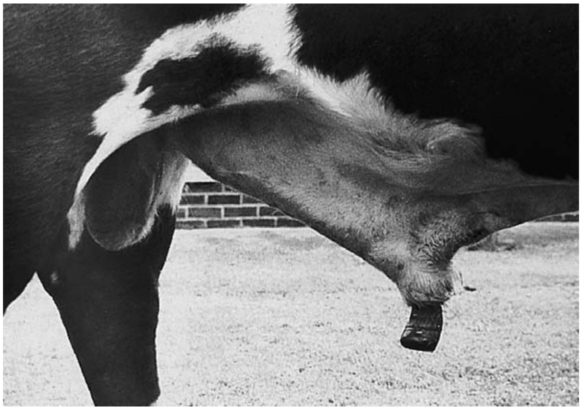


Fig. 30.14 Peripenile haematoma from the rupture of the CCP, close to the insertion of the retractor penis muscles in a 3-year-old Jersey bull.

abscesses (Figure 30.16(a)), while others develop fibrous adhesions between the penis and prepuce and within the fascial planes of the prepuce (Figure 30.16(b)). Diagnosis is relatively straightforward in recent cases, but neglected cases must be differentiated from preputial trauma and abscessation, tumours and urinary infiltration of the prepuce after urethral rupture. Peripenile haematomata may also be caused by trauma to the ventral abdomen or from peripenile vessels (Noordsy et al., 1970).

The time taken for recovery is such that treatment is inappropriate for all but the most valuable bulls, so that, in many cases, slaughter for salvage of the carcass price should be recommended.


(a)


(b)

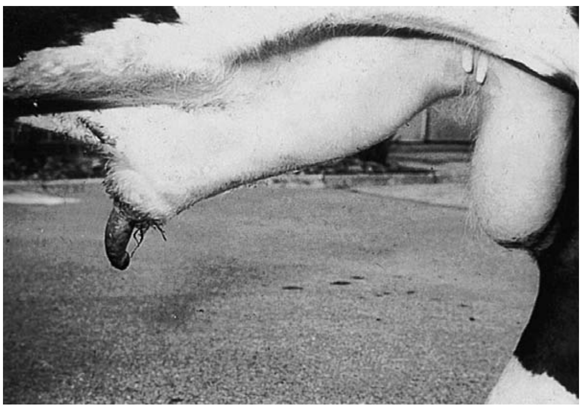
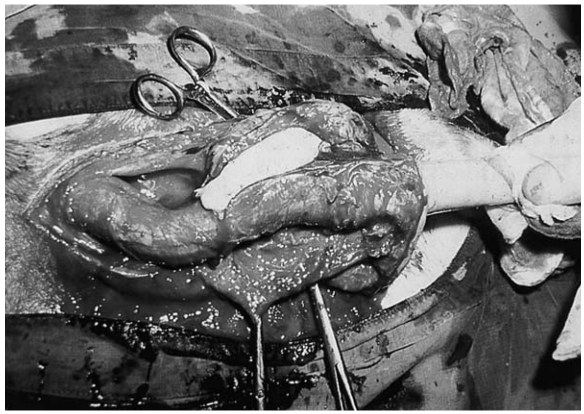

(c)

Fig. 30.15 (a) Prescrotal swelling due to haematoma formation after rupture of the CCP in a bull. (b) Prolapse of the preputial mucosa after rupture of the penis in a Hereford bull. (c) Prolapse of the penis secondary to rupture of the penis.


(a)

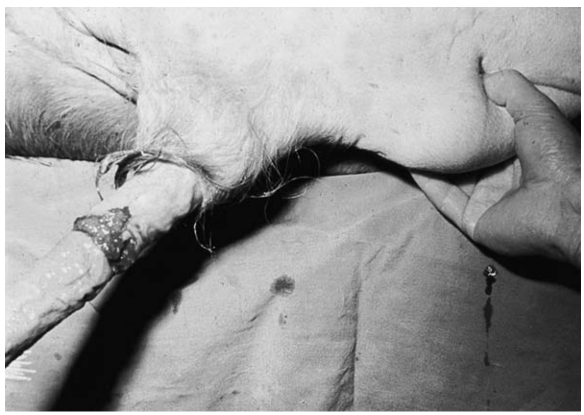

(b)

Fig. 30.16 (a) Secondary abscess formation in a periphenile haematoma in a bull. (b) Preputial fibrosis in a yearling bull with a small penile haematoma. The fibrotic lesion prevented protrusion and required resection.

Where treatment is an option, several alternatives need to be considered, whose relative merits and demerits are still the subject of debate (Pattridge, 1953; Vandeplassche et al., 1963; Metcalfe, 1965; Pearson, 1972; Walker and Vaughan, 1980; Cox, 1982). Conservative treatment, consisting of sexual rest for 90 days, with initial antibiotic therapy to prevent abscess formation in the haematoma, and daily massage of the affected area to limit formation of peripenile adhesions, has been reported in some surveys to allow as many as 50% of bulls to regain service ability. However, other surveys have indicated that a successful response to conservative therapy occurs in as few as 10% of animals. Selection of cases to which conservative treatment is applied may account for some of the variation between surveys, for bulls with relatively small, freely movable, circumscribed, haematomata that are barely larger than the diameter of the normal penis respond best to conservative treatment. Larger haematomata carry a greater risk of abscess formation and of resulting in peripenile adhesions. Surgical treatment of such cases is best confined to evacuation of the haematoma, with strict attention to asepsis (Figure 30.17). More radical surgery, in which the site of rupture of the tunica albuginea is isolated, resected and sutured closed, has been suggested, but appears to result in a high proportion of animals developing peripenile adhesions

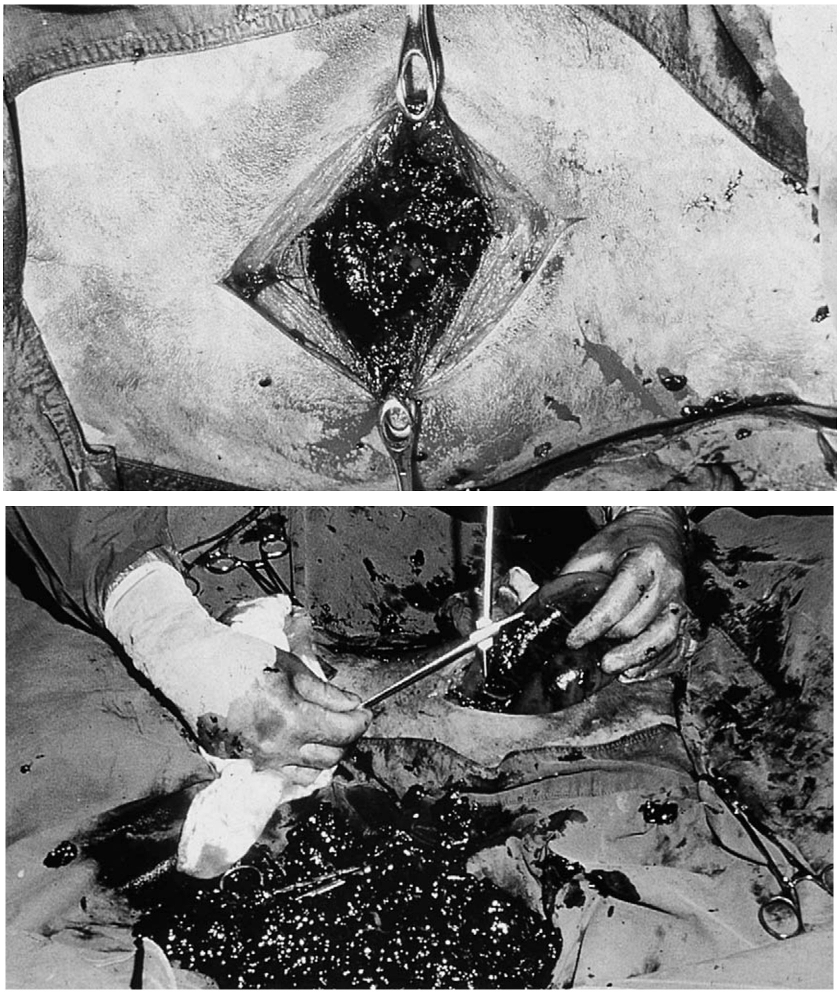


Fig. 30.17 Surgical evacuation of a peripenile haematoma in a bull.

and consequential inability to protrude the penis. In either case, surgery should not be delayed for more than 7 days after injury, as adhesions can become extensive thereafter. Sexual rest after surgery should only last for a few days, for a long period of inactivity also promotes peripenile adhesions. Affected bulls should therefore be teased regularly in order to induce penile movement and counteract the effect of contracting scar tissue. Mounting should, however, be prevented. Initially, protrusion of the penis is limited, but during ensuing weeks a progressively greater length of penis is protruded.

Sequelae of rupture of the CCP include abscessation of the blood clot, if this is not evacuated. Peripenile adhesions are likely to be more severe if the clot is not removed, but even quite small areas of fibrosis can prevent penile protrusion. A proportion of animals develop ectopic veins that drain the CCP through the site of rupture. Such veins prevent vascular engorgement of the CCP. In such cases, surgical location of the site of the venous drainage and closure of the vein are often feasible, for, unlike congenital venous drainage of the CCP, such acquired cases usually only possess a single abnormal vein.

Fig. 30.18 Persistent penile frenula in an 18-month-old Friesian bull.

Abnormalities of erection

Persistence of the penile frenulum. Persistence of the penile frenulum is most frequently encountered in young bulls (Ashdown, 1962; Carrol et al., 1964), in which it either limits the amount of penis that can be protruded or causes the protruded penis to be deviated ventrally (Figure 30.18). Transecting the frenulum after ligating the frenular blood vessels (if they are sufficiently prominent) gives a good prognosis for the recovery of breeding ability (Elmore, 1981; Cox, 1982). A familial predisposition has been suggested for the Angus and Beef Shorthorn breeds (Carrol et al., 1964), although the lesion occurs in all breeds of cattle. Persistence of the penile frenulum has also been occasionally reported in boars and dogs (e.g. Joshua, 1962).

Congenital abnormalities of the penis preventing protrusion. Considerable growth of the penis and changes in the relationships between the penis and the peripenile tissues occur during the prepubertal period. Failure of these developmental changes can result in failure of normal erection. For example, failure of the penis to undergo normal growth causes a congenital shortness of the organ, such that normal intromission cannot be achieved. Alternatively, if such failure of growth is confined to the sigmoid flexure, it may be impossible to exteriorise the penis (Roberts, 1986). Similarly, the retractor penis muscles can fail to develop, causing inability to protrude the penis. Although treatable by myectomy, this condition, like so many others affecting the bovine penis, is probably inherited (DeGroot and Numans, 1946), so correction should, perhaps, not be undertaken.

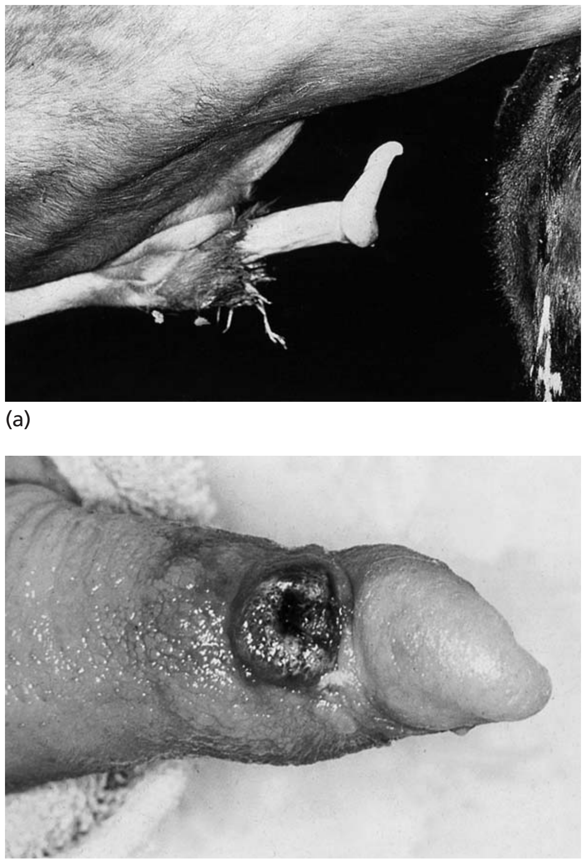
At birth, the integument of the penis and the penile part of the prepuce are fused but, during the prepubertal period, the bridging connective tissue normally breaks down (Ashdown, 1962). Where the connective tissue remains substantially intact, the penis cannot properly be protruded, such that only a few centimetres appear through the preputial orifice. This condition is most frequently encountered in bulls that were reared in isolation, which have never had the opportunity to indulge in the calfhood riding behaviour, which normally causes stretching and dislocation of the connective tissue between penis and prepuce (Cox, 1982). The condition is frequently self-limiting once sexual activity begins, although it may result in persistence of the frenulum. It must be differentiated from other causes of adhesions between the penis and prepuce.

Deviation of the penis. Ventral deviation of the penis, often referred to as 'rainbow' deviation,

Fig. 30.19 Localised fibrosis responsible for ventral deviation of the penis. The animal was treated by excision of the lesion.

can arise through a number of underlying conditions, of which the most common is persistence of the penile frenulum. In the absence of a persistent frenulum, the condition usually arises from defects in the fibrous architecture of the tunica albuginea. Such defects can be congenital, but can also arise from injuries to the penis that result in scar formation within the tunica (Figure 30.19). True ventral deviation of the penis must also be differentiated from partial failure of erection, when the penis appears deviated ventrally due to its flaccidity. Lateral deviation of the penis is also often attributed to injuries to the tunica, but may arise from inadequate development of the dorsal apical ligament of the penis or congenital defects of the tunica albuginea. Treatment is occasionally successful but is generally unrewarding (Milne, 1954; Walker, 1970; Boyd and Henskelka, 1972; Walker and Vaughan, 1980), due to the difficulty in identifying the site of the causal lesion. For bulls of moderate value, attempting treatment is therefore rarely worthwhile, while for valuable sires, collection of semen for cryopreservation and artificial insemination is often the most viable option.

The most spectacular deviation of the bovine penis is the spiral deviation. Spiralling of the tip of the penis is a normal part of the process of ejaculation in the bull, occurring after intromission, during the ejaculatory thrust (Ashdown and Coombs, 1967, 1968; Seidel and Foote, 1967, 1969; Ashdown et al., 1968). If spiralling occurs prior to intromission, the latter cannot be achieved and the bull is described as suffering from a spiral deviation. Premature spiralling occurs in most afflicted bulls as the tip of the penis touches the hindquarters of the cow (Figure 30.20(a)), but may occur while the penis is still contained within the prepuce. In such cases, the spiralled penis can be clearly seen just behind the preputial orifice, through which it cannot pass. An affected bull may escape the farmer's attention for quite long periods of time, for bulls generally mount keenly enough but, unless they are observed carefully, the characteristic absence of the ejaculatory thrust may be missed. Moreover, bulls do not invariably display premature spiralling, such that some cows may be successfully served. Persuading affected bulls to undergo premature spiralling during clinical



(a)

(b)

Fig. 30.20 (a) Spiral deviation of the penis manifested after protrusion. (b) Traumatic ulceration of the glans penis, secondary to spiral deviation of the penis.

examination can also require some patience; the author's experience is that it may take a long time of quite intense teasing before the abnormality is displayed. The onset of the condition may be at any age, although it is least common in yearlings and most common in animals in their second or third season of use (Pearson and Ashdown, 1976). Continual premature spiralling can result in the penile integument becoming traumatised as it comes into contact with the perineum of the cow. The pain caused by the consequent ulcer on the glans penis (Figure 30.20(b)) may, in time, impair the libido of the bull to the extent that it will not mount.

The cause of premature spiralling of the penis is not clear. Originally, it was suggested to be due to deficiency of the dorsal apical ligament of the penis (Walker, 1970), but later investigations indicated that this was unlikely (Ashdown et al., 1968; Ashdown and Pearson, 1971). Given that spiralling is a normal part of copulation, it appears more probable that the cause is neural or behavioural rather than a defect of the architecture of the penis. The condition can be alleviated, and normal service behaviour allowed, by suturing the dorsal apical ligament to the tunica albuginea with alternating catgut and stainless steel sutures (Ashdown and Pearson, 1973a). However, increasingly strong evidence is accumulating for an inherited component in the aetiology of the condition (Ashdown and Pearson, 1981; Blockey and Taylor, 1984), so it is questionable whether surgical correction is justifiable.

Lesions of the prepuce

Adhesions between the peripenile tissues can arise from localised trauma, haemorrhage and/or abscessation in and around the prepuce. Infection of the penis (balanitis) or prepuce (posthitis) not only is painful, causing unwillingness to copulate, but also can result in development of adhesions between the two organs, preventing protrusion of the penis.

Preputial injuries. In cattle, intermittent protrusion of varying lengths of preputial mucosa is a normal occurrence, which normally coincides with non-erectile movement of the penis within the sheath (Long and Hignett, 1970; Long et al., 1970; Ashdown and Pearson, 1973b). Pathological eversion of the prepuce is associated with aplasia or hypoplasia of the retractor muscles of the prepuce, which normally stabilise the preputial mucosa during penile movement. It is most commonly observed in naturally polled members of breeds with a pendulous prepuce (Long, 1969; Lagos and Fitzhugh, 1970; Bellenger, 1971). The resulting damage to the prepuce occurs most commonly in the segment of preputial mucosa that is closest to the preputial orifice and therefore most likely to be everted. In such cases, eversion of the preputial mucosa is followed by acute inflammatory changes that cause local hyperaemia and oedema. Unless replaced soon after eversion, the mucosa may become permanently prolapsed, with severe and diffuse fibrosis and thickening, often with chronic fissure formation and granulation tissue. Penile protrusion is substantially impaired by such lesions, usually to the extent that, at most, only the tip of the glans protrudes at full erection.

Acutely affected animals may be treated by the application of emollient dressings and replacement of the everted preputial tissue (Wheat, 1951; Hattangady et al., 1968; Larsen and Bellenger, 1971; Walker and Vaughan, 1980; Roberts, 1986). However, chronically inflamed tissue generally requires surgical removal, with the objective of restoring free movement of the preputial mucosa during penile erection. Many variations of surgical procedure have been described to remove the effete and fibrosed preputial tissue (Wheat, 1951; Milne, 1954; Walker, 1966; Larsen and Bellenger, 1971; Pearson, 1972; Walker and Vaughan, 1980), which is generally achieved by either submucosal resection (Figure 30.21), or amputation of the prolapse if the fibrotic change extends deeply into the submucosal tissues. In principle, a circumferential incision is made in the outer layer of preputial mucosa, after which the dissection is then deepened so as to excise all the fibrotic tissue before the inner mucous membrane of the prepuce is cut. The removal of the submucosal lesions may cause considerable venous bleeding, such that careful haemostatis is essential. The inner layer of the mucosa is then sectioned in quadrants, so that suturing of mucosal frills can be performed without risk of retraction of the inner membrane into the preputial cavity. Interrupted sutures of


(a)


(b)

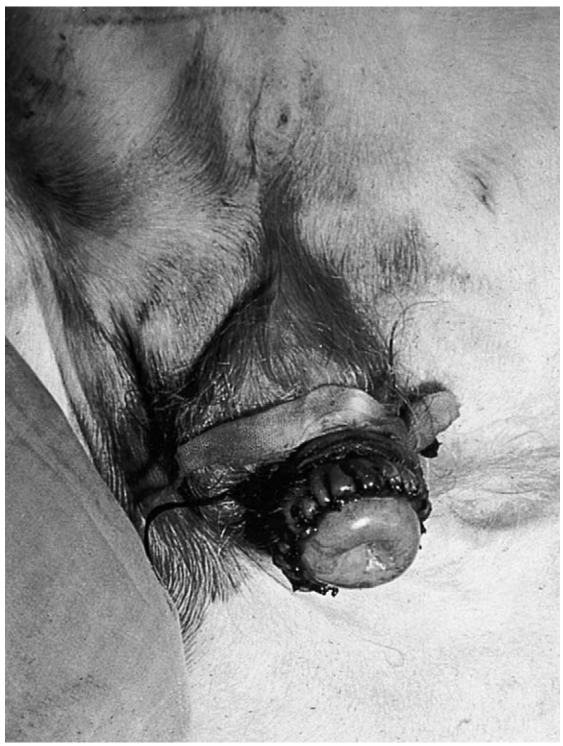

(c)

Fig. 30.21 (a) Chronic fibrosis in a prolapse of the distal prepuce in a Hereford bull. (b) Submucosal resection. (c) Final repair of the lesion.

catgut or polyglycolic acid are suitable. Resection of lesions around the preputial orifice inevitably reduces the effective length of the penis at erection, so the amount of tissue removed should therefore be limited to the minimum necessary to restore penile movement. Postoperative oedema may cause temporary protrusion of the sutured tissues, but this soon subsides. After 2 weeks' sexual rest, the bull should be teased as frequently as possible until penile protrusion is adequate for intromission. Such teasing should persist for at least 3 months after surgery before an animal is condemned.

Alternatively, preputial lesions may occur at the site of the reflection of the preputial mucosa on to the penile integument (Figure 30.22). This site is less common than at the preputial orifice, and presents greater difficulty in treatment. The cause of such lesions is not always clear, but may result from partial or complete avulsion of the prepuce from the penis during copulation or AV collection of semen. Alternatively, lesions may occur when the prepuce becomes lacerated deep within the preputial cavity, usually as a result of the ventral prepuce bursting during the ejaculatory thrust. The site of such lesions is not always clear on clinical examination, for the changing relationships between penis and prepuce make it difficult to correlate the apparent site of a lesion with its position when the penis is protruded under anaesthesia. Intrapreputial lesions present difficulties of exposure for surgery. Even under general anaesthesia it may be difficult to expose the penis fully without tearing the tissues at the site of fibrotic change. After the penis is extruded, forcibly if necessary, all fibrotic tissue masses are excised, down to the tunica albuginea, between two circumferential mucosal incisions at the proximal and distal extremities of the lesion. The mucosal edges are repaired as for distal resections and the animals are managed similarly in the postoperative period. Such surgery is surprisingly effective, although somewhat unpredictable. Much depends upon the length of mucosa that requires resection.

(a)

(b)

(c)

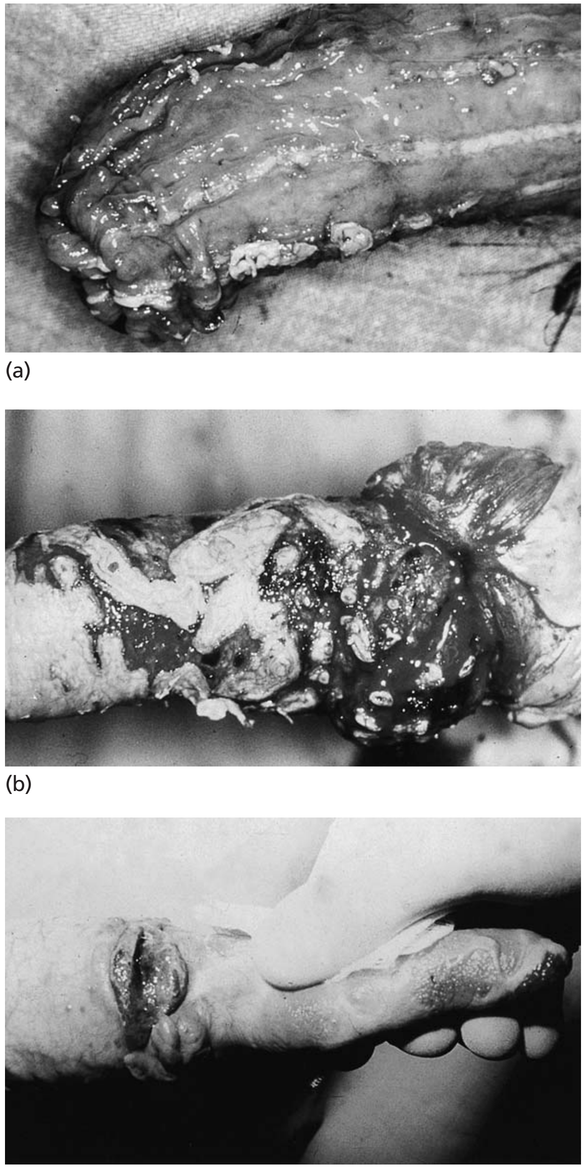
Fig. 30.22 (a) Severe preputial adhesion in a bull. (b) Preputial avulsion and fibrosis at the attachment of the preputial mucosa to the penis. (c) Mucosal tearing as the penis is forcibly withdrawn for the resection of the fibrotic tissues.

Balanoposthitis

Infections of the penis and prepuce are common in the dog, bull and ram, occasional in the stallion, and rare in the boar and cat. Low-grade infection of the preputial cavity is very common, and rarely causes clinical disease. Severe balanoposthitis can cause pain, unwillingness to mate, preputial stenosis, adhesions between penis and prepuce, and peripenile adhesions.

Mild balanoposthitis is particularly common in the dog, in which the prepuce almost always contains a mild seropurulent exudate that is rarely indicative of clinical disease. It does cause a great deal of anxiety to some dog owners, however, who find both the discharge of such material from the preputial orifice and their dogs' efforts to cleanse themselves of the material, offensive. Prophylactic treatment of these mild infections is also frequently requested by owners of stud dogs. Such requests should be received with some caution for, whereas the use of mild antiseptic douches or bland antibiotic or antiseptic ointments rapidly clears any infection, the loss of the normal bacteriological flora from the preputial cavity can predispose to the establishment of opportunist infections by organisms of clinical significance. Occasionally, a canine herpesvirus causes a more severe balanoposthitis (Roberts, 1986), which is characterised by ulceration of the penis and unwillingness to copulate. However, it is more common to find that a traumatic aetiology underlies the relatively infrequent cases of the condition that are of clinical significance.

Similarly, many organisms are able to colonise the preputial cavity or penile integument of the bull. Most of these do not cause any clinical problems, although there are also a number that produce venereally transmitted diseases. Most of these diseases do not cause any gross lesions of the penis, and the bull is a symptomless carrier. A spectacular exception to this generality is the condition caused by the infectious bovine rhinotracheitis–infectious pustular vulvovaginitis (IBR–IPV) virus (Studdert et al., 1964; and see Roberts, 1986; McEntee, 1990). IBR–IPV causes an acute, ulcerative inflammation of the penis and prepuce (Figure 30.23), accompanied by an initial period of pyrexia. Secondary bacterial infection of the ulcers results in a severe, purulent balanoposthitis, causing very considerable discomfort to the bull. Affected bulls may exhibit swelling and pain in the region of the penis and may appear dysuric. Treatment is symptomatic, and consists of sexual rest and infusion of oily suspensions of antibiotics into the prepuce. Healing occurs over 2–8 weeks. In



(a) (b) (c)

Fig. 30.23 Balanoposthitis in the bull. (a) Acute balanoposthitis, which prevented penile protrusion. (b) Acute balanoposthitis caused by infectious bovine rhinotracheitis (IBR) infection. (c) Diffuse fibrosis, following balanoposthitis.

neglected cases, fibrinous adhesions may develop between adjacent ulcers on the penis and prepuce, eventually resulting in an impaired protrusion of the penis. Pustular vulvovaginitis usually occurs in cows served by bulls in the early stages of the disease. A few cases have been recorded in which the bull was relatively asymptomatic, despite characteristic lesions of pustular vulvovaginitis in the cows. The virus is transmissible in semen, which presents considerable risk if bulls that either have active infection or are seropositive are used in AI programmes. For this reason, many regulatory authorities preclude the use of seropositive bulls in AI studs.

Bulls are also susceptible to granulomata formation on the penis, which is a non-transmissible and usually asymptomatic condition (Cox, 1982; Roberts, 1986). Occasionally it causes pain, producing the clinical sign of unwillingness to mate. The condition is characterised by hypertrophy of the lymphoid nodules of the penis, in the absence of an obvious, purulent balanoposthitis. Sexual rest and prophylactic infusions of oily suspensions of antibiotic into the prepuce usually produce a resolution of clinical signs. Tuberculous balanoposthitis has been described in areas where the disease is epizootic (Williams, 1943). Its signs include enlarged, granulomatous lesions of the penis that may bleed, peripenile adhesions and secondary phimosis. This condition must be differentiated from actinomycosis of the prepuce, which causes a similar syndrome.

Balanoposthitis is a relatively common condition of the male sheep, in which it is colloquially known as 'pizzle-rot'. Bacterial balanoposthitis is caused by a number of organisms, of which *Corynebacterium renale*, an organism that grows readily in alkaline urine, is the most important. The disease is more common in wethers than in rams, due to the incomplete development of the penis and prepuce in castrates. The condition is most common in animals that are fed on lush pastures containing high levels of protein. From the urea contained in the urine, the bacteria produce ammonia, which scalds the preputial region. Ulcers and scabs develop over the preputial area, followed by necrosis of the preputial mucosa. Eventually the preputial orifice may become blocked, leading to urine retention (Figure 30.24). Treatment of advanced stages of the disease is seldom successful, but mild lesions resolve simply by reducing the urea load in the urine by restricting access to lush grass (Bruere and West, 1993) and shearing the underside of the ram. The condition can be prevented in intact rams by keeping the belly and preputial region shorn. In wethers, especially the older animals that are kept for wool rather than for meat, testosterone implants are used prophylactically with considerable success. Balanoposthitis lesions are also produced by infection with the orf (contagious pustular dermatitis) virus. Lesions may be present upon the preputial orifice, prepuce and glans penis, where the shallow ulcers are generally covered by a scab. Lesions may also be present in the other sites characteristic of orf, namely the lips, nostril and feet and, in females of the same flock, the vulva and teats. The disease is spread by contact, including venereal contact. Affected rams are generally unwilling to achieve intromission, but their libido is often maintained sufficiently to allow mounting to continue. Thus, the condition may escape notice for quite long periods of time, until it is realised that there is a very high incidence of returns to oestrus amongst the ewes. In consequence, the primary lesions are often healed by the time animals are presented for clinical examination, with little evidence, other than the scars left on the glans penis by the healing ulcers (Figure 30.25), that infection has occurred.

Fig. 30.24 Balanoposthitis in the ram. Advanced lesions causing necrosis of the prepuce and characteristic staining of wool around the preputial orifice (photograph by courtesy of D. M. West).

The most serious cause of balanoposthitis in the stallion is the notifiable disease of dourine (Bowen, 1987; De Vries, 1993). Dourine is caused

Fig. 30.25 Scars left on the ovine penis by healed orf lesions.

by the protozoan parasite *Trypanosoma equiperdum*, and occurs in the Middle East, North Africa and South America. Pockets of infection also occur in the Balkans and South Africa. Infection is predominantly venereal, but can also be transmitted through infected AI equipment and can be passed to foals through vaginal discharges from the mare. The initial sign is oedema of the prepuce, penis, scrotum and surrounding skin, which may be sufficiently severe to cause paraphimosis. Inguinal lymph nodes are often enlarged, and a mucopurulent urethral discharge may also be present. Death may occur rapidly, but a chronic condition is more common outside Europe. Death occurs as the consequence of vascular degeneration causing peripheral nerve degeneration, muscle wasting, emaciation and paralysis. The condition can be treated in its early stages by trypanocidal drugs, although affected animals are slaughtered in many countries, such as those of Northern and Western Europe, the USA, Canada and Australasia, where eradication policies are in force.

In parts of the world where *Habronema muscae* occurs, larval infection produces granulomata in the equine penile and preputial integument. These fungoid growths are infiltrated with eosinophils, bleed easily, cause pruritus and may interfere with urination. In cool climates, lesions may temporarily regress during the winter. Treatment, by administration of systemic insecticide and corticosteroids, generally causes resolution of the disease (Wheat, 1961; Vaughan, 1993). Occasionally, surgery is required to relieve urethral obstruction or to remove the scar tissue that may form during healing (Stick, 1981).

A more common condition of stallions is equine coital exanthema, or 'horse pox' (Cox, 1982; Couto and Hughes, 1993; De Vries, 1993). The herpesvirus (equine herpesvirus 3) that causes the disease is dissimilar to both the equine rhinopneumonitis and equine cytomegaloviruses and, while its mode of transmission is predominantly venereal, it may also be transmitted by vectors or vomites. Lesions occur mainly on the free part of the penis, consisting of papules that are surrounded by an area of hyperaemia and oedema, and which progress to form ulcers. Where the density of lesions is sufficiently great, large ulcerated areas may appear. Mild infections do not impair libido, although lesions with severe secondary infection may do so. Control is achieved by preventing affected animals from mating, while individual animals may need affected areas to be washed weekly with antiseptic solution to limit the establishment of secondary infection.

Other conditions of the penis and prepuce

Phimosis. Phimosis indicates a stricture of the preputial orifice that prevents the penis from being protruded. It has been recorded in most of the domestic species, and may arise from the injuries described above. It may also be a congenital defect, particularly in dogs of the German shepherd and golden retriever breeds. Severely congenitally affected puppies may be unable to urinate adequately, with the consequential balanoposthitis leading to septicaemia and death (Johnston, 1986). Where dogs are affected by congenital lesions or a simple stricture of the preputial orifice, they may be treated by removing a wedge of preputial skin, fascia and mucosa, from just behind the ventral aspect of the preputial orifice. Mucosa and skin are then sutured together. Mild urine scalding may occur after surgery, as urine does not run away freely (Burke, 1986; Allen, 1992). In afflicted bulls and rams, a wedge of tissue should similarly be removed from the ventral aspect of the prepuce.

Paraphimosis. Inability to withdraw the penis into the prepuce results from congenital or acquired strictures of the prepuce, paralysis of the penis and, occasionally, from balanoposthitis. Although not strictly constituting paraphimosis, some coital injuries to the penis also prevent its return to the prepuce and thus, giving similar clinical signs, are considered under the same heading. The condition is most common in the dog and the stallion, but is also reported occasionally in most domestic species.

Paraphimosis following copulation or spontaneous erection is relatively common in the dog. It may also occur when the preputial opening becomes constricted by a band of hair, thereby preventing return of the penis to the prepuce (Johnston, 1986). In cases that have occurred recently, the penis can often be returned to the prepuce with careful manipulation and plentiful lubrication. If neglected, the penis initially becomes oedematous, then swollen and inflamed, and suffers damage to its increasingly friable integument. In such cases, the preputial orifice may need to be surgically enlarged before the penis can be replaced (Chaffee and Knecht, 1975; Walker and Vaughan, 1980; Johnston, 1986). If the condition is left untreated, the penis can become strangulated within a relatively short space of time. The prognosis for cases that have not been treated promptly is therefore guarded, depending upon the severity of trauma and the degree of necrosis the penis has sustained. Unfortunately, the dorsal nerves of the penis are highly susceptible to ischaemic damage and, as these are required for the ejaculation reflex to be operative, inability to ejaculate is a relatively common sequel of relatively minor penile damage.

In stallions, prolapse of the penis is the sequel to many conditions. For example, it generally occurs transiently after the administration of phenothiazide tranquillisers (Pearson and Weaver, 1978; Lucke and Sansom, 1979). On occasion, this prolapse is irreversible (Figure 30.26(a)). Penile prolapse can also follow exhaustion and severe systemic illness and can occur in the terminal stages of disorders of the central nervous system. It may be seen secondary to the preputial oedema that follows castration or other inguinal surgery (see Vaughan, 1993). However, it most commonly occurs after injury to the penis, such as occurs during copulation (Figure 30.26(b)), due to the use of ill-fitting stallion rings, malicious damage to the penis, accidents (Figure 30.26(c)), or injuries sustained during fighting. The pathology of the condition is similar to that of the dog: namely, development of swelling, oedema and inflammation, followed by trauma, ischaemia and necrosis.

The earlier treatment is instigated, the greater the chance of obtaining a satisfactory outcome. Treatment (Walker and Vaughan, 1980; Cox, 1982; Vaughan, 1993) must aim to reduce oedema, prevent trauma to the penile integument and provide support for the penis until it can be returned to the prepuce. In the early stages, oedema may be dissipated by the use of cold water hosing, cold packs and exercise, whereas in the later stages the use of anti-inflammatory drugs and diuretics may also be helpful. The surface of the penis must be protected by the use of ointments that prevent drying or by antiseptic ointments if open wounds are present. Finally, the penis must be supported. Arguably, providing adequate support for the penis is the most important aspect of treatment of this condition, for the drainage of fluid from the penis is greatly facilitated by reducing the tension on the lymphatics that is caused by the weight of the swollen, pendulous penis. Effective supports have been made out of nylon stocking material or U-section plastic guttering, appropriately slung around the horse's hindquarters. Where the condition fails to resolve, amputation of the penis may become necessary. However, recourse to this option should not be taken at too early a stage, for resolution of a case of paraphimosis may take a considerable time, especially where it was initially severe.

Strangulation and necrosis of the penis. Strangulation may occur as a consequence of paraphimosis or as a result of constriction of the penis by hair or maliciously placed objects. It is most common in long-haired breeds of dog and long-woolled breeds of sheep (Figure 30.27). As with paraphimosis, the prognosis depends upon the duration of the vascular constriction and the degree of necrosis that has ensued. Restoration of penile anatomy is relatively easy to achieve, for, providing gross necrosis has not occurred, the ability of the organ to heal is relatively good.

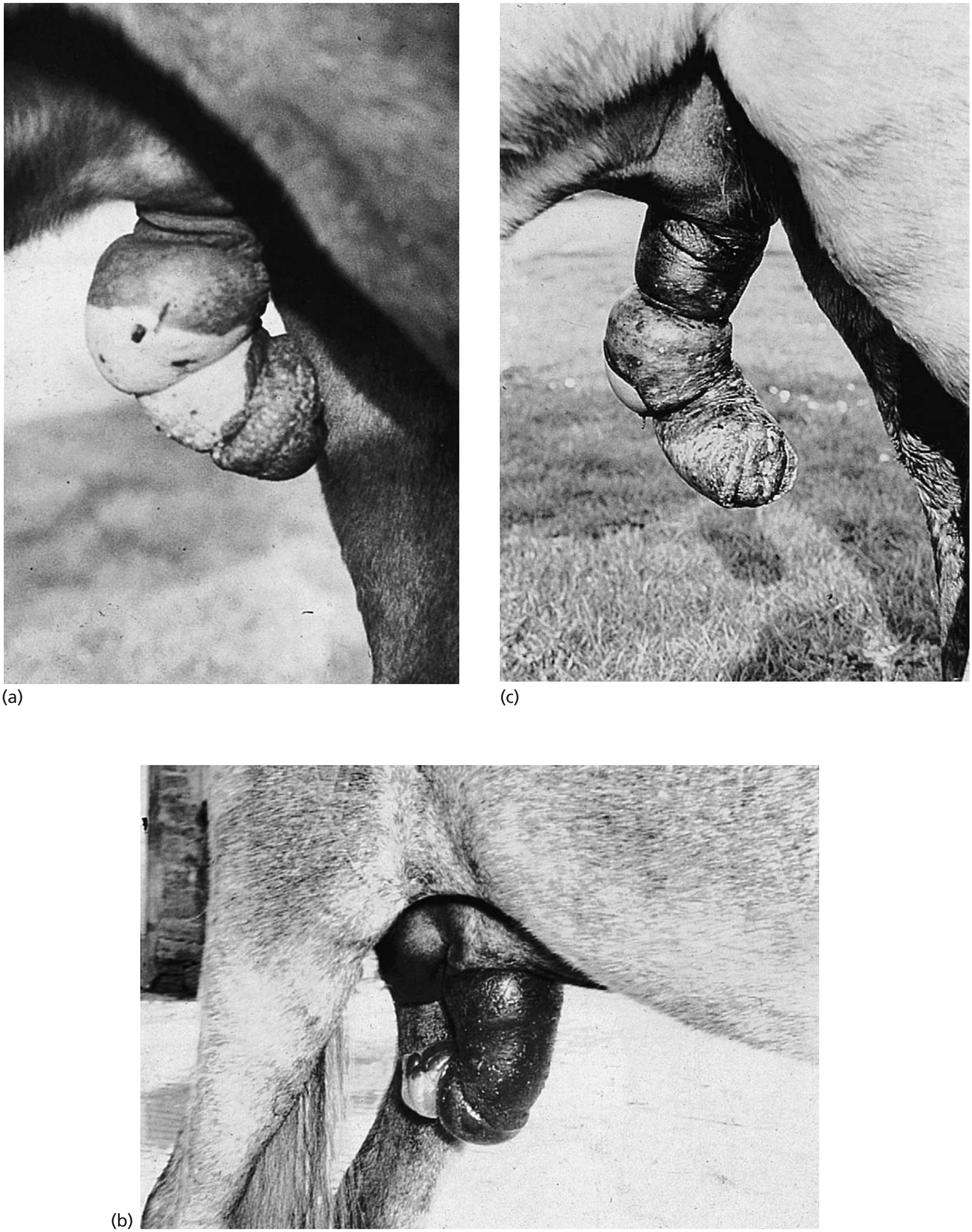


Fig. 30.26 (a) Priapism, 9 days after administration of neuroleptanalgesia for castration. (b) Penile prolapse in a horse after a kick at service. (c) Penile prolapse in a horse after an inguinal stake wound.

Fig. 30.27 Strangulation of the penis of a ram after long strands of fleece became wrapped around the glans during successive copulations.

However, loss of function, particularly impairment of the ejaculation reflex, occurs after relatively short periods of ischaemia. The prognosis should therefore always be guarded in the first instance. Where gross necrosis has occurred, amputation of the penis may be indicated. Again, recourse to this option should not be taken at too early a stage, for, as with the stallion, resolution of even a severe case of paraphimosis may occur over several weeks.

Necrosis of the penis, which does require immediate action, occurs in the ram following obstruction of the urethra by urethral calculi. This condition is seen most commonly in ram or wether lambs that have been growing very rapidly on diets that contain a high proportion of concentrate feeds, but it may occur in any male sheep. At first, the calculi may lodge in the vermiform appendage and, if caught at this stage, the condition can easily be resolved, without impairing the fertility of the animal, by amputation of the appendage. However, calculi rapidly build up thereafter in the urethra, with penile necrosis rapidly ensuing. In such cases, amputation of the necrotic tissue, probably accompanied by perineal urethrostomy, is often the only recourse. In neglected animals, urethral rupture may occur, leading to infiltration of urine into perineal tissues, the prepuce and the scrotum. Alternatively, rupture of the bladder may occur. In both situations, extreme uraemia occurs and, where urine has infiltrated into tissue, necrosis and sloughing of the tissue invariably follow. The best that can be hoped for is the recovery of the carcass value of the animal, but even this is rarely achievable in neglected cases.

Penile neoplasia. Virally induced fibropapillomata of the skin, genitalia and alimentary tract are common in young cattle. The penile integument, particularly its terminal 5 cm, is a common site for such tumours, which may be single or multiple, sessile or pedunculated (Figure 30.28). Tumours can be found in intact and castrated animals, but rarely persist beyond 3 years of age. Clinical effects vary according to the size and the morphology of the lesions. Haemorrhage and ulceration are the most common sequelae, the pain caused by the latter sometimes being sufficiently severe to impair libido. Large lesions can prevent retraction of the protruded penis back into the sheath, resulting in the tumour-bearing segment remaining outside the preputial orifice and becoming traumatised and infected. Multiple or large lesions can cause complete irreducible prolapse of the penis, which then undergoes secondary changes of venous congestion and oedema. Rapid growth of penile tumours within the preputial cavity can result in compression of the urethra, which may even rupture, allowing extensive infiltration of urine into peripenile tissues.

Fibropapillomata sometimes undergo necrosis and sloughing, others become detached during coitus, while yet other lesions regress spontaneously. However, such resolutions cannot be assumed, so recourse to surgery is frequently indicated (Pearson, 1972). Single, pedunculated lesions can sometimes be removed during coitus, but most tumours require careful removal with the bull very heavily sedated or under general anaesthesia. Pedunculated lesions can be ligated relatively easily, but excision of sessile lesions often leads to profuse haemorrhage, which may be difficult to control if a large area of mucosa has had to be removed. However, although such haemorrhage may persist for some time, it is rarely dangerous and, provided the tumour does not recur, the wounds generally heal quickly. Care should be exercised to avoid incising the urethra, for its highly vascular mucosa may continue to bleed at ejaculation for long periods afterwards. Penile fibropapillomata do not metastasise, but a small proportion of tumours exhibit a remarkably

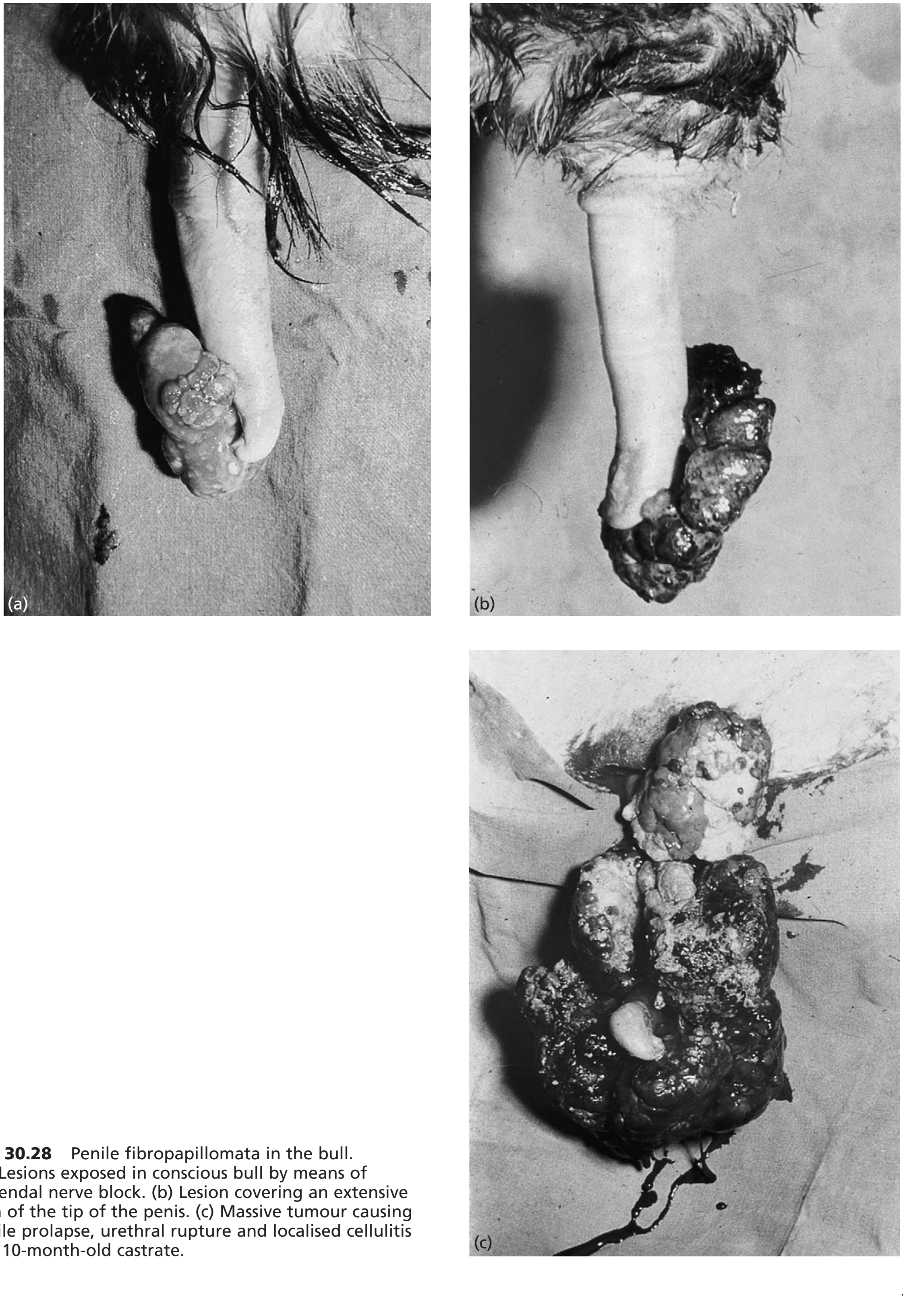


Fig. 30.28 Penile fibropapillomata in the bull. (a) Lesions exposed in conscious bull by means of pudendal nerve block. (b) Lesion covering an extensive area of the tip of the penis. (c) Massive tumour causing penile prolapse, urethral rupture and localised cellulitis in a 10-month-old castrate.

aggressive recurrence after surgical removal. There is debate over the incidence of such recurrences, but possibly 10% of lesions recur with sufficient rapidity to be obvious again within 3–4 weeks of surgery (Pearson, 1977). Cryotherapy of the affected area usually prevents further recurrences (Pearson and Lane, unpublished findings), while administration of an autogenous tissue vaccine markedly reduces the incidence of recurrences (Desmet et al., 1974). The prognosis for use as breeding animals is generally good, although libido may take some time to recover in animals that have had long-standing, painful, ulcerated lesions.

Penile tumours are also common in the horse. Squamous cell carcinomata of the glans penis (Figure 30.29(a)) or preputial ring occur in aged geldings, in which they develop as large, fungating tissue masses that may cause bloody preputial discharge or penile prolapse. The tumour develops in response to the carcinogenic properties of the smegma that accumulates around the penis of geldings (Plaut and Kohn-Speyer, 1947). Its growth is relatively slow and it is slow to metastasise, although it does spread within the preputial cavity and, eventually, metastasises to local lymph nodes. However, local lymphadenitis may also occur due to the secondary infection which invariably occurs with these tumours, so care must be exercised in giving a prognosis. Occasionally, penile carcinomata behave in a malignant, invasive manner and rapidly destroy the body of the penis (Figure 30.29(b)). Penile carcinomata are best treated by radical amputation of the penis, with a urethrostomy on the penile stump within the sheath or directly to the preputial skin (Walker and Vaughan, 1980). Some lesions of the more loosely attached preputial mucous membrane can be dealt with by simple or reefing excision.

Sarcoids can develop on the preputial skin and mucosa in both castrated and entire horses, donkeys and other Equidae. Sarcoids also occur on the scrotal integument. In most circumstances, such lesions can be extirpated without difficulty, using a variety of surgical methods (Vaughan, 1993). However, the presence of multiple, recurrent sarcoids on the inner or external surfaces of the prepuce (Figure 30.29(c)) may necessitate its complete ablation and amputation of the penis (Cox, 1992). Grey horses may develop melanomata of the penis or prepuce (Figure 30.29(d)). Their progress is generally slow and only produces local invasion of tissue, but aggressive lesions that metastasise also occur from time to time.

In the dog, the most common penile tumour is the transmissible venereal tumour (Jones and Joshua, 1982; Roberts, 1986). This tumour, which is mostly found in the tropics, is spread by coitus and has an incubation period of 5–6 weeks. A fetid, bloody preputial discharge may be present and, on exteriorisation of the penis, characteristic fleshy, greyish red, nodular masses are observed (Bloom et al., 1951). The tumours ulcerate easily and are friable (Figure 30.30), so bleed when handled. In neglected cases, spread to local lymph nodes occurs. Treatment, where this is possible, is by removal of the penis and prepuce. The recurrence rate is relatively high. The use of chemotherapy has also been advocated (Wittrow and Susaneck, 1986).

Penile papillomata (Figure 30.31) also occur in the dog but, unlike those of the bull, are generally ulcerative, locally keratinised and poorly circumscribed (Arthur et al., 1989). They may also be completely sessile, with a sharply demarcated, ulcer-like border. Such lesions bleed profusely with sexual or other excitement, but their development may be remarkably slow, even over a number of years.

Miscellaneous conditions of the penis. Complete avulsion of the preputial mucosa from its attachment to the glans penis is an occasional injury of bulls that usually results from careless use of an AV (Roberts, 1986). Diaphallus, or duplication of the penis, is a rare condition of the bull. Hypospadias and epispadias are congenital abnormalities of the penis, in which the urethra opens in the perineum or on the ventral or dorsal surface of the penis. The consequences of such lesions depend upon the site of the urethral opening. Where this is close to the distal end of the penis, the condition may be undetected and have no effect upon fertility. However, more proximal lesions adversely affect fertility.

The retractor penis muscle may undergo disuse atrophy after the penis has been unable to achieve erection for a long period. However, calcification of the retractor penis muscle, which occurs in aged bulls, does not affect erection. Finally, some bulls exhibit profuse arterial haemorrhage at the

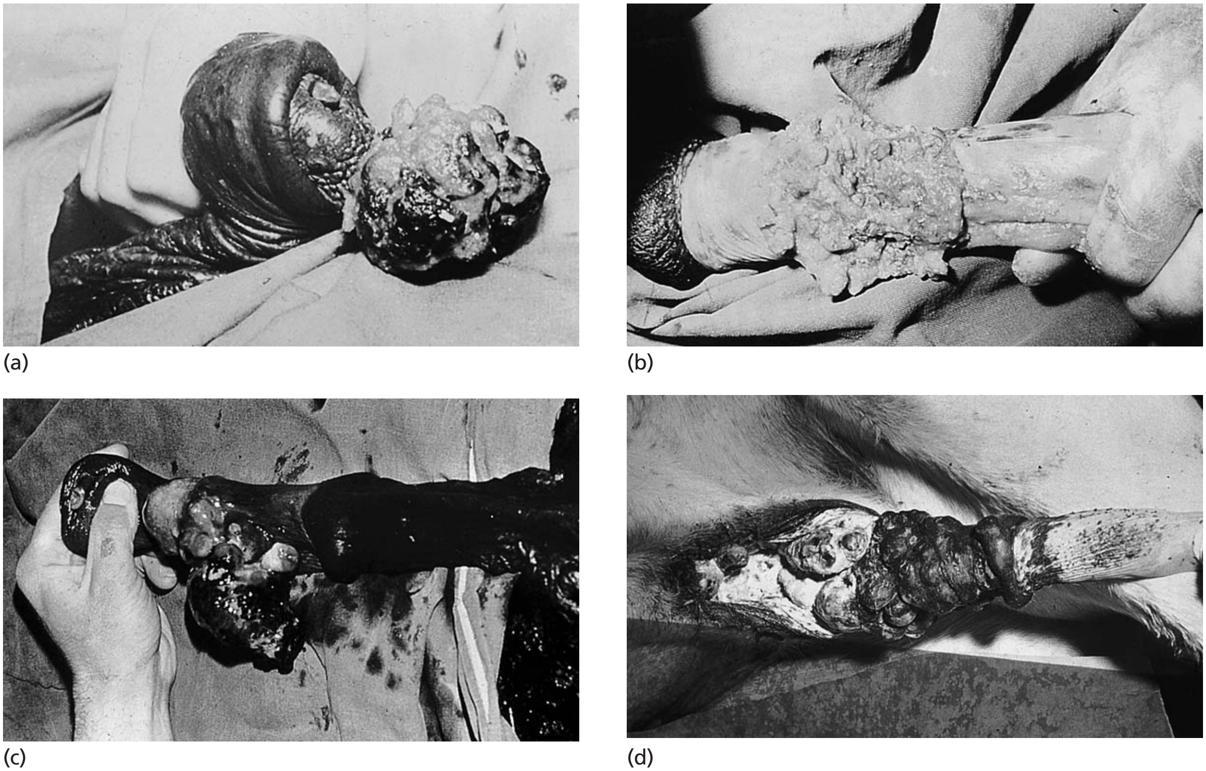


Fig. 30.29 Penile and preputial tumours in the stallion. (a) Squamous cell carcinoma of the penis in an aged gelding. (b) Squamous cell carcinoma of the penis associated with tissue infiltration and destruction. (c) Multiple sarcoids of the penis and prepuce. (d) Multiple melanomata formation in the prepuce of an aged grey gelding.

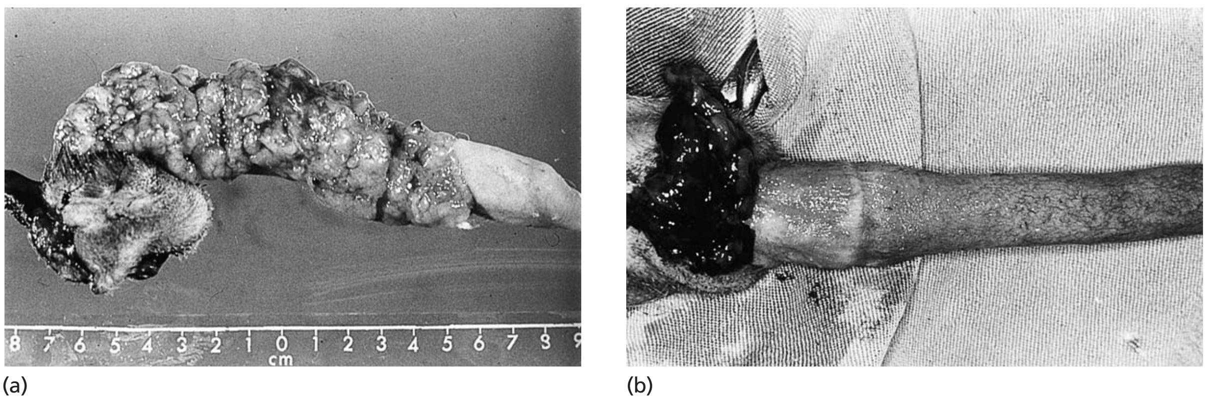


Fig. 30.30 Transmissible venereal tumour of the dog. (a) Tumour formation over most of the penile integument. The characteristic fleshy, greyish red, nodular masses of the tumour are readily apparent. (b) Tumour developing in the preputial fornix.

time of ejaculation. This condition, which is difficult both to investigate and to treat, may be due to unhealed traumatic lesions of the penile integument but, more commonly, is due to leakage of blood from the corpus spongiosum penis into the urethral lumen (Ashdown and Majeed, 1978).

(a)

(b)

Fig. 30.31 Penile papillomata of the dog. (a) Localised lesion and (b) diffuse 'florid' lesion.

Conditions causing failure of ejaculation

There are a few conditions in which ejaculation does not occur, despite otherwise normal intromission and copulation. Such conditions can be broadly divided into those where the ejaculation reflex is impaired and those in which localised pain makes the animal unwilling to ejaculate. The former conditions generally occur when some damage has occurred to the neural pathways between the glans penis and the spinal cord. Strangulation of the penis, with ensuing damage to the sensory dorsal nerve of the penis, causes ejaculation failure, while in older bulls, compression of the spinal nerve roots by age-related exostoses can also preclude ejaculation.

Localised pain is effective in preventing ejaculation. Localised peritonitis in the caudal abdomen of ruminants causes pain during the ejaculatory thrust, so affected animals are often willing to mount but less willing to ejaculate. Animals with back pain behave similarly, although they may be less willing to mount. Finally, some painful conditions of the penis, notably orf in rams, make the animal unwilling to achieve intromission and ejaculate, despite their willingness to mount.

Conditions causing failure of fertilisation

Fertilisation failure, despite normal copulation, generally characterises diseases of the testis (including abnormalities of spermatogenesis), epididymis and accessory glands. Many of the conditions causing failure of fertilisation can be diagnosed by an examination of the external genitalia of the sire, but many more can only be diagnosed by semen evaluation, which is therefore an essential component of one's clinical examination. However, a diagnosis of failure of fertilisation on the part of the male animal always requires careful differentiation from the many female factors that can present with apparently identical signs. For this reason, it is generally advisable to undertake an evaluation of the group of females with which the apparent infertility of a sire has been manifest, before examining the sire.

Furthermore, it is important to separate causes of fertilisation failure that represent pathologies of the reproductive tract from causes that are non-pathological. In the latter category are two important factors. Firstly, the time of year when the sire was used must be considered in relation to the endogenous breeding season of the species. Secondly, the number of females the sire was required to impregnate and the system of mating under which he was being used must also be considered, in relation to the normal reproductive expectations of the species and breed.

Conditions affecting the testis and epididymis

Cryptorchidism. Cryptorchidism occurs when the normal process of testicular descent is perturbed, such that one or both testes fail to complete their descent into the scrotum. Spermatogenesis is generally markedly impaired or absent in testes that are not scrotal, due to high intratesticular temperature. Animals that have a single cryptorchid testis are usually fertile, although the inhibition of spermatogenesis in retained testes means that the sperm density is often below expectation for the species. Where both testes are cryptorchid, the ejaculate is either aspermic or very severely oligospermic. Testosterone secretion is unaffected by a cryptorchid position, so the libido of affected animals is normal. Indeed, it is far more common for cryptorchidism to be detected in supposedly castrated animals that continue to exhibit masculine behaviour than as a cause of subfertility in intact males.

Cryptorchidism occurs most commonly in the stallion (Hayes, 1986), the boar (Huston et al., 1978) and in some breeds of dog (Patterson, 1977). It is uncommon in other species, except as an iatrogenic condition of bulls and rams. In these species, unskilled use of rubber rings for castration can result in one testis being forced back either into the inguinal canal or, more commonly, into a subcutaneous position cranial to the scrotum. Occasionally, a high incidence of cryptorchidism has been recorded in individual flocks of sheep. Such flocks, together with the clear breed incidences of the condition in other species (e.g. Red Danish cattle – Blom and Christensen, 1947; Angora goat – Warwick, 1961), indicate the probability of an inherited basis for the disease. For this reason, the use of cryptorchid animals as sires should be avoided, and their castration recommended. In the dog and the stallion, retained testes exhibit a similarly high incidence of neoplasia to that reported in men, a further reason for the castration of such animals (Willis and Rudduck, 1943; Pendergass and Hayes, 1975).

Testes may be retained in the abdomen or inguinal canal. The incidence of different sites of retention in the horse is shown in Table 30.2. In supposedly castrated animals, diagnosis of the presence of a testis in the inguinal canal may be achieved by careful palpation of that region, but many cases require demonstration of the presence of male hormones for confirmation. All affected stallions exhibit testosterone concentrations that are not only higher than in castrated animals, but also responsive to LH stimulation (Cox et al., 1973; Cox, 1975). Thus, after collection of an initial blood sample, 3000 IU of hCG are given intravenously, then a further blood sample collected 40 minutes later. Both are assayed for testosterone concentrations. In older animals (4 years and over), the presence of oestrone sulfate in the blood also confirms the presence of testicular tissue. Removal of retained testes from the horse may be effected by initial surgical exploration of the inguinal canal. Many testes will be found in this site, while others can be withdrawn from an abdominal position through the inguinal ring without much difficulty. Other abdominal testes can be withdrawn through a parapenile abdominal incision (Arthur, 1961; Walker and Vaughan, 1980; Cox, 1982). Providing the gubernacular attachments of the testis are not disrupted during surgery, it is very uncommon to find an abdominal testis at a site other than near the internal inguinal ring.

Table 30.2 Sites of testes in the cryptorchid stallion (compiled from Hobday, 1914; Silbersiepe, 1937; Stanic, 1960; Arthur, 1961; Wright, 1963; Bishop et al., 1966; Fessler, 1978 and Cox et al., 1979)

	Position of right testis			
Position of left testis	*Abdominal*	*Inguinal*	*Scrotal*	*Total*
Abdominal	125 (7.0)	12 (0.7)	545 (30.7)	682
Inguinal	2 (0.001)	69 (3.9)	254 (14.3)	325
Scrotal	291 (16.4)	446 (25.1)	—	
Total	418	527		1744

Testicular degeneration. The seminiferous epithelium of the tesis is highly susceptible to damage, with a wide variety of agents causing reversible or irreversible degeneration. Testicular degeneration occurs in response to raised intratesticular temperature, toxins, endocrine disturbances and infection (Humphrey and Ladds, 1975; McEntee, 1990). Many of the causes of testicular degeneration do not manifest themselves in infertility immediately, due to the protracted time taken for spermatogenesis. There is therefore normally a lag interval, which may be of several weeks, between the time at which the testis is damaged and the time at which effects upon semen quality are first noted.

Raised temperature in the testis can itself emanate from many causes. Many animals exhibit a period of relative infertility during and after high summer temperatures. This phenomenon is well recognised in the boar (Crabo, 1986), even in Northern European conditions, and is sufficiently important in the bull for some AI studs to provide air-conditioned accommodation for the sires to limit summer maxima of temperature (Roberts, 1986). Temperature-induced summer infertility of males is a very important cause of infertility in European breeds of domestic animals that are imported into tropical and subtropical climates. In rams, raised scrotal temperature can result from excessive amounts of wool over the scrotum, or from leaving animals unshorn during the summer (Hulet et al., 1956). Thus, the practice of showing rams in full fleece in midsummer accounts for the very high proportion of such animals that become infertile during the subsequent autumn breeding season. Excessive deposition of fat in the scrotum, such as occurs in rapidly grown bulls and rams, can prevent heat loss from the scrotum and result in infertility (Jubb and Kennedy, 1970). Local inflammation to the scrotal skin or other structures in the scrotum can also raise the testicular temperature sufficiently to impair spermatogenesis (Rhodes, 1976; Burke, 1986; Roberts, 1986). In the ram, scrotal mange, caused by *Chorioptes bovis* (Figure 30.32), raises scrotal temperature sufficiently to cause testicular degeneration. Large numbers of rams can be affected in a flock, such that examination for the presence of the condition before the start of the breeding season is a wise precaution (Bruere and West, 1993). Trauma and abcessation of the scrotum (especially in rams after shearing), or inflammation of one testis or epididymis almost invariably cause a temperature-dependent degeneration in the opposite testis.

Fig. 30.32 Scrotal mange of a ram. The inflammatory reaction that results in the crusty lesions of the scrotal skin raises intra-testicular temperatures to a sufficient degree to result in substantial degeneration of the testis. (Photograph courtesy of DM West).

Abnormalities of the testicular circulation, such as occurs in varicocoele, perturbs the heat exchange mechanism responsible for maintaining the testis at a temperature below that of the body, again resulting in temperature-dependent testicular degeneration (Ott et al., 1982). This condition is rare in domestic species other than the sheep; however, in the Merino breed it is sufficiently common to warrant routine examination of the spermatic cords during fertility examination of rams (Bruere and West, 1993). Degeneration results from inguinal or scrotal hernias, for the same reason of thermal damage. Finally, prolonged pyrexia, such as occurs with systemic infections, can cause testicular damage, although short periods of illness are unlikely to be detrimental. Conversely, scrotal frostbite can also result in testicular degeneration (Faulkner et al., 1967).

Toxic causes of testicular degeneration are, likewise, many (Humphrey and Ladds, 1975). Heavy metal or radiation contamination are well-recognised causes of testicular damage, but many other materials have been implicated at various times. Establishing causal relationships between such substances and infertility is particularly difficult, given the time interval between their ingestion and appearance of infertility. Stress-related degeneration occurs largely due to the inhibition of LH secretion by the corticosteroids that are released during stress (Welsh et al., 1981). Stress-related degeneration has been described in beef bulls after movement (Knudsen, 1954; Jackowski et al., 1961) and in other animals due to chronically unsuitable housing and management (Clarke and Tilbrook, 1992). Hormonal degeneration is described in dogs whose gonadotrophin secretion is impaired either by a primary lesion of the anterior pituitary or due to the presence of oestrogenic tumours, such as Sertoli cell or adrenal tumours (Roberts, 1986). Aged animals undergo a progressive, irreversible degeneration, initially characterised by increased percentages of sperm with primary abnormalities, with later oligospermia and testicular fibrosis (Bishop, 1970; McEntee, 1990).

Many infectious causes of degeneration have also been described. The most severe of such infectious causes, orchitis, is considered under a separate heading, but many other, more mild conditions affect the efficiency of spermatogenesis. Ascending infection by enterocytopathic bovine orphan (ECBO) viruses and infection by IBR or, where it occurs, Epivag virus have been specifically associated with testicular degeneration, although viral causes are frequently implicated even when a causal organism cannot be conclusively demonstrated (Humphrey and Ladds, 1975; Roberts, 1986). Bacterial contamination of such testes may well lead to an inherently mild bout of degeneration progressing to purulent or necrotic orchitis.

Clinical signs of infertility and oligospermia usually supervene 4–8 weeks after the onset of the cause of the degeneration. Ejaculate volume is usually unaffected, but the number and motility of spermatozoa fall, while the proportion of sperm exhibiting abnormal morphology rises (Figure 30.33). In severe cases, the ejaculate may become virtually aspermic, with such sperm as are present having such a bizarre morphology as to be scarcely recognisable. After such an acute phase, the condition may resolve in one of two main ways. In many animals, resolution of the degeneration occurs over a period of weeks or months, with an eventual

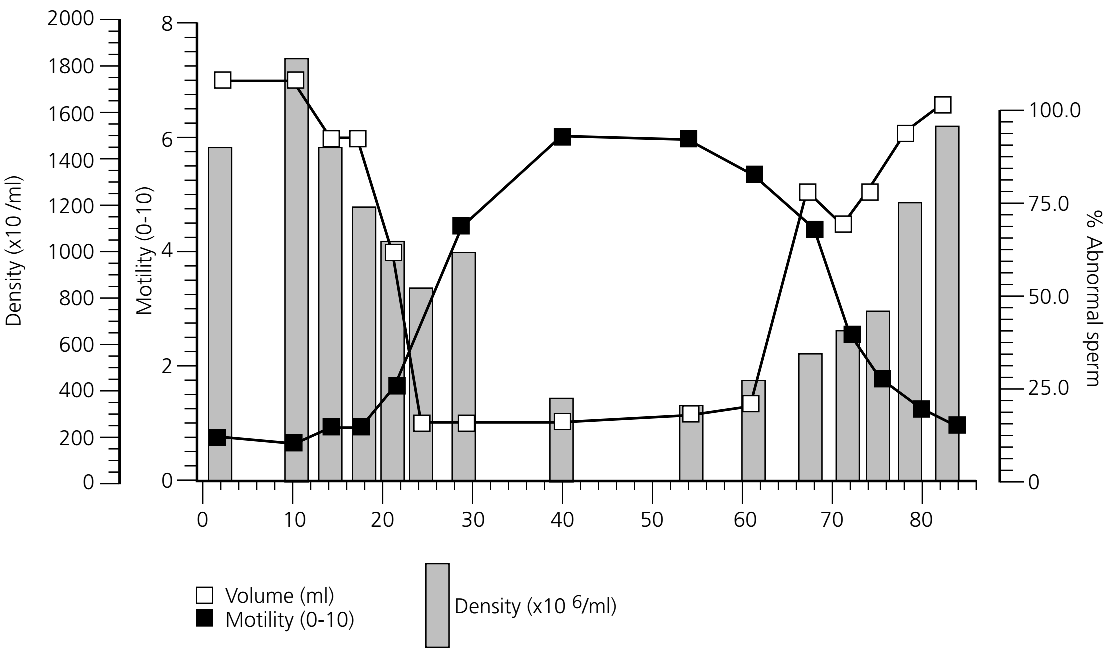
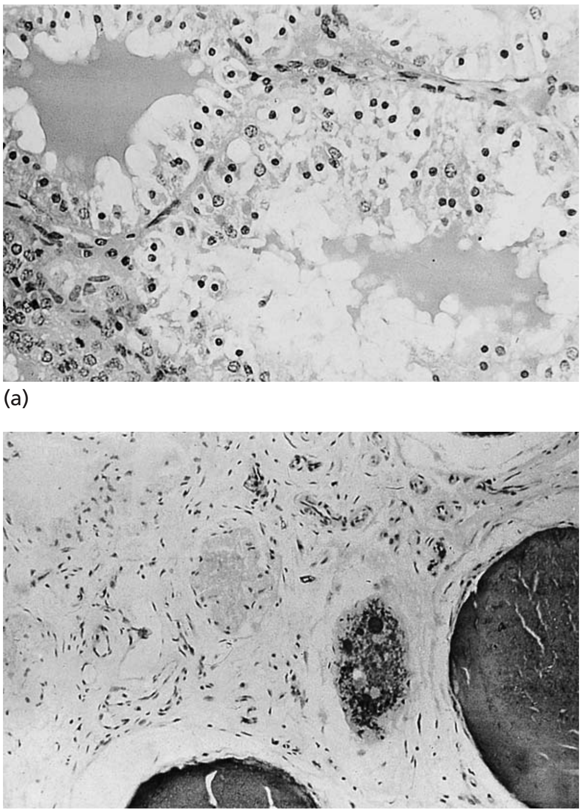


Fig. 30.33 Changes in semen quality of a bull during a period of testicular degeneration and the subsequent restoration of normal spermatogenesis. The cause of the degeneration was presumed to be high summer temperatures (redrawn from Parkinson, 1991, with permission).

recovery of semen parameters back to, or close to, normality. Some residual oligospermia or increased proportion of morphologically abnormal sperm may be present. In more severe cases, permanent loss of seminiferous tubules occurs, with fibrosis and calcification (Figure 30.34) of the testis following. Such animals never regain normality, but present with shrunken, firm, irregular testes and virtually aspermic semen. It is important to note that the severity of the initial changes in semen quality is of very little prognostic value; the author has seen animals with very severe changes in semen quality that have returned to normal fertility in a matter of months, whereas other animals with quite mild initial seminal changes have never again produced acceptable semen. Testicular biopsy can be a valuable aid to prognosis, although great care is needed if such samples are to be collected from ruminant species, due to the very considerable risk of severe haemorrhage into the testis or between the vagina tunics (Gassner and Hill, 1955). However, biopsies can be collected from the dog (Soderberg, 1986) and stallion (Threlfall and Lopate, 1993), although, even in these species, considerable damage to testicular parenchyma can result in individual animals. Intact basement membranes of the seminiferous tubules, the presence of spermatogonia within the tubules and the patency of the lumina of the tubules all indicate a good prognosis for restoration of fertility (Kenney, 1970). Where collection of biopsies is not undertaken, the progress of the animal must be monitored by regular collection of semen until signs of resolution of the condition are observed. An early sign of resolution that occurs in some animals is that the grossly abnormal morphology of the spermatozoa is replaced by relatively high percentages of cells with cytoplasmic droplets at the distal end of the midpiece.



(a)

(b)

Fig. 30.34 Histology of the testis (a) from a bull with moderate testicular degeneration. Healing may completely restore normal testicular architecture, or may result (b) in loss of seminiferous tubules, together with fibrosis and calcification of interstitial tissue (reproduced from Parkinson, 1991, with permission).

Orchitis and epididymitis. Orchitis ranges from a mild infection of the testis, scarcely distinguishable from testicular degeneration, through to gross suppurative or necrotic destruction of the organ. Orchitis can arise from a primary infection or by haematogenous spread of bacteria into the testis superinfecting pre-existing traumatic viral or parasitic damage. Primary testicular damage can arise from ECBO infection in bulls (Humphrey and Ladds, 1975), but in rams it is likely that many infections become established after fighting injury to the testis. *Brucella* species cause orchitis in many domestic animals, *B. abortus*, *B. canis*, *B. melatensis* and *B. suis* affecting bulls, dogs, goats and boars, respectively (reviewed by Plant et al., 1976; Jones and Joshua, 1982; Roberts, 1986; Smith, 1986). In cattle, the role of *Mycobacterium tuberculosis* as a bacterial pathogen should not be overlooked, especially if granulomatous orchitis is discovered (see Adeniran et al., 1992, for a recent example). However, the majority of isolates from cases of orchitis are either non-specific bacteria and mycoplasma, or are the particular pyogenic organism for the animal species (e.g. *A. pyogenes* in cattle). Ascending infection from the urinary tract is postulated, but haematogenous spread seems more probable.

Orchitis is more commonly unilateral than bilateral and may involve the epididymis. During the acute phase of the disease, the affected testis is inflamed, with consequent hyperaemia, heat and swelling (Figures 30.35 and 30.36). The testis may become grossly enlarged, up to two or three times its normal size. The testis is often very painful, so that the animal resents it being touched. The pain may be sufficiently severe to produce an altered gait. A systemic pyrexia may occur. The localised inflammation usually causes temperature-dependent degeneration in the unaffected testis. If the condition progresses to the chronic phase, the testis becomes shrunken, fibrotic and adherent to the tunic and scrotum. Abscesses may break through the scrotal skin.

Orchitis invariably causes a great deal of destruction of the affected testis. The infection may be purulent, with large, coalescent abscesses occupying much of the testicular parenchyma, or it may be necrotic, when the substance of the testis is almost entirely destroyed. Because of the degree of destruction that occurs, the prognosis for saving the affected testis is hopeless. If it is hoped to salvage an affected animal for breeding, removal of a unilaterally affected testis should therefore be advocated at the earliest possible stage of the disease, to limit degeneration of the unaffected testis. If bilateral orchitis occurs, the prognosis for future breeding is hopeless, and castration should be performed as soon as it is safe to do so.

Epididymitis can also occur as a primary infection or by spread from an infected testis (Humphrey and Ladds, 1975). Orchitis in the associated testis can also occur following a primary epididymitis. The general signs of epididymitis are similar to those of orchitis: namely, heat, swelling and pain of the affected organ. Any inflammation of the epididymis causes obstruction of the single, highly convoluted tube of which the organ is composed, so a loss of function


(a)

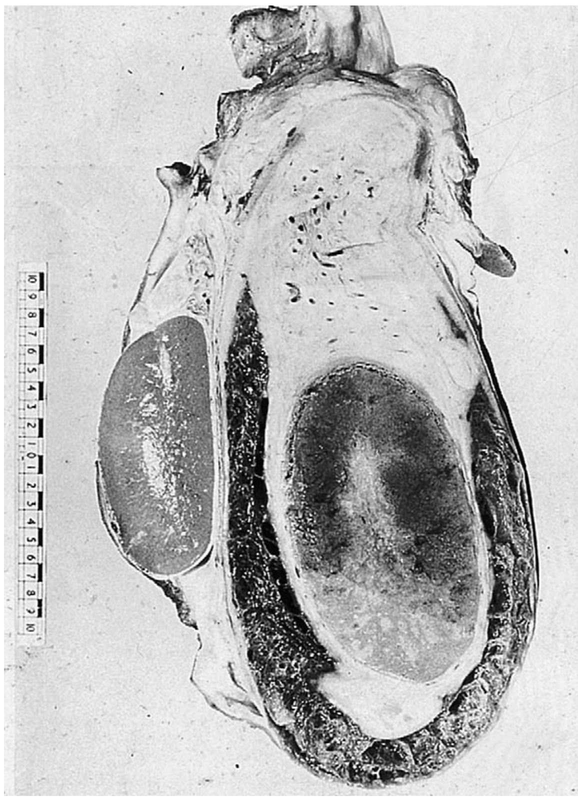

(b)

Fig. 30.35 Orchitis in the bull. (a) Simmental bull with acute orchitis. (b) Longitudinal section of the scrotum, showing inflammation within the substance of the testis and in the vaginal tunics. Degeneration of the contralateral testis has caused a reduction in its size (reproduced from Parkinson, 1991, with permission).

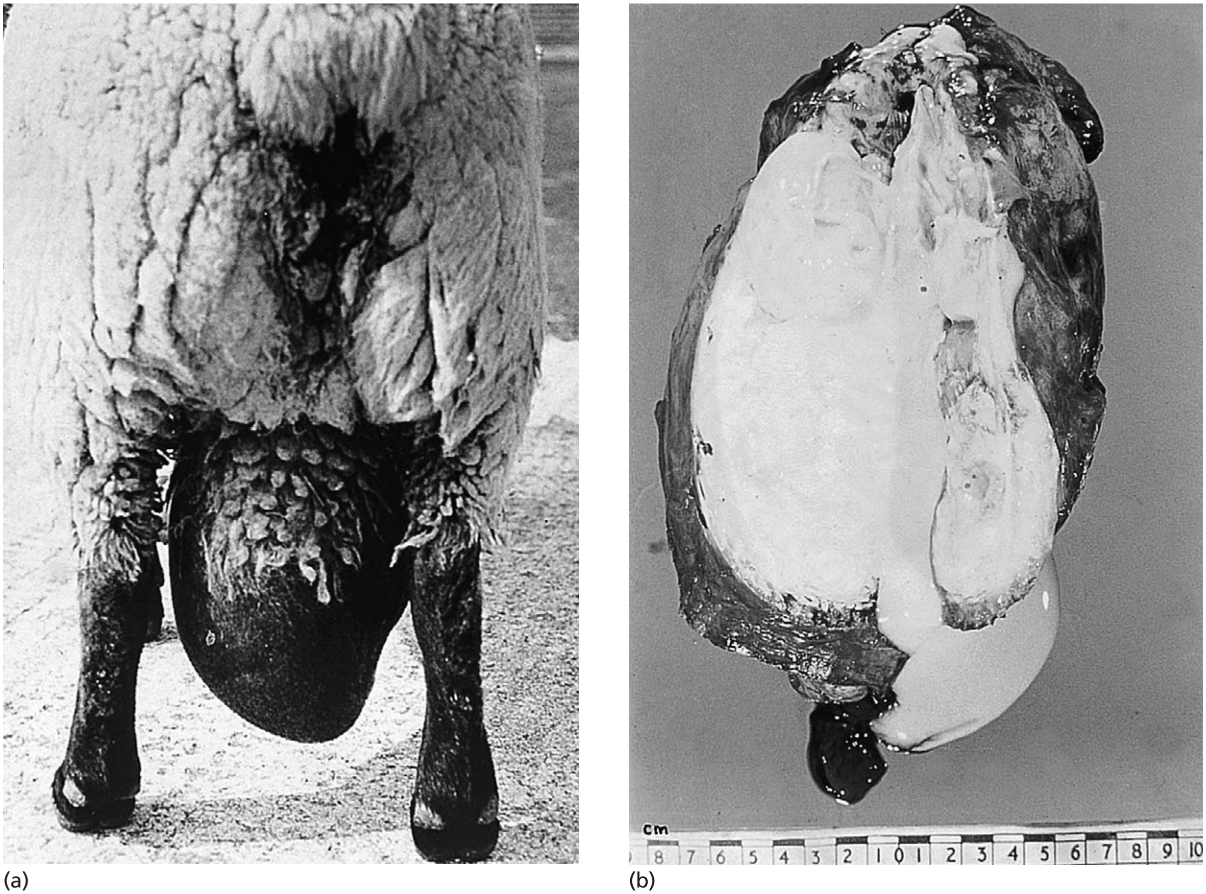


Fig. 30.36 Orchitis in the ram. (a) Ram with acute orchitis. (b) The affected testis after removal.

normally ensues. Unilateral epididymitis therefore results in reduced fertility, whereas bilateral obstruction results in sterility. Furthermore, as with orchitis, unilateral epididymitis causes temperature-induced degeneration in the contralateral testis, so early removal of the affected epididymis and its associated testis should be recommended.

In Australia, North and South America and central Europe, *Brucella ovis* causes an epididymitis, which causes epididymal obstruction and granuloma formation (Bruere, 1986; Bruere and West, 1993). In these countries, it is a significant cause of infertility in rams. In an Australian (Queensland) survey, epididymitis was present in 58% of culled rams (Foster et al., 1989). Even where *B. ovis* infection is not associated with clinical evidence of epididymitis, its presence in the semen of rams is associated with impaired semen quality (Kott et al., 1988). The organism is transmitted between rams by homosexual behaviour (Jebson et al., 1954) and may be transmitted between ewes and rams as a true venereal disease (Hartley et al., 1955). It also affects stags and can be transmitted from rams to stags by direct (rather than environmental) contact (Riddler et al., 1999; West et al., 1999).

Ovine epididymitis also occurs as a consequence of infection with the pleiomorphic Gram-negative organisms of the *Actinobacillus seminis–Haemophilus sommis–Histophilus ovis* group (Bruere and West, 1993). Infection with these organisms is common in virgin rams and ram lamb-producing flocks (Bagley et al., 1985), but *A. seminis* is also recovered from older rams with epididymitis. Typically, however, these organisms affect rams in the peripubertal period, when the organism can be recovered from a high proportion of rams (Walker and LeaMaster, 1986). Fortunately, only a relatively low proportion of animals progress to develop epididymitis. The possibility of venereal transmission has been suggested and *A. seminis* undoubtedly infects ewes, causing abortion (Foster et al., 1999) and, at least experimentally, mastitis (Alsenosy and Dennis, 1985). The infection is found in AI stud rams (Low et al., 1995) and anecdotal evidence exists for transmission between teasers, ewes and AI stud rams (T. J. Parkinson, unpublished). *A. seminis* was first isolated from rams with epididymitis in the UK in 1991 (Heath et al., 1991). The measures for controlling the disease are poorly understood. Avoiding holding ram lambs in dirty yards and reducing pasture stocking densities have been suggested (Bruere and West, 1993), while culling animals with clinical epididymitis reduces the incidence but does not eliminate the disease (Bagley et al., 1985). Lesions occur in both the head and tail of epididymis, where they are often associated with spermatocoeles (Walker et al., 1986). Figure 30.37 illustrates an *A. seminis* epididymitis in the epididymal head of a 4-year-old ram.

It should also be noted that *H. somnus* infection is associated with orchitis (Corbel et al., 1986; Plagemann and Mutters, 1991) and infertility in cattle.

Obstruction of the epididymis can occur following localised rupture of the duct and leakage of sperm into the surrounding stromal tissue. The granulomata formed in response to the presence of foreign (sperm) antigen in the tissue cause an obstruction of the epididymes (Parkinson et al., 1993; Figure 30.38). Leakage of sperm into the tail of the epididymis also occurs in animals that have been vasectomised. In vasectomised rams, the epididymal tail can be nearly as large as the testis (Figure 30.39), where innumerable small sperm granulomata have formed. Surprisingly, this condition does not appear to be painful, and the libido of such vasectomised rams is normal.

Testicular hypoplasia. Testicular hypoplasia implies an incomplete development of the germinal epithelium of the seminiferous tubules, due to inadequate numbers of germinal cells within the testis. Lack of germinal cells may arise through

Fig. 30.37 *Actinobacillus seminis* infection of the head of epididymis of a 4-year-old Suffolk ram. Inspissated material was present in several large, abscess-like lesions (arrows) within the heads of both epididymes.

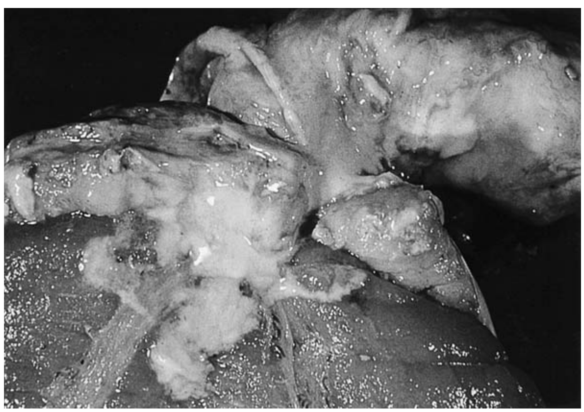


Fig. 30.38 Abscess-like sperm granuloma formation in the epididymal head of a Devon bull after rupture of the epididymal duct (reproduced from Parkinson et al., 1993, with permission).

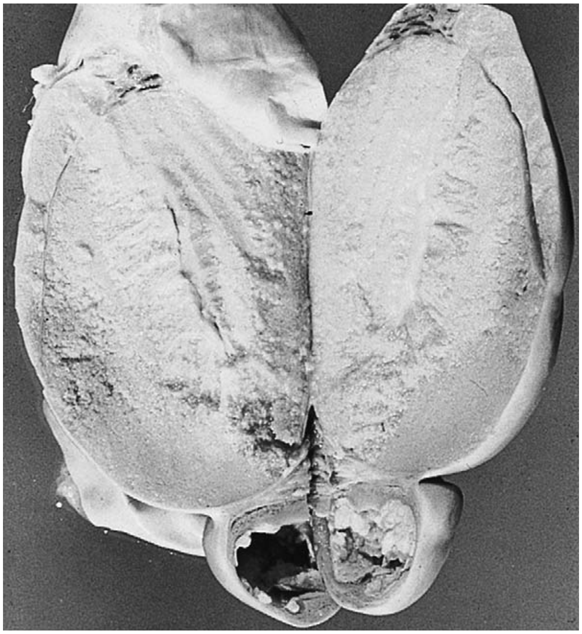


Fig. 30.39 Multiple sperm granuloma formation in the epididymal tail of a vasectomised ram.

partial or complete failure of the germinal cells to develop in the yolk sac, failure to migrate to the genital ridge, failure to multiply in the developing gonad, or widespread degeneration of embryonic germinal cells within the primitive gonad (Roberts, 1986). Mild cases may exhibit moderate oligospermia or poor sperm morphology, but severe cases may be aspermic. A hereditary form of hypoplasia exists in Swedish Highland cattle (Lagerlof, 1936, 1951; Eriksson, 1950), affecting the left testis more commonly than the right. Formerly, in the UK, many cases were detected when bulls were licensed at 10 months of age; however, the abandoning of such licensing means that the frequency of animals with relative hypoplasia is likely to increase. A high incidence of hypoplasia occurs in the Welsh Mountain pony (Arthur et al., 1989), in which the right testis is most commonly affected, and in which an inherited aetiology is probable. Sporadic cases of hypoplasia occur in all species, occasionally, but not often, with a clear familial predisposition (e.g. Gunn et al., Holst, 1949; 1942; Soderberg, 1986; Siliart et al., 1993). The condition (Figure 30.40) is relatively common in rams (Bruere, 1986).

Fig. 30.40 Testicular hypoplasia in a ram. The scrotal circumference of 22 cm was well below the 30–35 cm expected for a ram during the breeding season.

Klinefelter's syndrome (karyotype XXY) is a sporadic cause of testicular hypoplasia in bulls (Logue et al., 1979), and has been reported in rams, boars and dogs (Breeuwsma, 1968; Bruere et al., 1969; Clough et al., 1970). It is also particularly associated with male tortoiseshell and calico cats (Smith and Jones, 1966; Centerwall and Benirschke, 1975; Long et al., 1981). The spermatogonia of such animals fail to develop, so the seminiferous tubules are virtually devoid of spermatogenic cells. The semen of such animals is therefore aspermic, although the Leydig cells being unaffected, libido is normal.

Diagnosis of the condition is by measurement of scrotal circumference, a value below acceptable limits for the species and breed being diagnostic. Palpation of the testes reveals one or both to be small and flabby, but regular in outline and freely movable in the scrotum. Semen analysis may reveal aspermic or oligospermic ejaculates, sometimes with markedly abnormal morphology or motility characteristics of such sperm as are present. By contrast, libido is generally normal and, for this reason, the condition may escape the owner's attention until the failure to achieve satisfactory pregnancy rates is noticed.

Because of the probable inherited basis of testicular hypoplasia, attempting to breed from an affected animal should be avoided. Attempts at treatment with exogenous hormones are invariably unsuccessful, so castration and (for meat animals) slaughter for recovery of the carcass value should be recommended.

Testicular neoplasia. Testicular neoplasia (reviewed by Humphrey and Ladds, 1974; Roberts, 1986; McEntee, 1990; Schumacher and Varner, 1993) is rare in the bull, ram and boar and, although common in dogs, rarely presents as a cause of infertility. Interstitial cell tumours are the most common tumour of the dog, and are recorded occasionally as incidental findings in aged bulls. They are very rare in stallions. Seminomata, the next most common canine testicular tumour, are also occasionally found in bulls (and stallions), while Sertoli cell tumours rarely occur in species other than the dog. In cryptorchid stallions, a further testicular tumour is found relatively commonly: the teratoma, a benign growth that contains many different tissue types, including hair, bone, teeth and cartilage (Figure 30.41). Overall, testicular tumours account for over 10% of tumours in male dogs, with a considerably increased incidence in animals with cryptorchid testes.

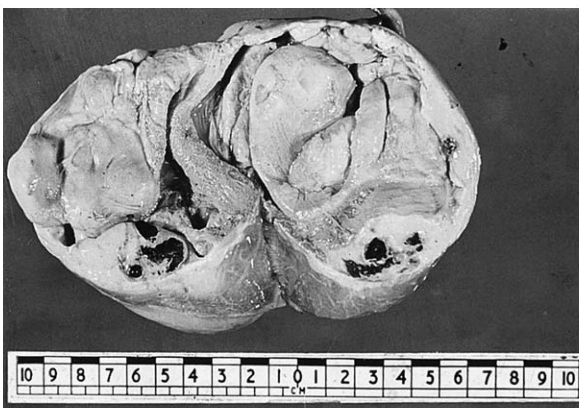


Fig. 30.41 Testicular teratoma in the undescended testis of a shire colt.

Interstitial cell tumours usually occur in scrotal testes of aged dogs, but are usually too small to be palpated. They may result in increased circulating concentrations of androgen and, thereby, predispose to androgen-related disease, such as prostatic hyperplasia and circumanal gland adenoma. In bulls, interstitial cell tumours may cause irregular texture of the testis and, sometimes, enlargement. They cause no clinical signs in bulls and no impairment to fertility.

The incidence of seminomata in cryptorchid dogs is about 20 times that of dogs with scrotal testes. Seminomas may become large but are generally innocuous in scrotal testes. They often grow slowly for long periods, but may undergo a sudden increase in the rate of growth, for no apparent reason. The tumours may become necrotic or haemorrhagic, whereupon affected dogs may exhibit lameness, pain, crouching or hunching. Occasionally they metastasise to local lymph nodes.

Canine Sertoli cell tumours are usually characterised by feminisation, in response to the tumour's oestrogen-secreting properties. Feminisation is typified by gynaecomastia, symmetrical alopecia, penile atrophy, a pendulous prepuce and attraction to male dogs, and occurs more often and to a greater extent if the neoplastic testis is inguinal or intra-abdominal than scrotal. If the tumour is unilateral, the contralateral testis is generally markedly atrophied. The oestrogenic secretion of the Sertoli cell tumour causes squamous metaplasia of the prostate gland, which may be of sufficient magnitude to cause an obstructive uropathy. Metastases are uncommon but, when they occur, they retain the oestrogen-secreting properties of the parent tumour.

Teratomata (Figure 30.41) occur most frequently in the stallion and very occasionally in other species. The tumour is most commonly found in cryptorchid testes, particularly of draught horses. They are either solid or cystic and may have many tissue types identifiable within their substance. Hair, bone, teeth, fat, cartilage and nerve tissue appear most frequently. The tumours can become very large, so that their removal may be quite difficult to achieve. However, they rarely metastasise.

In addition to their hormonal and malignant effects, tumours of undescended testes predispose

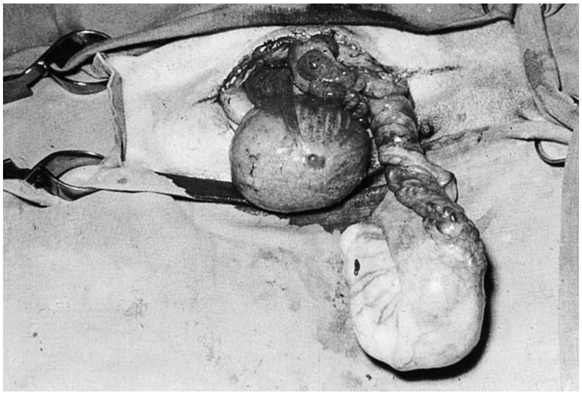


Fig. 30.42 Torsion of the spermatic cord of an undescended testis.

the spermatic cord to undergo torsion, which results in gradual testicular infarction. In exceptional cases, both spermatic cords may be tightly twisted through several rotations (Figure 30.42). Torsion of normal scrotal testes occurs occasionally (Young, 1979), but the condition is most frequently associated with neoplastic changes in undescended gonads (Pearson and Kelly, 1975). The susceptibility of such testes to tumour formation is therefore a strong justification for their removal.

Aplasia of the mesonephric ducts. Segmental aplasia of the mesonephric ducts (Blom and Christensen, 1951) is most commonly manifested as an absence of parts of the epididymis (Figure 30.43). In the bull, the condition is probably inherited (Konig et al., 1972).

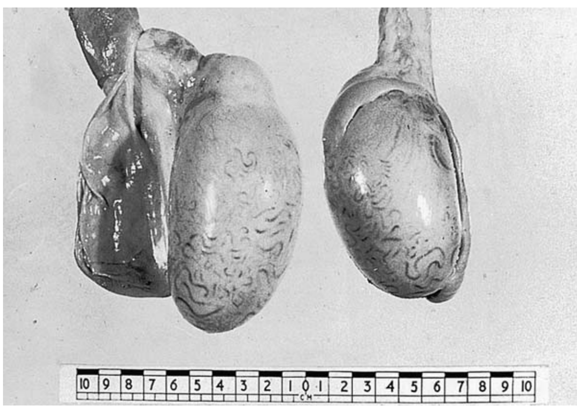


Fig. 30.43 Aplasia of the mesonephric ducts in the bull. Absence of the entire epididymis.

Absence of the head or tail of epididymis can be determined relatively easily by careful palpation of the scrotum, but the medially sited epididymal body is rarely palpable. Only in thin-skinned bulls and rams can it occasionally be felt; even then the scrotum must have little fat within it. Epididymal aplasia also occurs sporadically in other species (McEntee, 1990). Oligospermia occurs if one epididymis is aplastic, aspermia if both are affected. Parts of the epididymis distal to the site of obstruction are often enlarged and tense, but the testis undergoes degenerative atrophy. Stasis of secretory material within the epididymis and the possibility of sperm leakage into the surrounding tissue predispose to secondary pyogenic infection.

Aplasia of the vas deferens is less common. In bulls (Blom and Christensen, 1951), the condition may sometimes be diagnosed by rectal palpation, when strictures, or dilations distal to the site of stricture, may be discerned. As with epididymal aplasia, unilateral aplasia usually does not affect fertility, whereas sterility is the consequence of bilateral lesions.

Lesions of the accessory glands

Vesicular glands. Infection of the vesicular glands (seminal vesicles) is relatively common in bulls. Incidences range between 0.2 (Blom, 1979) and 9% (Bagshaw and Ladds, 1974), depending upon the country and class of bull in which the survey has been undertaken. Bacteriological examination of infected glands usually reveals the presence of *A. pyogenes*, although a wide variety of organisms, including *Corynebacterium renale, Actinobacillus actinoides, Escherichia coli, Pseudomonas aeruginosa*, streptococci and staphylococci, can be recovered. It is, however, unlikely that these are the causative organisms, for these are more commonly regarded as secondary infections of a previously damaged organ. Primary causative organisms may include *B. abortus, Chlamydia spp.*, and the Epivag, ECBO and IBR–IPV viruses. *Mycoplasma bovigenitalium* has also been implicated as a primary cause of vesiculitis (Al-Aubaidi et al., 1972). Seminal vesiculitis occurs most commonly in young bulls of less than 2 years old and in aged bulls. It has been suggested that the shape of the pelvis of individual animals predisposes them to the development of vesiculitis (Blom, 1979). Curiously, it is not a common disease of younger mature animals. The disease is rare in rams.

Infection of the vesicular glands also occurs in the stallion (Blanchard et al., 1988; Varner et al., 1993), from which a similarly mixed series of organisms have been isolated (*B. abortus, Klebsiella pneumoniae, P. aeruginosa*, streptococci and staphylococci). It may be one of the differential diagnoses of colic in the entire stallion (Freestone et al., 1993). Transrectal ultrasonography is a useful adjunct or, indeed, substitute for rectal palpation in the diagnosis of such cases. Treatment may be attempted with antibiotics (Freestone et al., 1993), although such therapy is by no means always successful (Blanchard et al., 1988), possibly depending upon the pharmacokinetics of the antibiotic selected.

In bulls, during the acute phase of the disease, localised peritonitis may occur in the caudal abdomen, producing the signs associated with that syndrome. One sign of localised peritonitis that itself affects reproductive performance is the unwillingness of affected animals to undertake movements that cause stretching of the area of inflammation. Foremost amongst such actions are mounting and, more particularly, the ejaculatory thrust. Hence, in the early stages of vesiculitis, animals may present with these signs. Later, animals generally present as being infertile, despite normal service behaviour. Occasionally, abscesses form in infected glands, which can burst, causing generalised peritonitis, or fistulate, generally into the rectum. Infection is more commonly unilateral, but may be bilateral.

The main consequence of infection of the vesicular gland is a decline in semen quality, which exhibits a decrease in motility, accompanied by elevated pH, low fructose concentrations and the presence of polymorphonuclear leucocytes. In moderate or severe cases, the semen may appear overtly purulent and may be tinged brownish, due to the presence of degenerating blood from the damaged gland. In most cases these changes in semen quality lead to a decrease in fertility, although cases have been reported in which affected bulls have maintained normal conception rates. Diagnosis of the condition can be confirmed by rectal palpation of the vesicular glands (Figure 30.44), which are characteristically enlarged, tense and painful during the acute phase, or lobular, fibrous and sometimes shrunken in the chronic phase.

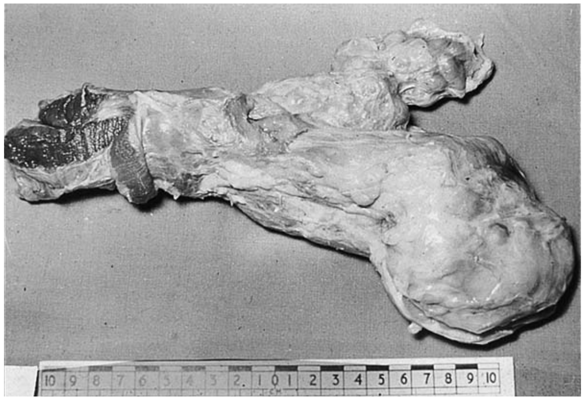


Fig. 30.44 Seminal vesiculitis in the bull.

Treatment is sometimes possible if the disease is noticed in its earliest stage, by the administration of very large intravenous doses of bacteriocidal antibiotics (Roberts, 1986; Arthur et al., 1989; Varner et al., 1993). However, in many cases antibiotic treatment is ineffective For such animals, amputation of a unilaterally infected gland is the only possible means of restoration of fertility (McEntee, 1962; King and McPherson 1969). In bilaterally infected animals, the prognosis is hopeless. No treatment should be attempted in animals infected with *Brucella*; instead the bull should be slaughtered. Hence, a very cautious prognosis should be given in the first instance for any bull with vesiculitis.

It would be natural to assume that a sire with an infection of the vesicular glands would transmit the infection venereally to females. Cases of such transmission have been reported in bulls infected with streptococci (e.g. Webster 1932), associated with herd infertility, a yellowish white post-coital discharge and pronounced cervicitis. However, other cases of streptococcal infection have had no associated signs in cows. Nevertheless, it would be prudent to advise that a bull with vesiculitis should not be used for service.

Prostate. Prostatic disease is rare in species other than the dog, in which prostatic infection and hyperplasia are common (Barsanti and Finco, 1986). Tumours and senile atrophy of the canine prostate are also rare. The route of infection is generally ascending, with *B. canis, E. coli, Proteus spp.* and *Streptococcus spp.* commonly being recovered. Prostatitis and prostatic hyperplasia often occur together, the prostate undergoing a diffuse or local suppurative reaction, with a tendency to abscess formation. Polymorphonuclear leucocytes, bacteria and blood are often found in the urine of affected animals. During the acute phase of the diseases, affected animals may show systemic signs of infection, constipation and abdominal pain, especially on digital examination per rectum. Prostatic hyperplasia is a common age-related change, with the gland forming numerous small, or a few large, cysts. Constipation, but not pain or signs of systemic illness, characterises this condition. Neither condition is typified by changes in fertility. Prostatitis may be treatable with broad-spectrum antibiotics, whereas hyperplasia, being androgen-dependent, is best treated by the administration of oestrogens or by castration.

ABNORMALITIES OF SEMEN

Semen examination

Semen can be collected by the methods described earlier in this chapter, and assessed so as to provide information about the fertilising potential of the ejaculate. Classically, assessment of the numbers of sperm, their motility and morphology, and the presence of extraneous material in the ejaculate, has been used to give an approximate indication of the quality of the ejaculate. However, while such assessments are adequate to identify ejaculates of low fertilising potential, correlations between the parameters of classic semen evaluation and conception rates of individual sires have been only mediocre. Hence, such assessments are a valuable part of the evaluation of potentially infertile sires but, if accurate predictions of fertility are required, more sophisticated examinations are needed.

Much care is needed in the handling of semen, if the results of its examination are to be meaningful. Spermatozoa are very sensitive to cooling, so the semen must be maintained at temperatures close to that of the body (above 30°C) prior to and during assessment, which should be undertaken as promptly as possible after collection. Furthermore, any microscope slides or material in which the semen is to be diluted must also be maintained at around 30°C. Semen is initially inspected for the presence of urine, fresh or changed blood, pus and extraneous material. The colour and consistency are noted, for watery samples are usually oligospermic, and samples that are not homogeneous often contain pus. Some normal bulls have semen that is bright yellow in colour, due to excretion of grass pigments into the seminal plasma.

Motility

Sperm motility is markedly influenced by temperature, so temperature control during this stage of semen examination is critical. Ideally, this is achieved by using prewarmed slides on a heated microscope stage, but in the field improvised methods are needed. The use of a small burner to warm the slides, or placing a small, flat-sided, clear glass bottle full of warm water on the microscope stage beneath the slide, have proved effective methods. Temperatures must also be kept below 50°C, above which sperm rapidly die. For the bull and ram, a drop of semen is placed on the slide and examined under low power. At low magnification, individual sperm cannot be seen, but mass sperm movement can be observed in the form of recurrent swirling waves. It is important to differentiate between true movement and the apparent movement exhibited by dead sperm that are being moved passively by living sperm beneath them. For other species, it is essential, and for the bull and ram desirable, that a small drop of semen, either neat or diluted (in warm 0.9% saline or 2.9% sodium citrate solution), is placed on a slide, covered and observed at higher power for assessment of individual sperm motility. Progressive, forward motility, with a characteristic swing of the head and tail, is the ideal, but other forms of motility are seen. Moderately damaged sperm may swim around in circles or backwards, while more severely damaged and dying sperm roll from side to side, alternately presenting the broad and narrow edges of their heads.

Sperm count

Ranges of sperm density and ejaculate volume are given in Table 30.3. For field use, sperm density

Table 30.3 Semen characteristics of domestic animals

	Species				
Characteristic	*Bull*	*Ram*	*Stallion*	*Boar*	*Dog*
Volume (ml)	4 (2–10)	1.0 (0.5–2.0)	60 (30–250)	250 (125–500)	10 (2–19)
Fractionated	N	N	Y	Y	Y
Density (× 10^6/ml)	1250 (600–2800)	2000 (1250–3000)	120 (30–600)	100 (25–1000)	125 (20–540)
Motility (motile sperm, %)	> 70	> 90	> 60	> 60	> 85
Normal spermatozoa (%)	> 75	> 85	> 60	> 60	> 90

Figures in parentheses indicate the normal range. Compiled from Arthur et al. (1989), Roberts (1986) and Morrow (1986).

can be estimated most readily using a haemocytometer. Ram and bull semen should be diluted 1:100 in 0.9 saline/0.02% formalin solution; other species, whose semen is less dense, may require lower dilution factors. The total sperm count is then derived as the product of volume and density. Where a large number of semen samples require evaluation, such as occurs in AI studs, estimation of sperm density can be facilitated by the use of spectrophotometry, in which the optical density of the sample is compared with a calibration curve (Salisbury et al., 1943). Alternatively electronic particle counters can be used, although the small size and flattened shape of the sperm head make it a relatively difficult cell to count.

Live: dead ratio and sperm morphology

A further estimate of the proportion of dead sperm in an ejaculate can be obtained by the use of a vital stain, such as eosin B (Lasley et al., 1942). This stain is most commonly used as part of a combined stain, eosin–nigrosin, which is used to evaluate both the proportion of dead sperm and sperm morphology (Swanson and Bearden, 1951). For vital staining to be effective, great care has to be taken of temperature control, and conditions must be standardised. Semen that has been frozen is difficult to assess with eosin, as cryoprotectants, such as glycerol (Mixner and Saroff, 1954), enhance penetration of the vital stain into the cells, thereby giving artificially high percentages of dead cells. Also, until considerable experience has been obtained, repeatability of live: dead ratio counts is low.

Assessment of sperm morphology is, by contrast, a useful and important aspect of semen examination. Nigrosin, a simple background stain, is adequate for most purposes, but specialist sperm stains, such as aniline blue plus eosin B (Casarett, 1953), are also widely used. Defects of the acrosome are often difficult to see in stained preparations, although specialised stains such as that of Wells and Awa (1970) are used to visualise acrosomal vacuoles. More commonly, phase contrast or differential interference contrast microscopy of wet preparations is used to examine acrosomal defects (Aalseth and Saacke, 1985).

Sperm function tests

Semen analysis provides enough information to recognise sires of very low fertility, but has been increasingly considered to be a poor discriminator between moderate and high fertility levels (Watson, 1990). In order to attempt to improve the accuracy of semen assessments, a number of tests of sperm function have been employed, with varying success. The simplest of such tests incubate semen at various temperatures (typically 4 or 40°C) and, by relating the duration of sperm survival under these conditions to survival in the female genital tract, produce reasonable correlations with fertility (Roberts, 1956). Other tests utilise additional measurements upon the semen, such as pH, adenosine triphosphate (ATP) content or aspartate transaminase concentration (Salisbury et al., 1978). These have been moderately successful, but have not been of sufficiently greater value than conventional semen assessment to justify their use. In medical practice, much value is placed upon the ability of sperm to penetrate cervical mucus and the behaviour of the sperm at the interface between semen and mucus (Linford, 1974; Blasco, 1984). Failure of mucous penetration is frequently a sign of failure of sperm function and occurs in sperm that have been damaged by cryopreservation or in the presence of anti-sperm antibodies.

Of more widespread use in veterinary practice is computer-assisted analysis of sperm swimming characteristics. In medical practice, such analyses are regarded as a useful prognostic tool in assessment of fertility because high correlations have been demonstrated between such measurements and fertility. The most important swimming characteristics are rate of forward progress, lateral movement of the sperm head and characteristics of the flagellar beat. Although the use of sperm motility analysis in veterinary practice at present is largely confined to thoroughbred stallions (Amann, 1988) and AI stud bulls (Budworth et al., 1988), it is probable that the use of such systems will rapidly increase as the cost of analysis programs decreases.

Assessments of sperm viability have also been improved in recent years. Fluorescent markers that stain live, but not dead, sperm have been used and high correlations with fertility demonstrated (Garner et al., 1986). Assessment of the proportion of sperm with intact acrosomes has been highly correlated with fertility (Saacke, 1972). The most recent innovation in assessment of sperm function has derived from the development of in vitro fertilisation (IVF) procedures, in which sperm from different sires were observed to have widely differing fertilisation success rates. Subsequently, the ability of sperm to undergo acrosome reaction in vitro was identified as a critical stage in the IVF procedure and, in the bull, tests of sperm function based upon in vitro induction of acrosome reactions have been found to have very high correlation with fertility in the field (Ax and Lenz, 1987; Whitfield and Parkinson, 1994; Whitfield and Parkinson, unpublished data; Figure 30.45).

Abnormalities of spermatozoa

Three main classifications of sperm morphology have been proposed. Firstly, defects can be classified according to their site on the sperm. By this classification, sperm are classified into head, midpiece and tail defects and sperm bearing protoplasmic droplets. A rather more useful classification is that based upon the site within the genital tract where the sperm defect has arisen

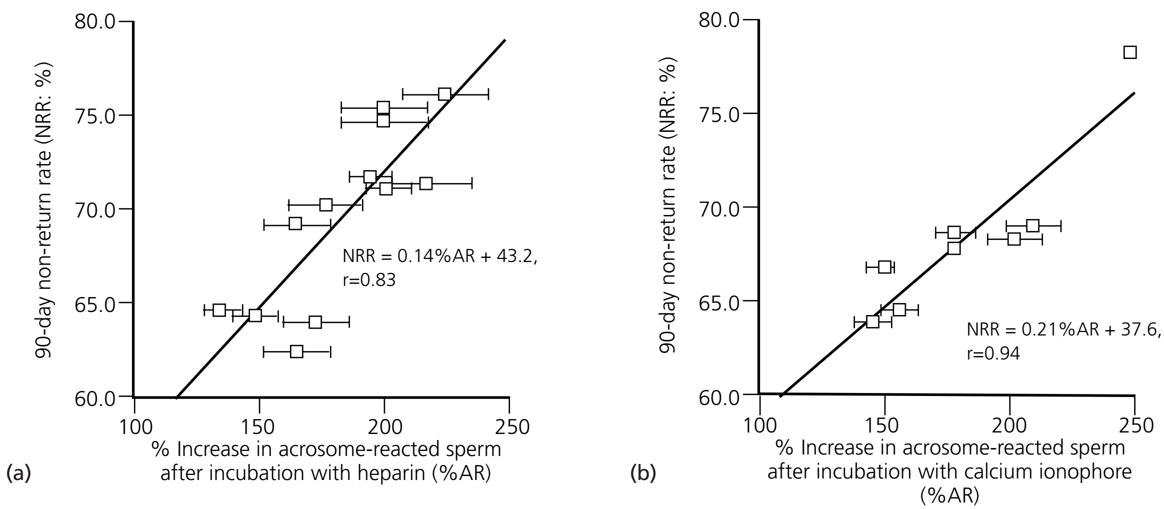


Fig. 30.45 Relationship between acrosome reactions induced in bovine semen in vitro by (a) heparin and (b) A23187 and fertility, as expressed by the proportion of cows not represented for service 90 days after AI (90 day non-return rate, or NRR).

(Blom, 1950). By this classification, defects are divided into primary abnormalities, which arise during spermatogenesis, secondary defects, which arise within the epididymis, and tertiary defects, which arise after ejaculation (e.g. from inadequate temperature, pH or osmotic control during handling of the semen). Thus, defects of the head and midpiece are mostly primary, protoplasmic droplets secondary and looped tails tertiary. The final classification (Blom, 1983; Figure 30.46) categorises defects, according to empirical observations upon their effects on fertility in the bull, into major and minor abnormalities. Major abnormalities include most defects of the head, proximal protoplasmic droplets and congenital acrosomal defects, while most other defects, including, somewhat surprisingly, detached heads, are classified as minor abnormalities.

Using the principles of the effect of specific abnormalities upon fertility, criteria have been established for maximal percentages of each class of sperm abnormality in an ejaculate. In the UK, a maximum of 20% total sperm abnormalities, with not more than 5% of any individual class, is allowed in bovine semen for use in AI. In bull studs in the USA, a maximum of 10% major abnormalities or 20% minor abnormalities is allowed. However, in bulls destined for use in natural service, different criteria would be applied, which may need to take into account the frequency of use of the sire and the use to which its progeny (i.e. slaughter or breeding) are to be put. In other species, the criteria for acceptance of semen are also different. For example, equine, porcine and canine semen can exhibit quite high percentages of abnormal sperm without materially affecting fertility, whereas in the ram only a very low percentage of abnormalities is acceptable.

Abnormalities of the sperm head

Two aspects of the morphology of the sperm head appear to be essential for normal fertility. Firstly, the shape of the sperm head is critical, as small changes in the overall size, acrosomal area and width at the base of the head markedly reduce the ability of sperm to fertilise (reviewed by Barth and Oko, 1989). Secondly, the morphology and stability of the acrosome are also important. Therefore, most abnormalities of the sperm head are major defects, i.e. having relatively serious effects upon fertility (Blom, 1950, 1980; Wilmington, 1981). The majority of such defects arise within the testis as abnormalities of spermatogenesis (primary abnormalities). Such defects include heads that are narrow at the base, pear-shaped, small and misshapen, and grossly abnormal and bizarre (Figure 30.47). Less serious defects of the head include giant heads (which have a diploid chromosome complement), double heads, narrow heads and small, normally shaped heads. The diadem defect (Figure 30.47(c)) represents pouches in the nuclear material. This defect is common at low percentages, may be present at high percentages for short periods of time after testicular damage, or may be continuously present at high percentage as an inherited defect (Barth and Oko, 1989).

Acrosomal defects are also of serious consequence for fertility. Many acrosomal defects arise as primary abnormalities of spermatogenesis, although acrosomal damage may also arise during epididymal transit and storage, or even after ejaculation. Many of the acrosomal defects that arise during spermatogenesis are present at a high percentage in the ejaculate, in which case they are usually inherited, but identical abnormalities can be found at low percentages in most ejaculates, indicating that they can also arise spontaneously. Furthermore, the significance of these defects depends upon the species. For example, the fertility of bulls is impaired by single-figure percentages of the knobbed acrosome defect, but percentages have to be much higher before the fertility of stallions or boars is affected. However, in general, all defects of the acrosome should be regarded as serious, and careful consideration given to the likelihood of inheritance of the condition before use of the sire is sanctioned. Defects of the acrosome can be difficult to see in stained preparations, so the use of phase contrast or differential interference microscopy upon wet smears is often needed. Some acrosomal defects can be seen if smears are stained with nigrosin alone, while others can be readily observed when the stain of Wells and Awa (1970) is used. Acrosomal defects with a suspected or known heritable basis include the knobbed sperm defect and the presence of vacuoles in the acrosome, whereas

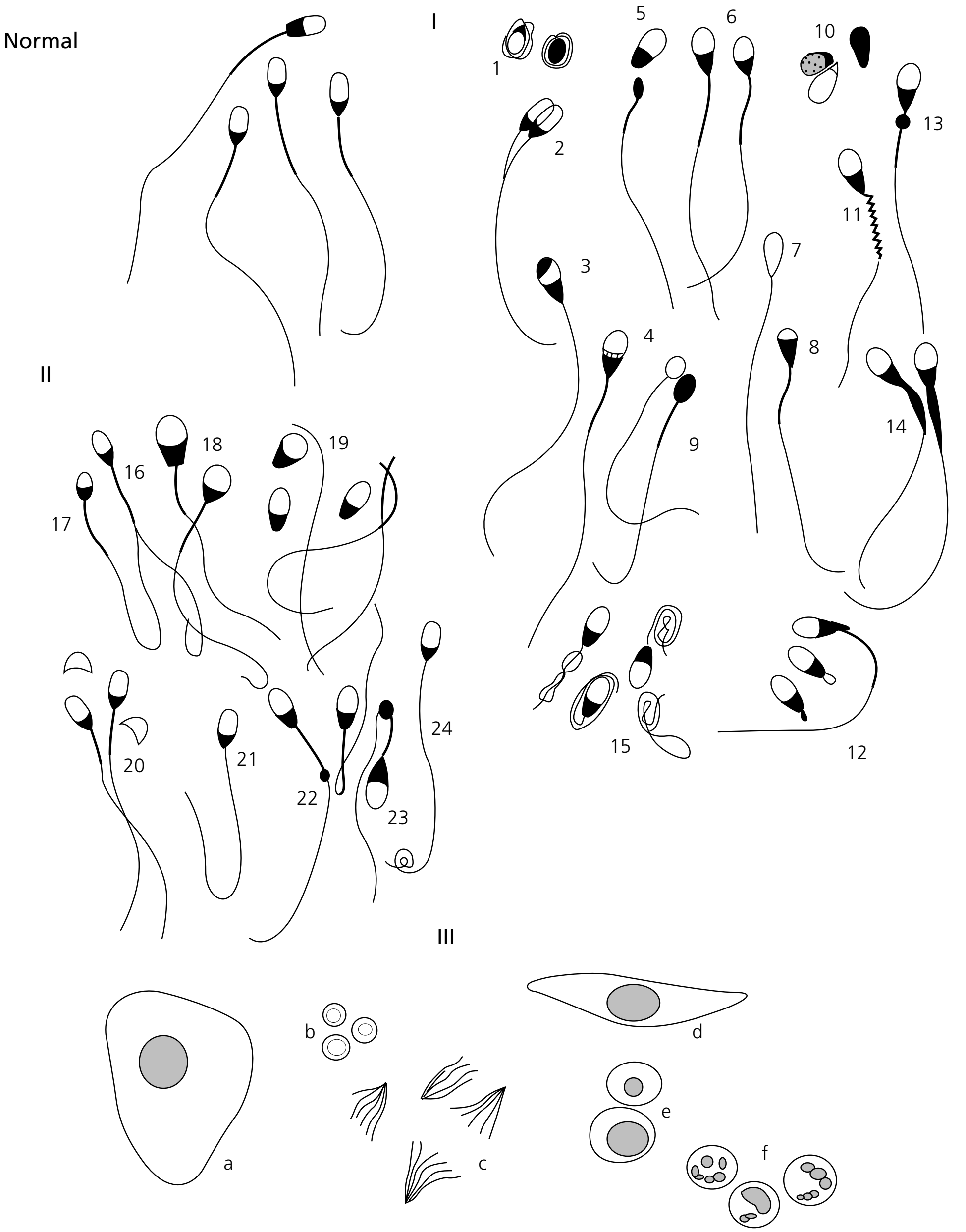
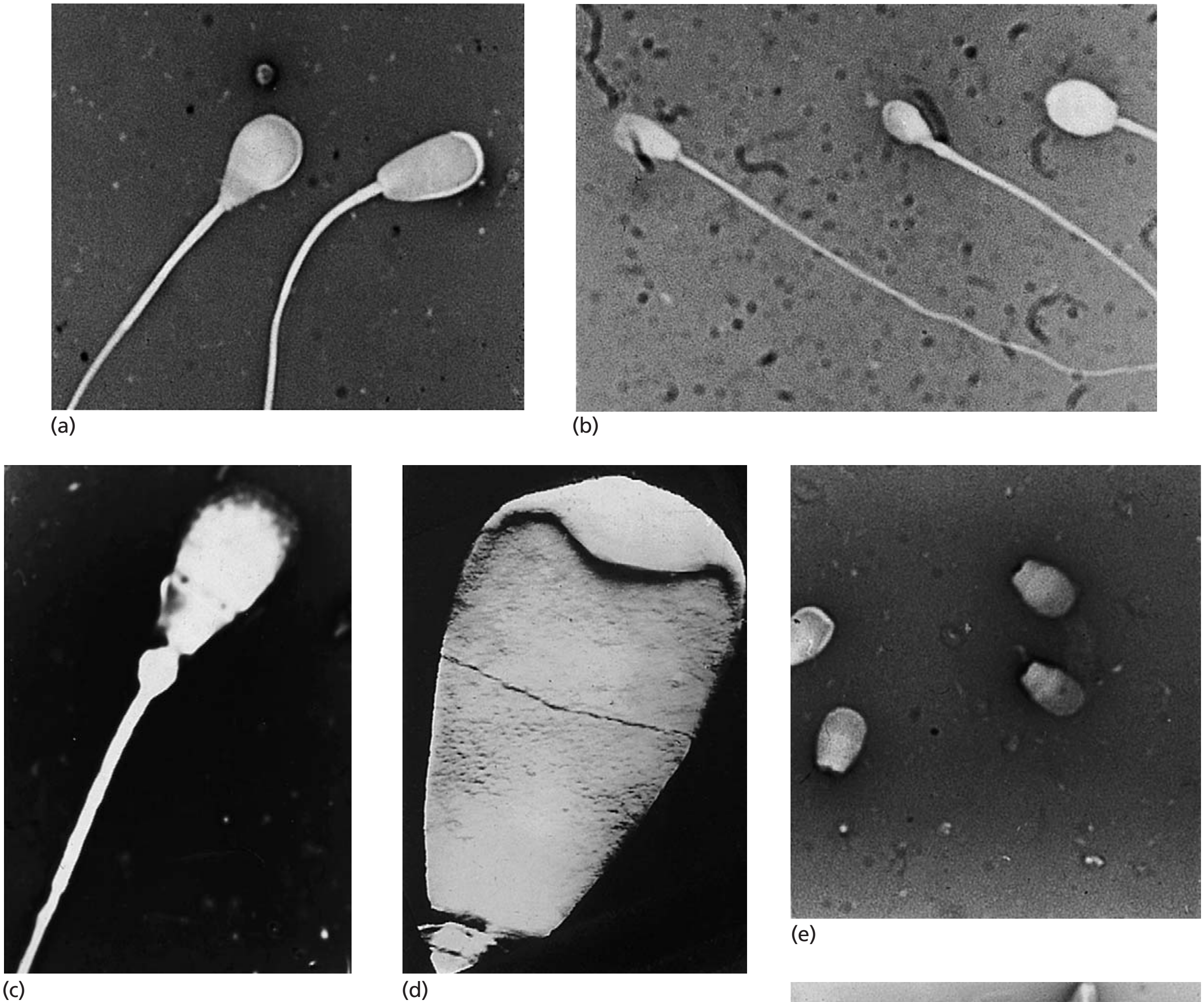


Fig. 30.46 Classification of spermatozoal abnormalities into major and minor defects according to their effect upon fertility. Major defects (I) include: 1, underdeveloped cells; 2, double forms; 3, acrosome ('knobbed sperm') defect; 4, diadem defect; 5, decapitated sperm defect (the tails appear active); 6, pear-shaped heads; 7, heads that are narrow at the base; 8, heads with an abnormal contour; 9, small abnormal heads; 10, free (detached) abnormal heads; 11, the 'corkscrew defect' of the midpiece; 12, other midpiece defects; including the 'tail-stump' defect and accessory tails; 13, proximal cytoplasmic droplet; 14, pseudodroplet and other thickened midpieces; 15, coiled or strongly folded tails (including 'Dag defect').

Minor defects (II) include: 16, narrow heads; 17, small, normal heads; 18, giant and short, broad heads; 19, detached normal heads; 20, detached acrosomal membranes; 21, abaxial implantation of the tail; 22, distal droplet; 23, simple bent tail; 24, terminally coiled tail.

Other cellular elements that may also be present (III) include: a, epithelial cells; b, erythrocytes; c, medusa formations; d, boat cells; e, mononuclear cells; f, neutrophils (redrawn and adapted from Blom, 1983, with permission).



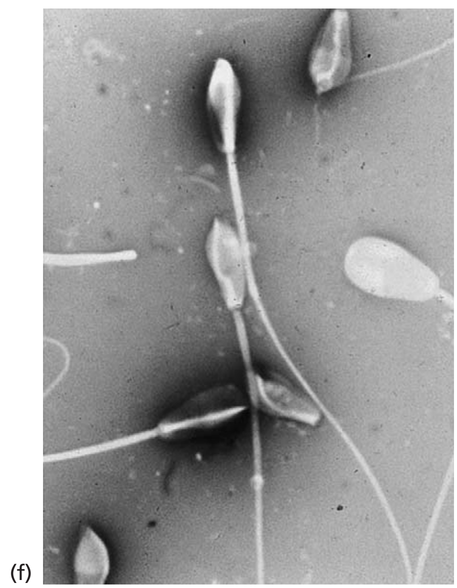


Fig. 30.47 Defects of the sperm head. (a) Pear-shaped head, (b) dwarf and giant heads, (c) 'diadem' defect, (d) 'knobbed sperm' defect, (e) detached normal heads and (f) abnormal heads.

simple ridges on the acrosome may be inherited or acquired. Acrosomal morphology may deteriorate preceding the appearance of major head abnormalities in cases of testicular degeneration, orchitis or epididymitis, so repeated evaluations of the semen may need to be undertaken.

Detached acrosomes may be observed in wet or nigrosin-stained smears, although observation under phase contrast or differential interference contrast microscopy offers the best means of evaluating the acrosome. The site of origin of such defects is not always clear, for this abnormality may arise at any time between spermatogenesis and insemination. In particular, it may be seen in frozen–thawed semen of animals whose sperm do not survive cryopreservation well. At low percentages, detachment of the acrosome has been regarded as a minor defect, with fertility only being impaired with higher levels of the abnormality. However, recent data from the bull indicate that the percentage of detached acrosomes may be related in a linear fashion with fertility, and therefore, the significance of this abnormality is being reassessed.

Abnormalities of the midpiece and the tail and of attachment of the head

Abnormalities of attachment of the sperm head are, generally, primary defects of spermatogenesis. Some are inherited defects of the centriole or axoneme, occurring at high percentages, while others are sporadic or occur as acquired defects. Surprisingly, many such defects have minor effects upon fertility, unless present at high percentages. Detached heads are generally an acquired defect, occurring particularly during testicular degeneration. The separated tails are usually immotile. However, an inherited condition of Guernsey and Hereford bulls (Blom and Birch-Anderson, 1970; Blom, 1977) occurs, in which most sperm are decapitated and the detached tails are motile. The semen of such bulls exhibits apparently normal wave motion. Detached heads may be present in the semen of animals that have not ejaculated for a considerable period of time, as a senescent change in the sperm. It is also relatively common in the semen of aged bulls. Sperm with fractures of the attachment between head and tail ('fractured neck') may arise from senescent changes, or due to congenital weakness of the attachment.

Abaxial implantation of the tail is generally of minor significance (Bishop and Hancock, 1955) and some degree of abaxial implantation may be regarded as normal in the stallion. Some bulls with abaxially implanted tails exhibit a curious, additional, vestigial tail (Figure 30.48(d)) beside the main flagellum, which causes a serious impairment of fertility if the abnormality is present in a high percentage (Williams and Savage, 1925). Most other defects of development of the midpiece and tail are of serious consequences for fertility, deformity of the tail precluding motility. The coiled tail defect (Figure 30.48(a)) is a primary abnormality that is commonly found during testicular degeneration. The somewhat similar 'Dag' defect (Blom, 1966) is usually of inherited origin, especially in Jersey bulls, in which it is relatively common. It has also been seen sporadically in most other domestic animals, either as a permanent defect – in which case it is probably inherited – or transiently, as a response of the testis to some insult. The apparently loose coils of the sperm tail in the Dag defect represent a serious perturbation of the genesis of the flagellum (Figure 30.48(b)), resulting in immotile sperm. The 'tail-stump' defect occurs as an inherited condition of several breeds of bull (Blom and Birch-Anderson, 1980), in which morphologically normal heads are attached to a vestigial structure that appears like a protoplasmic droplet (Figure 30.48(f)). On electron microscopy, this droplet-like structure can be seen to consist of small segments of flagellar material and represents a vestigial tail. Affected bulls are sterile.

Other less spectacular, but nevertheless serious, defects of the midpiece occur. These include the corkscrew defect, so-called because the loose arrangement of the helix of mitochondria gives the appearance of a corkscrew to the midpiece of the sperm; it may be inherited when present at high percentages. Various thickenings of the midpiece also occur that arise from other malformations of the mitochondrial helix.

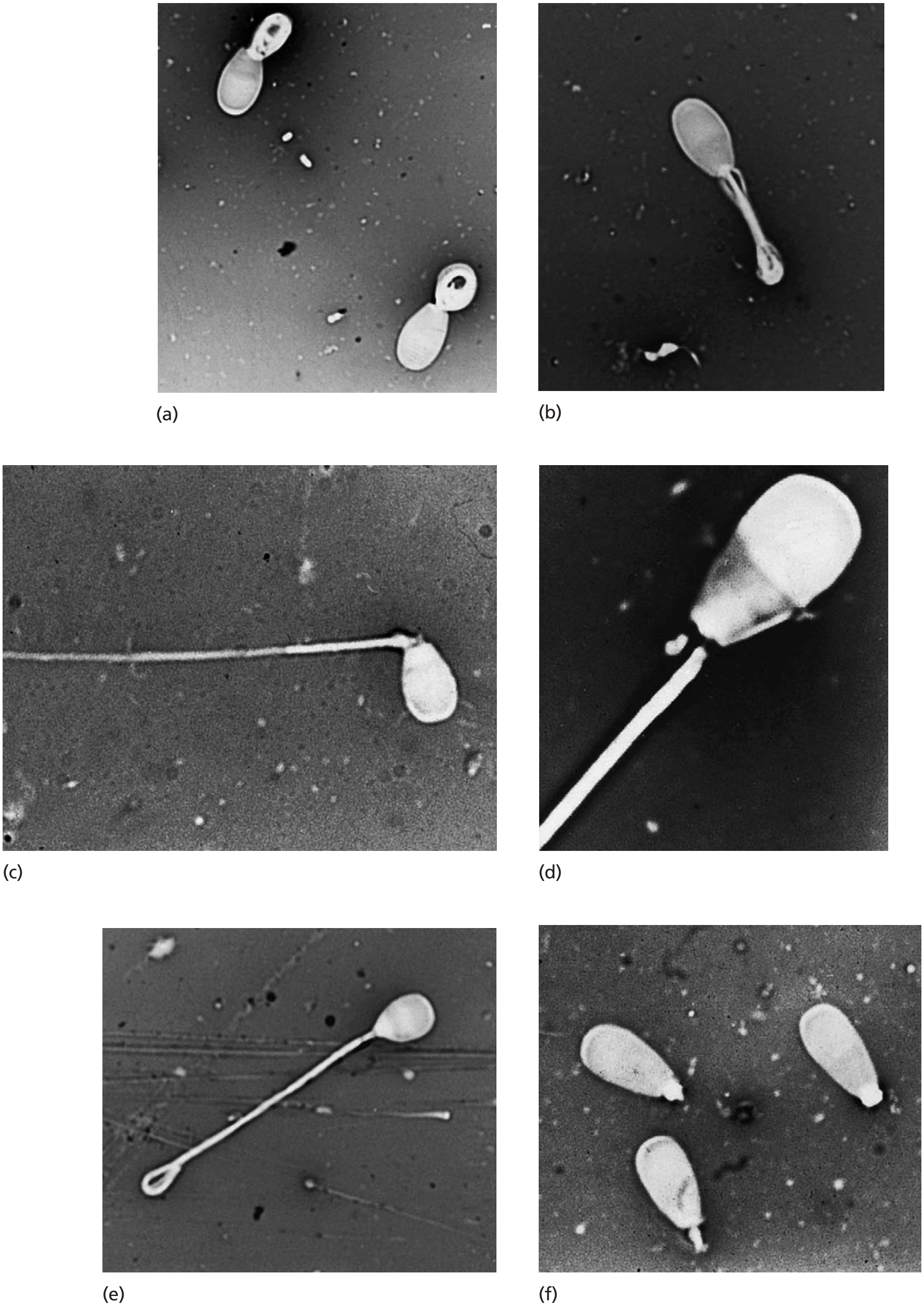
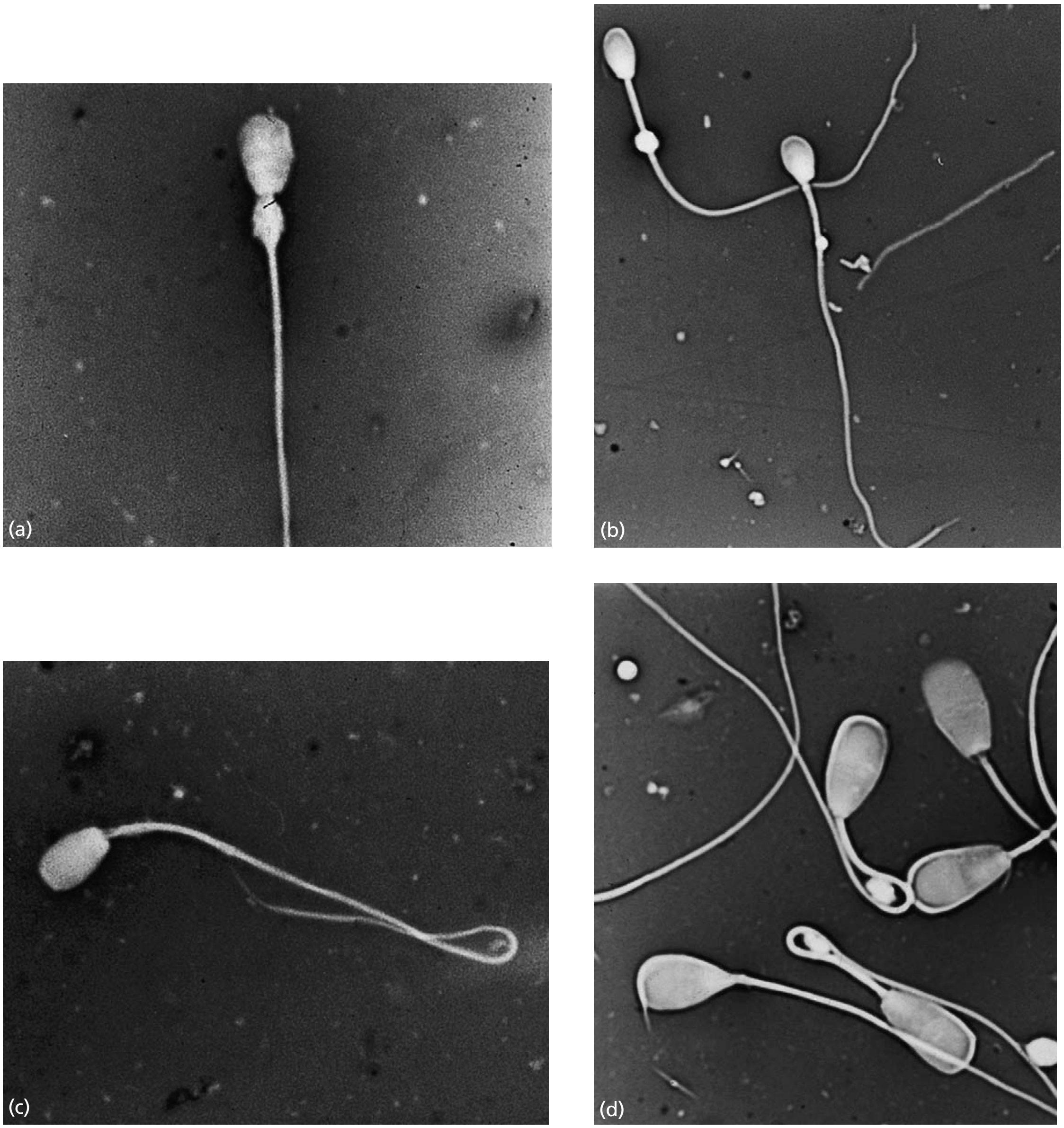


Fig. 30.48 Defects of the sperm midpiece and tail (1). (a) 'Coiled-tail' – a defect of formation of the midpiece, (b) 'Dag' defect, (c) fractured neck, (d) accessory tail, (e) terminally coiled tail and (f) the tail-stump defect.

Tail defects are, by contrast, generally minor defects. These include looped tails and terminally coiled tails. Care should be exercised in the interpretation of the presence of looped tails, for looping of the tail is a common response of sperm to noxious stimuli. Thus, while looped tails can arise as defects of spermatogenesis or epididymal function, they occur more commonly in response to poor temperature control of the semen, or in response to hypotonic stress such as may occur if the semen becomes contaminated by water. Departure of seminal pH from its normal range can also cause looped tails and, as such, may be an early indicator of the increase in pH that occurs during infection of the accessory glands.

Protoplasmic droplets

The residual cytoplasm that remains at the end of spermiation is removed during the passage of



sperm through the epididymis, as a maturational change. The presence of sperm with protoplasmic droplets (Figure 30.49 (a) and (b)) therefore indicates that epididymal maturation is incomplete by the time of ejaculation. Sperm with droplets close to the head (proximal droplets) are more immature than those with droplets at the distal end of the midpiece (distal droplets), although it has recently been argued that proximal droplets also arise as defects of spermiation (i.e. as a primary abnormality).

Protoplasmic droplets are often observed in sires that are being overused. In young animals, daily sperm production rates are lower than in fully mature animals and, in addition, the epididymis has not fully developed to its final length. Hence, if a young sire is overused, not only does the number of sperm in the ejaculate decline, but also the withdrawal of sperm from the tail of the epididymis means that the sperm that are ejaculated are often functionally immature. The fertility of such animals therefore can decline spectacularly. Where young sires are heavily used, such as in AI programmes, careful monitoring of the percentages of sperm with protoplasmic droplets is therefore advisable.

Semen changes during testicular degeneration

The initial changes in semen quality during testicular degeneration are a decrease in motility and an increase in the percentage of abnormal sperm (see Figure 30.33), particularly sperm with proximal droplets. If the semen is being cryopreserved, a precipitous decline in post-thaw motility may occur at this stage. Subsequently, sperm numbers generally start to decline, although ejaculate volume is usually unaffected. As sperm numbers decrease, the proportion of abnormal sperm increases, with high percentages of primary defects occurring (Figure 30.50). These include abnormalities of the head, detached heads and coiled tails. Bizarre abnormalities occur, including small, abnormal heads, acrosomal defects and the presence of premeiotic cells and stellate forms in the ejaculate. Sperm numbers may decline to the extent that the ejaculate becomes virtually aspermic.

During recovery, sperm morphology and motility tend to improve before sperm numbers. The percentage of sperm with distal droplets frequently increases during the recovery phase. Recovery may occur almost immediately after the nadir of semen quality, but may be protracted. The extent and severity of semen changes cannot be correlated with either the duration of illness or the likelihood of recovery.

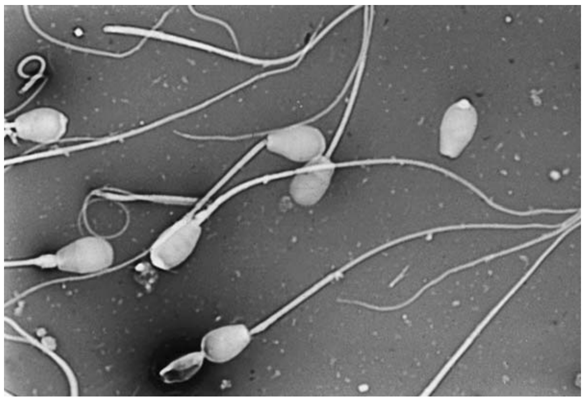


Fig. 30.50 Sperm morphology from a bull with severe testicular degeneration. Many abnormal cells are present, including sperm with abnormal heads, detached heads, defects of the midpiece and sperm with proximal droplets. The ejaculate was also characterised by oligospermia and low sperm motility.

Fig. 30.49 Defects of the sperm midpiece and tail (2). (a) Proximal cytoplasmic droplet, (b) distal cytoplasmic droplet, (c) looped tail and (d) looped tail with a cytoplasmic remnant enclosed in the loop.

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31 Artificial insemination

The successful use of artificial insemination (AI) as a means of animal breeding relies upon three major premises: firstly, that spermatozoa can survive outside the body; secondly, that they can be reintroduced into the female genital tract in a way that results in an acceptable conception rate; and thirdly, that the fertile period of the female can be identified.

The degree to which these underlying premises can be fulfilled dictates, to a large degree, the success with which AI can be applied to an animal species. For example, in cattle, the spermatozoa can (after cryopreservation) be preserved outside the body almost indefinitely. A technically straightforward intrauterine insemination means that the number of spermatozoa for each insemination dose is low; hence, each ejaculate can be used for breeding many females. The conception rates thereby achieved are identical to those of natural service, while the oestrous behaviour of cows means that detection of the fertile period is not difficult. Hence, in this species, in which all three premises are fulfilled, the use of AI is widespread. Conversely, in many other species, where one or more of the premises are less adequately fulfilled, AI is less successful and, therefore, less widely used.

AI regimes have been developed for most domestic and many semi-domestic species. It is routinely practised in cattle, sheep, pigs, goats, fowl, turkeys, salmon and trout, and is used in dogs, domestic foxes, buffalo, horses and even bees. Of these, cattle and sheep/goats account for the vast majority of mammalian inseminations. The use of AI in turkey breeding is essential, as natural mating is not possible in this species, so that very large numbers of inseminations are performed. AI in salmonid farming is also very widespread. The use of AI in pigs has been surprisingly low, with estimates of around 9% of the national herd being typical for Western Europe and the USA (Iritani, 1980). The discussion of AI in this chapter will be limited to the major domestic mammals.

ADVANTAGES AND DISADVANTAGES OF AI OVER NATURAL BREEDING

Artificial insemination offers several potential advantages over natural service. Of these, the reason most commonly advocated is as a means of genetic improvement. In most food-producing animals, each ejaculate can be divided into many insemination doses, such that each AI sire can potentially be used to breed a very large number of females. Hence, the total number of sires needed is reduced. In consequence, the selection intensity that can be applied to the male side becomes very much greater than for natural service. In dairy cattle, only the best 1% of cows are selected as potential bull mothers, and only about the best 1% to 3% of their male progeny eventually become sires of the next generation. In beef cattle and pigs the selection intensity is not quite so great but, nevertheless, very much more intense than can be achieved in natural breeding.

Direct genetic selection of sires is not, however, the most widely used application of AI for achieving genetic improvement. More common is the use of AI to allow rapid dissemination of new breeds. In the UK, AI was one of the main means whereby the Friesian breed of cattle displaced the indigenous British dairy breeds. Subsequently, AI has also been the means by which the Friesian has been displaced by the Holstein. In such breed substitution programmes, AI can be used to change the gene pool of a national herd rapidly, a technique that is also used for upgrading unimproved cattle in remote areas. In this process, AI has the advantage of being both cheap and simple, for local distribution of extended and chilled semen from small numbers of imported sires is within the economic capabilities of even the poorest countries.

International trade in livestock is also facilitated by AI. Improved stock can be imported in the form of semen for AI, rather than having to move animals themselves. By this means, many of the problems of acclimatisation, lack of resistance to local diseases, etc., can be eliminated. Importing semen also allows the importing country to exert a far greater level of effective control over the health status of the donor sires than if the livestock itself were imported.

The second major advantage of AI is the reduction of the numbers of sires that individual farmers need to maintain. The males of agricultural species generally require accommodation in which they can be segregated from the breeding females, so that breeding can be controlled, often in buildings, which also preclude, as far as possible, injury to farm staff. The significant housing and labour costs involved with keeping such animals can therefore be obviated by the use of AI; moreover, farmers generally have access to genetic material through AI centres, which would be far beyond their pockets to buy outright.

The third major advantage to AI is the control of venereal disease. A major impetus to the development of cattle AI in the UK during the 1940s was the need to control the epizootic venereal pathogens *Trichomonas fetus* and *Campylobacter fetus*. In the UK, in common with most countries in which bovine AI was introduced in the face of trichomoniasis and campylobacteriosis, these pathogens were virtually eliminated by the use of AI (see Chapter 23). However, the converse is also true: namely, that uncontrolled use of sires in AI can disseminate disease. Many diseases are transmissible through semen, including not only the classic venereal diseases, but also other conditions that would not generally be regarded as primarily venereal (Roberts, 1986). Rigorous monitoring of the health of AI donor sires is therefore regarded in many countries as an integral part of national disease control programmes.

Nevertheless, although AI carries many advantages over natural breeding, the technique is not without drawbacks. Detection of the fertile period in the female oestrous cycle is potentially the most problematic aspect of AI programmes. In cattle, the prominent homosexual behaviour of oestrous females allows relatively accurate human identification of the fertile period, but in most other species its detection is less easy. In such species, detection of oestrus therefore requires the presence of infertile (e.g. vasectomised) males, or the timing of oestrus must be controlled by pharmacological (e.g. oestrus synchronisation/induction regimens) or managemental (e.g. timing of weaning in sows) procedures. Thus, for ewes, which do not normally display any signs of oestrus in the absence of a male, AI requires either the presence of vasectomised rams to detect oestrus, or pharmacological manipulation of oestrus to define the timing of the fertile period. Hence, detection of the fertile period of the ewe is, to a greater or lesser extent, a costly procedure, thereby detracting from the appeal of AI in that species. It may therefore be considered that an economic 'trade-off' exists in such species, between the genetic advantages conferred by the use of superior AI sires on one hand and the costs of maintaining teaser males or pharmacological manipulation on the other.

Once oestrus has been identified, the female animal has to be restrained for insemination, which generally requires separation from the herd and holding in specialised pens. The process of insemination also requires trained personnel, which may require a limited degree of technical proficiency, as is the case in insemination of sows, or may be demanding, as in the case of laparoscopic intrauterine insemination of ewes.

It is also necessary to log insemination dates in an adequate recording system, in order to allow birth dates to be calculated and so that expected dates of return to oestrus are known, thereby allowing appropriate observations to be made. Secondly, the identities of the sires need to be recorded (and their pedigrees known) to avoid inbreeding. Some form of positive pregnancy diagnosis is advantageous, especially where males are not present in the herd, to ensure that non-returns to oestrus signify pregnancy rather than anoestrus.

The value of AI as a rapid means of transmission of the genes of superior sires has already been identified. However, a corresponding disadvantage exists: namely, that genetic faults can also be widely disseminated if they are present in an AI sire.

Dominant traits should rarely be transmitted in this way, but recessive traits may be very widely transmitted, especially if the recessive gene is present in the general population at such a low incidence that many breedings may have to be performed before the condition is expressed in a homozygous progeny. Hence, AI programmes should be underpinned by an efficient reporting system for monitoring abnormalities in the progeny, with clearly defined criteria for the withdrawal from use of sires that carry deleterious genes. For example, in cattle, achondroplasia is transmitted as a simple recessive gene (Marlowe, 1964) that, when present in the homozygous condition, causes failure of long bone development, resulting in the birth of so-called ‘bulldog’ (achondroplastic) calves (see Chapter 4). The incidence of this gene in the general cattle population is so low that the birth of one or two calves with this deformity is regarded as sufficient reason to slaughter the bull and withdraw all stocks of its semen. Spastic paresis is similarly transmitted and is dealt with in a similar manner (Keith, 1981). However, other defects may be less readily appreciated as such and may even result from breeding programmes. For example, the high incidence of dystocia in Friesian cattle has resulted from the selection of sires producing progeny with a level pelvis, which has also caused a lengthening of the pelvic canal (see Chapter 11). Likewise, an individual Canadian Holstein bull that was popular in the UK in recent years produced progeny with very straight hindlegs, considered desirable at the time, but many of which later developed severe hock malconformation. A further concern, which has frequently been expressed but has yet to prove of major impact, is the reduction in the gene pool of highly selected breeds. For Holstein cattle, concern has been expressed that the number of bloodlines from which sires are drawn is becoming progressively reduced; yet no unequivocal evidence of inbreeding depression has been identified in the breed so far.

PREPARATION OF SEMEN FOR USE IN AI

The methods for collection of semen from domestic mammals are described in Chapter 30. In most AI regimes, semen evaluation is limited to measuring sperm numbers, motility and, usually, morphology. More sophisticated analyses may be used in determining whether an individual sire produces semen of a sufficiently high quality for acceptance into an AI programme, but such evaluations are rarely carried out on day-to-day collections of semen. Unless the semen is to be directly inseminated without delay into a single female, it is then diluted and either cooled or frozen. Direct inseminations are performed most commonly in the bitch, usually in response to some incapacity of the sire that precludes normal mating (Roberts, 1986) or in the mare with chronic endometritis (Asbury, 1986).

Dilution

The ejaculates of most domestic animals contain more sperm than are needed for achieving a pregnancy. Hence, by diluting the semen, it can potentially be used for several inseminations. In species such as the dog and the horse, the whole sperm-rich fraction of the ejaculate is diluted and chilled, then used either for sequential inseminations of the same female over her extended oestrus period or after various determinations of the fertile period (Jeffcoate and Lindsay, 1989; Brinsko and Varner, 1993). In food animal species, the ejaculate is generally diluted so that it can be used to inseminate many females. In either case, the maximum degree of dilution is determined from the minimum number of spermatozoa and the volume of inseminate that is required to achieve acceptable pregnancy rates. These factors are themselves determined by the site of insemination, the survival of sperm in diluent and the idiosyncrasies of individual species and sires. In general, where an intrauterine insemination can be achieved, the minimum numbers of sperm are one or two orders of magnitude lower than for an intracervical insemination, which is itself one or two orders of magnitude lower than for an intravaginal insemination. Hence, where widespread use of sires is required, a great advantage exists in devising methods of achieving intrauterine insemination, even where, as in the ewe, this requires as complex a procedure as laparoscopic insemination.

The major properties of a semen diluent (Watson, 1979) are:

1. *Addition of volume*. Insemination doses must be prepared in a volume, which is a compromise between ease of handling and an appropriate volume for the site of insemination. Thus, for ovine intracervical inseminations, minimising volume is important to reduce retrograde loss from the cervix (Evans and Maxwell, 1987), while for porcine intrauterine inseminations, a minimum volume of 50 ml is required to spread the semen through that capacious organ (Reed, 1982).

Dilution of semen is not entirely straightforward, for mammalian sperm placed in simple diluents exhibits an initial increase in motility, which is then rapidly followed by a loss of motility and increase in vital staining (Mann, 1964). This phenomenon, known as the 'dilution effect', represents a loss of cell viability, probably through leaching of structural components of the cell membrane. Although it was of great concern amongst the early practitioners of AI, the use of diluents containing macromolecules such as proteins or polyvinyl alcohol was found to abrogate the dilution effect (Suter et al., 1979; Clay et al., 1984).

2. *Buffers.* Spermatozoa have a narrow range of tolerance to changes in pH, so provision of buffering capacity is necessary. Buffering is especially important where the semen is only to be chilled and not cryopreserved, as the metabolic activity of cooled spermatozoa remains appreciable (Salisbury et al., 1978). Whilst in many diluents, the major volume component is also the major buffering solution, buffers are a minor constituent of some diluents. Simple buffers are effective, with citrate being widely used (Willett and Salisbury, 1942). Phosphate-buffered saline is rather less suitable, as it predisposes to head-to-head agglutination of sperm. More recently, organic buffers have been used. Tris (tris(hydroxymethyl)aminomethane) is probably the most widely employed of such buffers (Davis et al., 1963), but the successful use of many similar materials (e.g. TES, HEPES Tricene) is described. The proteins contained in skimmed milk products also provide considerable buffering capacity to diluents.

3. *Maintenance of osmotic pressure*. Seminal plasma has an osmotic pressure of 285 mOsm, although sperm can tolerate a moderate range of tonicity (Foote, 1969). Some debate has centred on whether sperm respond better to a slightly hyperosmotic (Foote, 1970) or isosmotic diluent, with the former being generally favoured. Apart from the osmotic activity of the ionic component of diluents, a substantial contribution is made by proteins and, particularly, by sugars, which are added to provide nutrition for the sperm or to contribute to the cryoprotective properties (Watson, 1990) of the diluent.

4. *Energy substrate*. Most diluents make some provision of energy substrates for sperm. In general, simple sugars such as glucose, fructose, mannose and arabinose are suitable substrates, although the rate at which these sugars are metabolised varies substantially between species (reviewed by Bedford and Hoskins, 1990). Lactose, which is present in milk-based diluents, is not metabolisable to any appreciable extent. However, egg yolk, also a component of many diluents, provides many substrates for sperm metabolism (Salisbury et al., 1978). The provision of energy is relatively less important where sperm are to be frozen, for they will only remain active for a few hours, at most, before freezing suspends metabolic activity. However, if semen is to be used chilled, when sperm metabolism has to be sustained for several days, provision of energy is important.

5. *Antimicrobial activity*. Antibiotics are added to most semen diluents as a prophylactic measure against the transmission of pathogenic bacteria and to reduce the load of non-pathogenic organisms that contaminate the semen. In cattle AI, benzylpenicillin and streptomycin (Melrose, 1962) are the most widely used antibiotics, for these are efficacious against *C. fetus*. Most other antibiotics either fail to control this organism or are directly detrimental to sperm. Recently, concern over the potential transmission of *Mycoplasma* and *Ureaplasma* species in bovine semen has led to the incorporation of lincomycin and spectinomycin (Almquist and Zaugg, 1974) into semen diluents in an effort to control these organisms. There is evidence that the efficiency of antibiotics may be reduced in the presence of some components of diluents, notably egg yolk (Morgan et al., 1959), hence the practice in some bovine AI centres is to preincubate the raw semen with antibiotic cocktails

before the main dilution occurs. This procedure is virtually standard practice in the USA, but is rarely undertaken in Europe.

The life span of spermatozoa at ambient temperatures is generally short, but can be extended by inhibiting their metabolism and motility with carbon dioxide (VanDemark et al., 1965; Foote, 1967). For most species, the alternative method of inhibiting sperm activity, namely cooling, has been the method of choice. However, the spermatozoa of some species, notably the boar, do not tolerate cooling well, so ambient temperature diluents have been needed. The earliest of such diluents, the Illinois variable temperature (IVT) diluent, used glucose, citrate, bicarbonate and egg yolk and was gassed with carbon dioxide (Salisbury and VanDemark, 1961). Variations of this diluent formed the basis of diluents used in early pig AI programmes, although more modern diluents, such as the Guelph diluent (Haeger and Mackle, 1971) or the Zorlesco family of diluents (Gottardi et al., 1980), have now largely supplanted these. Such diluents allow boar semen to remain viable for 3–5 days at ambient temperatures.

The life span of spermatozoa of most other species can be prolonged more conveniently by either cooling or freezing. Cooling sperm, however, results in considerable damage to the cells, with the leakage of intracellular potassium, enzymes, lipoprotein and adenosine triphosphate (ATP) occurring (Salisbury et al., 1978). This phenomenon of cold shock is exacerbated by rapid cooling rates, but cannot be prevented even by slow cooling. The most effective way of protecting sperm against the detrimental effects of cooling is by the inclusion of lecithins, proteins, lipoproteins and similar complexes of large molecules that are found in egg yolk and milk (Blackshaw, 1954; Melrose, 1956; Blackshaw and Salisbury, 1957). Of these, lipoprotein appears to be the most critical, although its mode of action is poorly understood (Watson, 1990). It possibly prevents the leaching of similar materials from the sperm plasmalemma, or perhaps it mitigates and limits the consequences of such leaching when it occurs. Unfortunately, neither egg yolk nor milk adequately protects boar spermatozoa against cooling, nor does any other readily available or fully synthetic compound (Watson and Plummer, 1985). Furthermore, some of the constituents of egg are toxic to the sperm of some species, notably the goat, in which a toxic interaction occurs between yolk and components of the seminal plasma, causing sperm death (Corteel and Paquignon, 1984). Moreover, whole milk is also toxic to sperm, for it contains a protein, 'lactenin', which is spermicidal. Thus, milk for use as a semen diluent must be heat-treated (e.g. in the skimming process) to inactivate this toxic factor (Flipse et al., 1954).

The fertility of bovine semen stored at 5°C in such a diluent remains acceptable for 2–4 days (Foote et al., 1960), although that of ram semen only persists for 12–24 hours (Salamon and Robinson, 1962; Evans and Maxwell, 1987). The decline in fertility that occurs after this time is initially due to decreased motility and survival in the female genital tract rather than to sperm death per se. Short-term storage of semen by chilling to 5°C is, however, a very cheap and effective way of establishing an AI programme for cattle and is of value for on-farm collection and insemination of sheep, while the use of liquid boar semen at ambient temperatures remains, effectively, the basis of the technique in that species. Short-term 5°C storage is also widely used in the horse and the dog, for it avoids the unpredictable response to freezing that characterises the semen of those species.

Cryopreservation

Longer-term storage of semen is achieved through cryopreservation. Cryopreservation maintains the fertile life of semen virtually indefinitely, although a large proportion of individual spermatozoa fail to survive the considerable stresses of freezing and thawing. For sperm to survive freezing, they need to be extended in a diluent that contains not only substances that protect them against cold shock, but also cryoprotectants, such as glycerol, which protect them from the deleterious consequences of freezing.

The general responses of cells to freezing (reviewed by Farrant, 1980; Watson, 1990) were not understood until long after empirical methods of cryopreservation had become widely adopted. Initially, as the temperature of the external medium falls below its freezing point, crystals of pure water start to form. The concentration of solutes in the unfrozen part of the medium therefore rises as, in consequence, does its osmotic pressure. Ice crystals do not extend into the cell at this stage, as they are excluded by the cell membrane. Thus, the intracellular contents undergo a period of supercooling, during which the cell loses water to the unfrozen part of the extracellular medium by osmosis (Figure 31.1). A variable degree of cell dehydration follows, which is terminated by the formation of intracellular ice crystals. Thus, damage can occur to cells in one of two ways. Where a substantial degree of cellular dehydration occurs, the high concentrations of solutes in the residual intracellular water can be damaging, whereas, if only slight dehydration occurs, large ice crystals can form within the cell, which cause physical damage to its internal and bounding membranes. The degree to which each affects the cell is determined by the rate of cooling – the slower the rate, the more dehydration, the faster the rate, the greater the damage by ice formation – and the size of the cell, such that the larger the cell, the slower its inherent rate of dehydration.

Cryoprotective agents may either penetrate or remain outside the cell, but both act by binding water and therefore alter the availability of water either for dehydrative loss or for ice crystal formation. Penetrating cryoprotectants, such as glycerol or dimethyl sulfoxide (DMSO), appear not only to reduce the loss of water from the cell, thereby reducing solute damage, but also to bind it in a form that renders it unavailable for crystal formation, thereby reducing the effects of intracellular ice formation. Non-penetrating cryoprotectants, such as disaccharides or proteins, may hasten dehydration during very rapid cooling, thereby minimising intracellular ice formation. Notwithstanding the aforegoing, precise understanding of the mode of action of cryoprotectants still remains elusive, and much information relating to their practical use remains empirical.

Glycerol (Polge et al., 1949) is the main primary cryoprotectant used in preparing mammalian semen for freezing (Watson, 1990), despite the fact that it has some directly toxic effects upon sperm (Watson, 1979). Concentrations of glycerol depend upon the species and the other components of the diluent. For example, diluents for bovine semen that contain disaccharides can utilise lower percentages (3–4%) of glycerol than diluents that lack such disaccharides, which have a final glycerol concentration of at least 7% (Unal et al., 1978).

Whether the toxic effects of glycerol are exacerbated at high temperatures has been a matter of debate. Certainly Polge (1953) considered that the addition of glycerol at 28°C was more dam-

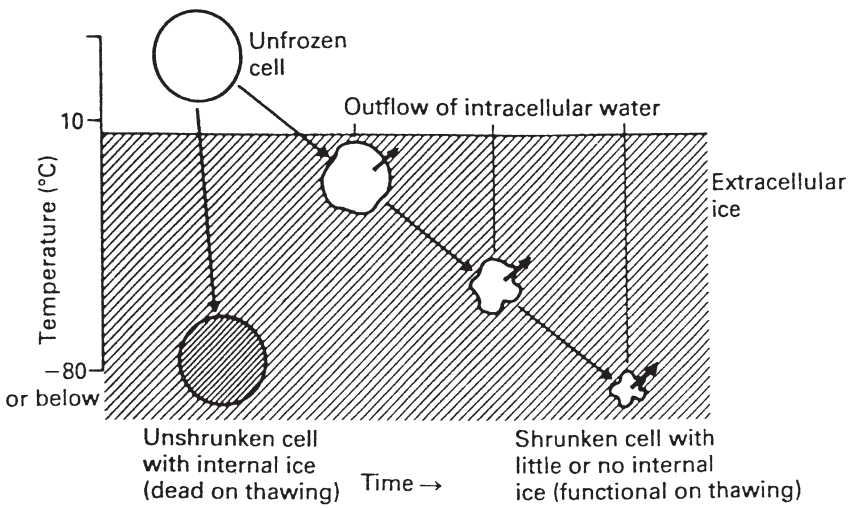
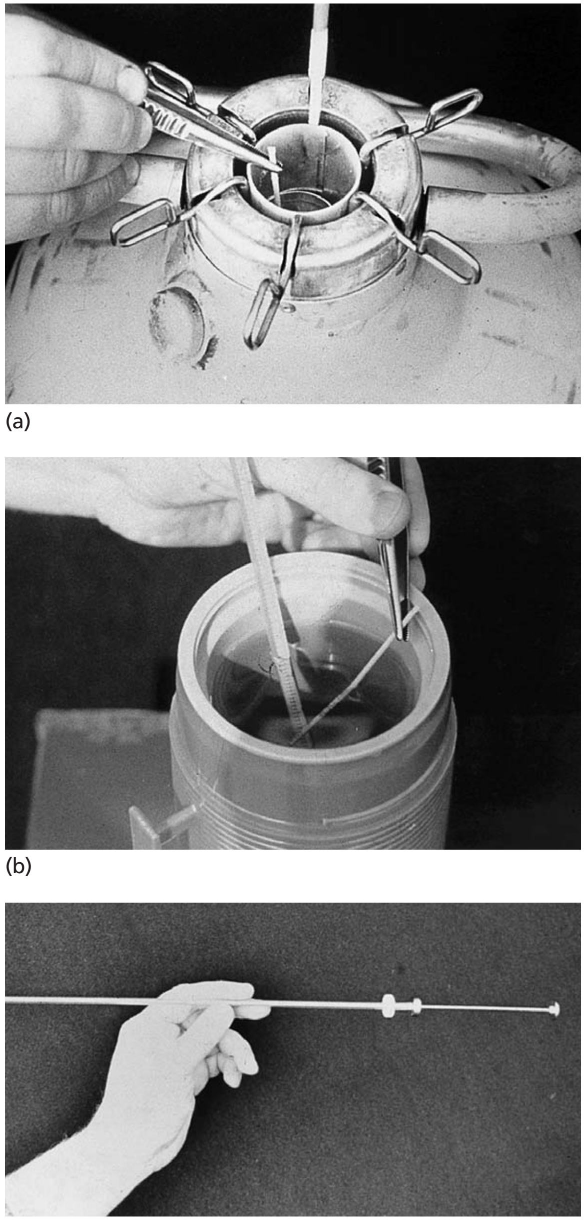


Fig. 31.1 Shrinkage of cells during cryopreservation. Extracellular freezing induces conditions that allow osmotically induced loss of water from cells during slow freezing. This correlates with survival on thawing. Rapidly cooled cells do not have time to shrink, form intracellular ice and are dead on thawing (reproduced from Farrant, 1980, with permission).

aging to bovine sperm than its addition at 4°C, although Salisbury et al. (1978), reviewing the (by then) copious literature, concluded that the effects of temperature of glycerolisation were equivocal. Nevertheless, normal practice in commercial bovine AI centres is that where the final concentration of glycerol is high (≥ 7%), a primary dilution of the semen is made with a diluent containing little or no glycerol, with glycerolisation being carried out after reducing the temperature to 4°C; whereas diluents that utilise lower final concentrations (<5%) are added in one step, at 30°C. With boar semen, by contrast, the toxicity of glycerol at high temperatures is much less equivocal, and low-temperature glycerolisation is desirable (Paquignon, 1985).

Originally, diluted semen was placed in glass ampoules for freezing in a mixture of alcohol and solid carbon dioxide at –79°C, or drops of diluted semen were placed directly on to the surface of a block of solid carbon dioxide where they froze in pellet form (see Salisbury et al., 1978). Long-term storage at –79°C was not satisfactory, however, as deterioration occurred at that temperature (Pickett et al., 1961; Stewart, 1964). Storage in liquid nitrogen at –196°C has subsequently become established as the standard medium for long-term preservation of semen and, over the 40 years for which it has been practised, has maintained sperm fertility unscathed. At the present time, semen is frozen in one of two main ways. Diluted semen is packed into thin, plastic tubes of 0.25 or 0.5 ml capacity, then, in the simpler techniques, these tubes ('straws' or 'paillettes') are suspended in the vapour of liquid nitrogen, which is at about –120°C, for about 10 minutes (Cassou, 1964; Jondet, 1964). The straws are then plunged into the liquid nitrogen (Figure 31.2(a)). More recently, a greater degree of control of freezing rate has been exercised by the use of microprocessor-controlled freezers, with improved sperm survival justifying the increased cost of the processing (e.g. Landa and Almquist, 1979; Parkinson and Whitfield, 1987).

Thawing of the semen needs to be rapid; slow thawing allows recrystallisation of ice within the cells, causing membrane damage (see Salisbury et al., 1978). In practice, the rate of thawing is rarely critical, the surface area-to-volume ratio of the 0.25 ml tubes being so great that any temperature



(a)

(b)

(c)

Fig. 31.2 Semen-handling for bovine artificial insemination. (a) Withdrawing a straw of frozen semen from the liquid nitrogen flask. The canister containing the semen should not be lifted above the level of the top of the neck of the flask. (b) Thawing. After checking the identity of the sire, the straw is thawed. Water temperature is not really critical, but placing the straw in water at 37°C for 10 seconds is a typical thawing regime. (c) The straw is placed in an insemination catheter, which is then covered with a plastic sheath. The catheter is then ready for use, but care must be exercised not to allow the semen to become chilled again before it is inseminated.

of the thawing water between 0 and 40°C will thaw adequately (Figure 31.2(b)). Of greater importance is the temperature control of the thawed semen. This should not be allowed to cool below the final temperature achieved during thawing; otherwise substantial sperm losses can occur. Rethawed spermatozoa are as sensitive to fluctuations in temperature as are their unfrozen counterparts (Roberts, 1986).

DISEASES TRANSMISSIBLE IN SEMEN

Many infectious agents can be transmitted through semen. Foot and mouth virus can be transmitted in the semen of all species that are susceptible to its infection (Callis and McKercher, 1980; Radostits et al., 1994). Indeed, control of foot and mouth transmission has, until recently, underpinned the UK legislation controlling AI of cattle, with broadly similar regulations in force throughout much of the developed world. Surprisingly, although cattle AI is generally closely regulated, AI of most other species, including many of agricultural importance, has generally received scant attention from regulating authorities.

Control of diseases in other species is generally much less stringent than in cattle. For example, for dogs, control of *Brucella canis* and leptospira infection is generally required for international shipments of semen, and pigs are generally screened for diseases such as Aujeszky's disease before entry to studs. Curiously, however, although a progressive trend towards increased disease security existed until the mid-1980s, the more recent trend has, particularly in the UK, been to reduce the legislative control of diseases that are transmissible through AI.

AI OF CATTLE

Collection, handling and storage of semen

Semen is usually collected by an artificial vagina, although electroejaculation is occasionally used. After assessment for motility, density and morphology, the semen is diluted into insemination doses. The degree of dilution and the diluents with which the semen is diluted depend upon its subsequent use. Most semen is cryopreserved, although some is used after simple extension and chilling to 4°C. The practice of using an ambient temperature diluent is virtually unique to New Zealand, where the practice is made possible by the low incidence of infectious disease and is required for the highly seasonal pattern of reproduction in its dairy cows.

For cryopreservation, or for use at 4°C, the semen is first extended with a diluent based upon either egg yolk or skimmed milk (see Table 31.1 for examples of widely used diluents; J. Willmington, personal communication, and see Salisbury et al., 1979), which contains antibiotics for the control of contaminating bacteria. The semen is then cooled to 4°C. If it is destined for use in this form, the motility of the sperm will be reassessed, then the semen released for use. If the semen is destined for cryopreservation, glycerol is also added, then the semen packed into 0.25 or 0.5 ml paillettes, or 0.5 or 1.0 ml glass ampoules. The semen is then equilibrated for 1–4 hours. It was originally considered that this was the period over which glycerol penetrated the sperm, although more recent observations indicate that the penetration of glycerol is very rapid and that most of the equilibration period is concerned with membrane stabilisation during exposure to low temperatures (Watson, 1979). The semen is then frozen in the vapour of liquid nitrogen or in a microprocessor-controlled freezer. The semen thereafter remains in liquid nitrogen until thawed for use. Freezing in alcohol and solid carbon dioxide or in pellets on blocks of solid carbon dioxide, although formerly used widely, has now virtually ceased.

The ability to perform an intrauterine insemination in cattle means that a relatively low dose of sperm is required to achieve acceptable pregnancy rates. Typically, of the 20–30 million sperm that are required in each insemination dose, 6–7 million survive freezing, a figure that is generally regarded as the minimum dose compatible with acceptable fertility (Milk Marketing Board, 1967; Sullivan and Elliott, 1968). Lower numbers of sperm can be used where unfrozen semen is used (Salisbury and VanDemark, 1961).

The use of ambient temperature diluents is precluded in most continental countries by the risk of

Table 31.1 Diluents for use in cryopreservation of bovine semen (from J. Wilmington, personal communication; Salisbury et al., 1979; Parkinson and Whitfield, 1987; Herman et al., 1994)

Constituent (litre)	*Skimmed milk*	*Egg yolk-citrate*	*Reading diluent*	*Egg yolk-Tris*	*CUE extender*
Egg yolk	100 ml	200 ml	200 ml	200 ml	200 ml
UHT skimmed milk	870 ml				
Fructose	12.5 g				
Lactose			82.8		
2.9% sodium citrate buffer[a]		770 ml			
Tris buffer[b]				800 ml	
Citrate-HCO^3 buffer[c]					753 ml
Glycerol			47 ml		47 ml
Stage 1	30 ml	30 ml		Nil	
Stage 2	110 ml	110 ml		140 ml	
Antibiotics	Typically, 1000 IU penicillin + 1000 μg streptomycin/ml				

[a] Trisodium citrate dihydride
[b] 30.28 g Tris, 17.30 g citric acid monohydrate/litre
[c] 14.5 g trisodium citrate dihydride, 2.1 g $NaHCO_3$, 0.4 g KCl, 3.0 g glucose, 9.4 g glycine, 0.9 g citric acid, 185 g raffinose/litre

Stage 1 diluents are added at 30–37°C, after which the semen is cooled to 4°C; stage 2 diluents are added at 4°C. Single-stage diluents are added at 30–37°C, after which the semen is cooled to 4°C

contamination of the semen with foot-and-mouth disease virus. However, it has many advantages over other methods of extension, for, whereas cryopreserved semen requires about 20 million spermatozoa per insemination, semen extended in ambient temperature diluents can achieve acceptable fertility with less than 2.5 million sperm per insemination (Shannon et al., 1984). Initial work on ambient temperature diluents for bovine semen was based upon the use of the IVT diluent (VanDemark and Sharma, 1957; Salisbury and VanDemark 1961; Melrose, 1962), but the development of ambient temperature dilution in New Zealand has subsequently been based upon the use of the Caprogen diluent (Shannon, 1965). With the subsequent modifications that have taken place, the Caprogen diluent (Table 31.2) is now capable of maintaining sperm viability and acceptable conception rates for up to 5 days, with an insemination dose of between 0.5 and 2.5 million sperm per insemination.

Table 31.2 Caprogen diluent for ambient temperature extension of bovine semen (from Shannon, 1965; Shannon and Curson, 1982)

Basic diluent (g/litre)	
Sodium citrate	20
Glycine	10
Glycerol	12.5
Glucose	3
Caproic acid	0.3125
Preparation of Caprogen diluent	
Basic diluent	80%
Egg yolk	20%
Nitrogen gas	Bubbled for 20 min at 5°C
Catalase	4.5 mg/litre

Insemination

Cows ovulate at about 12 hours after the end of the oestrus period. The ideal time for insemination is therefore 6–24 hours prior to ovulation (Roberts, 1986). Where the technician service provided by an AI centre is used, the optimum insemination times achieved in practice are on the same day (morning or afternoon) where oestrus is first observed in the morning, or on the morning of the next day, where oestrus is first observed in the afternoon (Olds and Sheath, 1954; Foote, 1979). However, it is claimed that it is possible to achieve a better timing of insemination in relation to the most fertile period of oestrus by farmers inseminating their own cattle at appropriate intervals after the first observation of oestrus. For this reason, and because of the cost of using AI centre technicians, technician services have fallen somewhat into disfavour, compared to farmers' own ('do-it-yourself') inseminations.

Cows are inseminated just into the short uterine body. Insemination into the cervix produces a lower fertilisation rate, while insemination deeper into the uterus runs the risks of either inseminating into the uterine horn contralateral to the ovulation site, or scoring the endometrium with the tip of the insemination catheter. Reduced fertility is the consequence of both of the latter two errors. The standard technique of insemination is to grasp the cervix through the rectum with the left hand. A catheter, into the tip of which a paillette of semen has been inserted (Figures 31.2(c) and 31.3(a)), is then passed into the vagina and manipulated into and through the cervix by the right hand. This technique, the rectovaginal method of insemination, requires considerable practice for success. The vulval lips are opened by downwards pressure from the arm in the rectum, while the circular folds of vaginal mucosa are obliterated by pushing the cervix forward. The catheter is initially inserted pointing upwards at an angle of about 30° to avoid entering the urethral meatus or fossa, and is then moved horizontally until it engages in the external os of the cervix. The left hand squeezes the anterior vagina on to the caudally projecting external os of the cervix, thereby obliterating the fornix of the vagina (Figure 31.3(b)) and facilitating entry of the catheter into the cervix. Entry into the external os is accompanied by a characteristic 'gritty' sensation. The catheter is then introduced through the

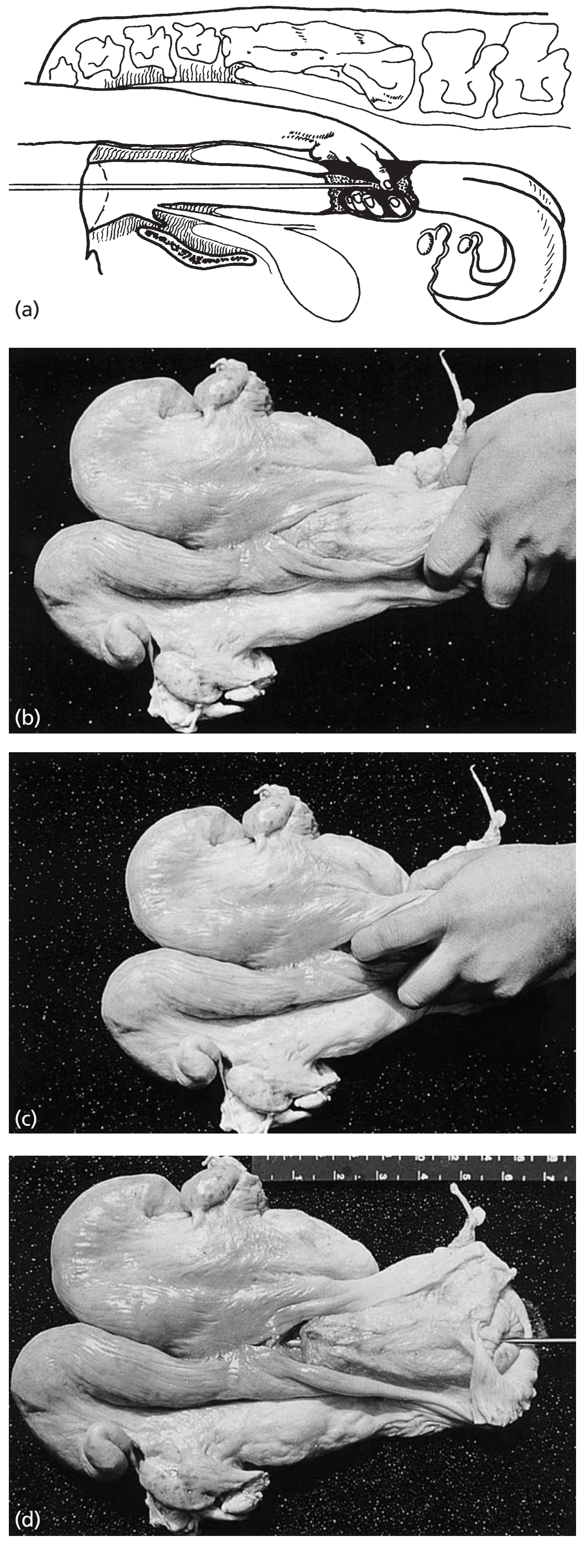
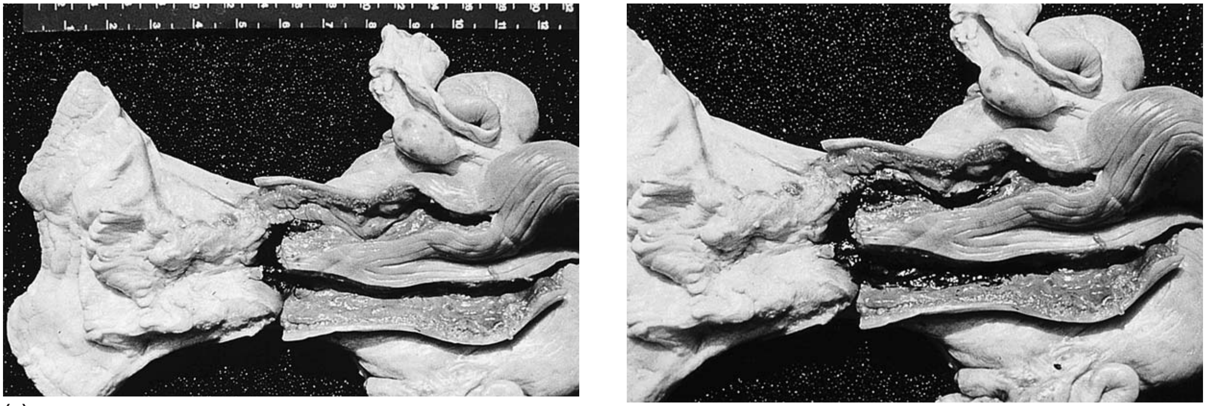


Fig. 31.3 Rectovaginal method of insemination. (a) General method. Partially dissected bovine uterus showing (b) grip on the cervix for the obliteration of the vaginal fornix, to facilitate entry of the catheter into the external os of the cervix; (c) second grip on the cervix to allow passage of the catheter through the tortuous cervical canal – the index finger is gently pressed over the internal os in order to feel the tip of the catheter as it emerges from the cervical canal; (d) site of deposition of semen in the uterine body – the recommended site is no more than 0.5 cm deep to the internal cervical os ((a) redrawn from Salisbury et al., 1978, with permission).



(a) (b)

Fig. 31.4 Distribution of semen in the cow after artificial insemination. A dark-coloured dye has been placed into the uterine body and runs equally into both uterine horns. As inseminators are trained not to palpate the uterus or ovaries, they should be unaware of the side on which ovulation will occur, so semen must have access to both uterine horns.

convoluted cervical canal by manipulation of the cervix through the rectal wall. One finger is placed over the internal os of the cervix, so that the tip of the catheter can be palpated as it emerges from the cervical canal (Figure 31.3(c)). As soon as the catheter has emerged, deposition of semen into the uterus begins; the catheter is advanced no deeper into the uterus. In this way, semen should be equally distributed between the two uterine horns (Figures 31.3(d) and 31.4).

No forward pressure should be exerted on the catheter with the right hand, for the uterine wall is friable and easily penetrated if the catheter moves suddenly. The most common fault of insemination (Figure 31.5) is twisting the cervix in the left hand, so that one uterine horn is partly occluded. Alternatively, the catheter may be partly withdrawn during the deposition of semen, resulting in a partially intracervical insemination. Penetration of the cervical canal of maiden cattle is difficult at oestrus, and virtually impossible at other stages of the oestrous cycle; such animals are therefore often beyond the capabilities of inexperienced inseminators. However, the cervix of parous cattle can, with greater or lesser difficulty, be traversed at most stages of the oestrous cycle and early pregnancy. It is therefore imperative that it is known whether an animal is likely to be pregnant before insemination is attempted, for abortion can be induced if an insemination catheter penetrates the fetal membranes or if infection is introduced into a pregnant uterus by poor insemination hygiene.

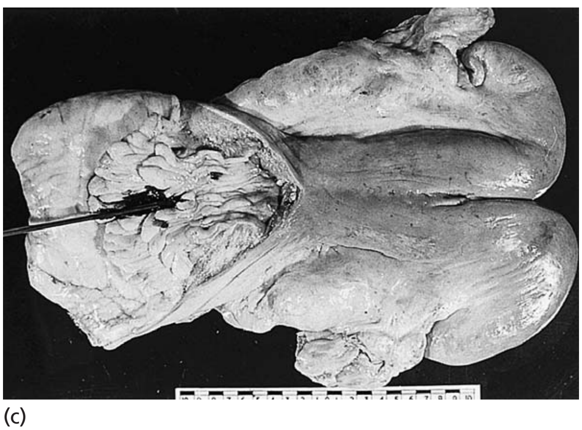
Management of insemination

Insemination can be performed at an observed or induced oestrus. The former is more common in dairy cattle, for considerable opportunity exists for the observation of oestrus, but in beef cattle and dairy or beef heifers the time required for observation makes the use of induced oestrus relatively more attractive. Oestrus can be induced and synchronised by the use of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or its analogues, progesterone-like hormones, or combinations of progesterone and a luteolytic agent. Most such regimens require the use of fixed-time insemination, although, particularly in the case of $PGF_{2\alpha}$, the accuracy with which the timing of oestrus can be predicted is sufficiently imprecise for some observation to be advisable, and reinsemination performed if animals exhibit signs of oestrus after fixed-time insemination has occurred.

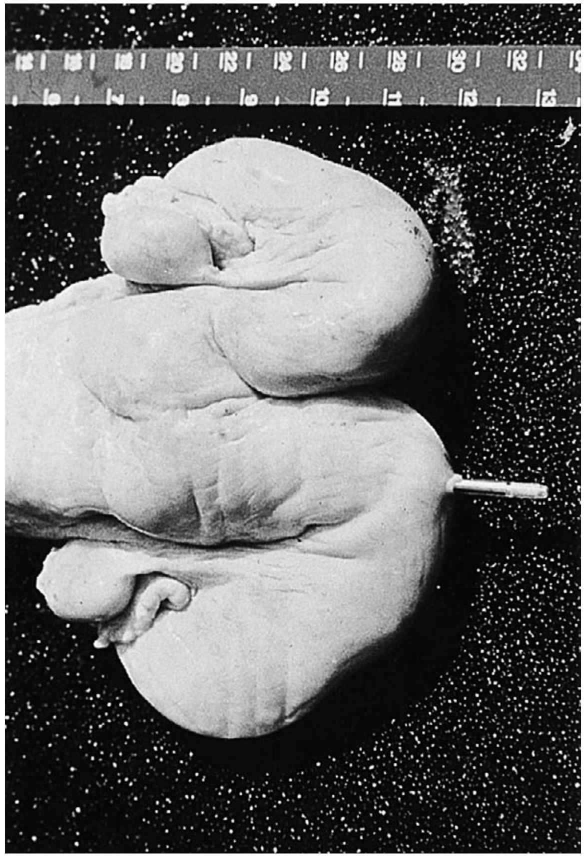
Fertility to AI is generally very similar to that achieved at natural service, with a calving rate to a single insemination of around 50% (e.g. Barrett et al., 1948). The true fertilisation rate is much higher than this, at around 90%, but subsequent embryonic losses bring the apparent figure to the lower value (Ayalon et al., 1968). In practice, AI

(a)

(b)



(c)



(d)

Fig. 31.5 Distribution of dye in the reproductive tract of the cow after faulty insemination. (a) Uterus twisted during insemination, occluding one uterine horn. The dye is present in only one horn; typically the right. (b) Catheter withdrawn into the cervix during semen deposition. Some dye is present in the uterine body, but most is within the cervical canal. (c) Failure to penetrate the cervix: semen deposited in the caudal cervical canal. (d) Penetration of the uterine horn following excessively deep insemination. Such penetrations are usually associated with the use of undue force on the catheter during passage of the cervical canal.

companies are generally unable to obtain complete data on the calving rates, so they estimate fertility from the proportion of cows which are re-presented for insemination by either 49 or 60–90 days after the initial service. The proportion of cows that are not re-presented is closely related to the proportion which actually have become and remained pregnant. The figure thereby obtained, the non-return rate (NRR), is an overestimate of calving rate, but is generally in fixed ratio to the calving rate, so is usable for monitoring fertility (reviewed by Salisbury et al., 1978). AI centres therefore use the 'non-return rate' to monitor both the fertility of their bulls and the results obtained by their technicians. Bulls or technicians that produce consistently low figures are generally slaughtered or dismissed, respectively. No such control exists over the technical proficiency of farmers who inseminate their own cattle, nor, necessarily, over the fertility of the bulls they use.

Regulation of cattle AI varies from country to country, but is generally under some form of state control. In the UK, AI centres are licensed by the Ministry of Agriculture, with the right to distribute semen, or to employ technicians to inseminate cattle, also being granted by ministry licence. Further licences grant individual farmers the right to inseminate their own cattle and to store frozen semen on their farms.

Control of infectious diseases

Even with increasing international trends towards deregulation of animal health issues, the control of the health status of semen used for bovine AI is usually under legislative control.

Control is exercised over the health status of donor bulls and the hygiene of technicians who, by travelling between farms, offer considerable risk as disease vectors.

Most of the serious viral diseases of cattle (foot and mouth, rinderpest, etc.) can potentially be transmitted through AI. Legislative regulation of bovine AI has, for many years, been based upon the primary precept of preventing such transmission from occurring. Many of the somewhat less serious viral diseases, such as infectious bovine rhinotracheitis–infectious pustular vulvovaginitis (IBR–IPV) can also be transmitted thus (Chapman et al., 1979; Kahrs et al., 1980). Recently, it has become apparent that the bovine viral diarrhoea (BVD) virus can be present in the semen of bulls (Barlow et al., 1986), potentially causing early embryonic death and abortions in inseminated cows (Grahn et al., 1984), as well as its more well-known ability to cause the birth of persistently infected progeny.

A number of bacterial diseases are transmissible in semen, including tuberculosis, brucellosis, leptospirosis and, possibly, Johne's disease (Roberts, 1986). *Haemophilus somnus*, to which the possibility of causing reproductive failure has been attributed, may also be present in semen (Humphrey et al., 1982). Many species of *Mycoplasma* and *Ureaplasma* are present as commensals of the prepuce of the bull, and are harmless when inseminated into cows, but some species are responsible for a granulomatous vaginitis in cows, which causes infertility and unwillingness to mate (Afshar et al., 1966; Radostits et al., 1994). Most importantly, the classic venereal pathogens of cattle, *T. fetus* and *C. fetus* (Garlick, 1939; Rasbech, 1951; Willett et al., 1955), are transmissible by AI; control of these two organisms remains the second major precept upon which legislation governing cattle AI is based.

Two other conditions warrant specific mention. Firstly, (enzootic bovine leucosis EBL) virus, which is generally considered not to be transmissible in semen (Radostits, 1979), rightly causes such anxiety to regulating authorities that infected bulls are excluded from AI studs. Secondly, blue tongue virus generally causes few or no clinical signs in infected cattle (Radostits et al., 1994). However, the disease that the virus causes in sheep is so severe that semen from potentially infected bulls (Bowen and Howard, 1984) is carefully excluded from most countries in which sheep production is of economic importance.

In cattle, control of these diseases rests upon three major strategies. Diseases that can be detected by serology, such as brucellosis, IBR, EBL, Q fever, etc., are controlled by exclusion of seropositive bulls from AI studs. Likewise, tuberculosis is controlled by exclusion of bulls that react to tuberculin testing. *Leptospira spp.* may be killed by freezing and thawing. The antibiotics that are added to semen diluents are intended to kill both pathogenic bacteria (including *C. fetus*) and the contaminant bacteria that originate from the penis and prepuce during semen. Antibiotics are also used to control *Mycoplasma* and *Ureaplasma* species. The final and most potent means of control of disease is the quarantine of semen after its collection. After semen has been frozen, it is placed in a container where it remains untouched for 28 days. If, during that period, the donor bull develops any disease, the semen is destroyed. If not, it is released for use.

AI OF SHEEP

Artificial insemination of sheep has recently been comprehensively reviewed by Evans and Maxwell (1987) and Chemineau et al. (1991). The sheep is less amenable to artificial insemination than is the cow, since oestrus cannot readily be detected without the presence of rams, insemination is less straightforward and ovine semen is less easy to freeze than bovine semen. In Eastern Europe, South America and Australasia, AI is widely used in sheep-breeding programmes, but its use is much less widespread in Western Europe and North America, mainly due to the high costs of handling and inseminating sheep compared with the costs of natural service.

Ewes normally display oestrous behaviour only in the presence of a ram. In order to determine the time at which AI should be performed, it is therefore necessary either to control the timing of oestrus or to detect it with male animals. In the former situation, pharmacological methods are used to induce and synchronise oestrus, so that the time of the fertile period is defined. In the latter situation, either raddled, vasectomised rams or intact rams with an abdominal apron to prevent intromission are used to detect oestrus. The cost:benefit ratio for the use of AI in sheep has therefore to be considered carefully. Where there are substantial costs associated with AI, either of maintaining rams, of drugs (plus their administration) for oestrus synchronisation, or of the procedures associated with insemination itself, these have to be set against the financial benefits gained from the superior carcass or wool characteristics of the progeny born to AI.

However, the most important limitation of the use of AI in sheep is in the method of insemination, since it is difficult to achieve an intrauterine insemination because the cervical canal of the ewe is so tortuous. Since intracervical AI results in both a lower conception rate and a lower number of lambs born per ewe than with natural service, a number of methods of insemination have been devised that try to circumvent the cervix. All of these attempt, with varying success, to achieve an economically viable compromise between fertility, technical difficulty and numbers of sperm needed for insemination. The methods in widespread use are:

- intravaginal
- intracervical
- transcervical intrauterine
- laparoscopic intrauterine.

These methods will be considered below.

Collection, handling and storage of semen

Most inseminations of ewes are performed using semen that was collected on the day of insemination. Such semen is normally extended by the addition of simple diluents, although direct insemination of raw semen is still practised in some regions. The semen is collected by an artificial vagina or electroejaculation and subjected to routine examination for motility and density. It is then diluted with a simple diluent, to a final volume and sperm content that depend upon the route by which it is to be inseminated. The number of sperm and the volume of diluted semen used for insemination depend upon the route of insemination, whether the insemination is undertaken during the natural breeding season or after induction of out-of-season breeding, and whether the semen is cryopreserved. Recommendations for sperm numbers and insemination volume are shown in Table 31.3.

Diluents that are in routine use for unfrozen semen include buffers that contain glucose, egg yolk–citrate or egg yolk–phosphate solutions, or

Table 31.3 Minimum numbers of motile spermatozoa for insemination of ewes at different sites (data from Evans and Maxwell, 1987 and Usboko, 1995)

Route	*Number of motile sperm ($\times 10^6$)*			*Insemination volume*
	Fresh	*Liquid*	*Frozen*	
Vaginal	300	400	2 × 450	0.3–0.5 ml
Intracervical	100	150	180	0.05–0.2 ml
Transcervical intrauterine		60		0.1–0.2 ml
Laparoscopic intrauterine	20	20	20	0.05–0.10 ml per horn

Table 31.4 Diluents for use with ram semen (from Evans and Maxwell, 1987)

	Final volume (g/litre)		
	Diluent 1	*Diluent 2*	*Diluent 3*
Tris	36.3		
Fructose	5.0		
Glucose		8.0	
Citric acid	19.9		
Sodium citrate			23.7
Egg yolk (ml)	140	200	
Skimmed milk powder			90.0

heat-treated cows' milk (Table 31.4). After dilution, the semen is cooled and stored at either +15°C or +4°C until used. The semen has to be used within 8 hours of collection, as fertility declines substantially after this time (Chemineau et al., 1991).

However, although such simple diluents are capable of supporting sperm viability for the relatively brief periods demanded for direct insemination, cryopreservation is required for long-term storage of semen. Unfortunately, cryopreservation of ovine semen is not particularly straightforward, since the process of cryopreservation causes a significant level of damage to ovine spermatozoa. Thus, they are less able to survive in the female genital tract than are unfrozen cells. In particular, the passage of the cervix appears to be very much more difficult for cryopreserved than fresh sperm, so, as a generalisation, insemination routes that require sperm to traverse a significant length of the cervical canal are not well suited to cryopreserved semen. Hence, frozen semen is not really appropriate for use in intravaginal insemination, unless large numbers of spermatozoa are used and a lower than normal conception rate is accepted.

There are also a number of difficulties encountered when semen is frozen for intracervical insemination. These arise as a relatively large number of sperm (150–200 million total sperm: Salamon and Robinson, 1962; Langford and Marcus, 1982) have to be contained within the limited volume of inseminate that can be placed within the ovine cervix. Since the anatomy of the cervical canal limits the insemination volume to below about 0.25 ml (Evans and Maxwell, 1987) the dilution rates are limited to between 1:1 and 1:4. In consequence, insufficient protection can be afforded to the sperm by the diluent against cold shock and freezing damage (Miller, 1986), generally resulting in mediocre post-freezing survival of functional sperm.

For intrauterine insemination, in which lower numbers of spermatozoa are required, far more satisfactory dilution rates of semen can be achieved, so cryopreservation is more successful. Furthermore, since the insemination site does not require sperm to traverse the cervix, the sperm do not have to survive in such a robust state as is needed for intracervical insemination. Hence, the conception rates that are achieved with the use of cryopreserved semen for intrauterine insemination are commensurate with those of natural service after oestrus synchronisation (see, for example, Davis et al., 1984).

As with most other species, it has been noted that there is much variation in the ability of the semen of individual rams to survive cryopreservation. However, the semen of most rams has been relatively successfully frozen in pellet form on the surface of blocks of solid carbon dioxide (Salamon, 1971), or in paillettes in the vapour of liquid nitrogen (Fiser and Fairfull, 1984), using diluents based upon egg yolk, skimmed milk, citrate and/or lactose. The problem of low dilution rates for semen that is to be used for intracervical insemination was addressed by Salamon (see Evans and Maxwell, 1987), by preparing a range of different diluents for freezing semen at dilution ratios of 1:1 to 1:4, in which the constituents were present in higher concentrations at the diluents to be used at lower dilution rates. In this way, compensation is achieved for the effects of the ratio between diluent and seminal plasma. Chemineau et al. (1991) dealt with the same problem by using two different diluents (an egg yolk–lactose diluent and a glycerolised skimmed milk diluent) in varying proportions depending upon the final dilution rate to be achieved.

Nevertheless, despite the use of higher numbers of cryopreserved sperm for intracervical insemination than when using fresh semen, conception rates are below those of natural service or fresh semen insemination (Colas, 1979). Conception rates of 65–80% are typical when this method is used in the ewes' breeding season, with a somewhat lower figure when for out-of-season breeding regimes. Embryonic mortality is generally considered to be similar with frozen and fresh semen (Evans and Maxwell, 1987), although some reports exist of higher embryonic losses after the use of frozen semen.

Insemination

Vaginal insemination deposits semen into the cranial part of the vagina, without attempting to locate the cervix. The requirements of this method, in terms of both technical proficiency and handling facilities for the sheep, are minimal. However, as previously described, this method requires large numbers of spermatozoa per insemination and is not really amenable for use with stored semen. Moreover, conception rates are also poor after pharmacological oestrus synchronisation, so intravaginal insemination is best suited to use after oestrus detection during the natural breeding season. The ideal timing of insemination is before ovulation, i.e. 12–18 hours after the onset of oestrus (Evans and Maxwell, 1987). Highest conception rates are therefore achieved when the timing of insemination is optimised by drafting ewes for insemination twice per day.

Intracervical insemination is best achieved with the hindquarters of the ewe elevated. After cleaning of the perineum, the vagina is opened with a duck-billed speculum and the cervix located (Figure 31.6(a)). The insemination catheter is then inserted as far as possible into the cervix. Penetration of the cervix is typically 0–2 cm. The conception rate is highly correlated with the depth of penetration (Table 31.5; Evans and Maxwell, 1987) and, hence, the technical proficiency of the inseminator. Conception rates achieved with the use of unfrozen semen by this method are adequate after pharmacological methods of oestrus synchronisation (Chemineau et al., 1991). The ideal time for insemination is 55 ± 1 hour after removal of progesterone sponges, or 15–17 hours after the onset of detected oestrus.

The method of direct intrauterine, laparoscopic insemination (Figure 31.6(b)) was developed to overcome many of the difficulties of intravaginal and intracervical insemination. In this method, ewes are restrained in a cradle and laparoscopy is per-

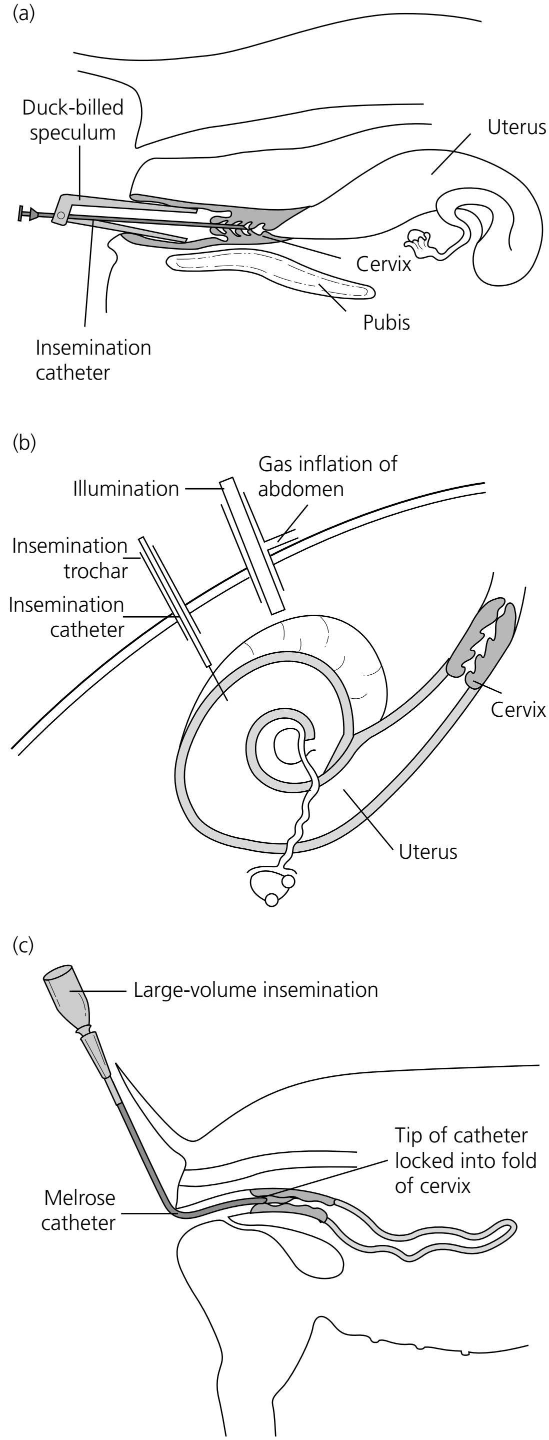


Fig. 31.6 Insemination of ewes and sows. (a) Intracervical insemination of the ewe. (b) Laparoscopic intrauterine insemination of the ewe. (c) Insemination of the sow, using a Melrose catheter.

Table 31.5 Lambing rates in relation to the depth of intracervical insemination (from Evans and Maxwell, 1987)

	Site of insemination		
No. spermatozoa per inseminate (× 10^6)	*On the entrance fold of the cervix*	*Up to 1 cm into the cervix*	*Deeper than 1 cm into the cervix*
400 (undiluted)	50%	68.8%	71.4%
100 (diluted)	43.8%	66.7%	71.4%
50 (diluted)	25.7%	60.7%	66.7%

formed close to the udder, whereupon the uterus is located and semen injected into the uterine lumen (Killeen and Caffery, 1982). The semen can be introduced to the uterus via a simple pipette (Evans and Maxwell, 1987) or by the use of specialised insemination equipment (Chemineau et al., 1991). With oestrus-synchronised ewes, the ideal timing of insemination is between 68 and 72 hours after withdrawal of progesterone sponges (Chemineau et al., 1991). Conception rates to frozen semen inseminated by this method are higher than for intracervical insemination, because of better cryopreservation of sperm and a site of insemination that avoids sperm having to traverse the cervix. Conversely, the laparoscopy is technically demanding and there are far greater implications for the welfare of the ewes than with intracervical insemination.

Use of laparoscopic AI is already widespread throughout Australasia, but the method has had only a limited uptake in Western Europe, mainly due to concerns for the welfare of its subjects and, as with sheep AI in general, the lack of a clear cost benefit. However, laparoscopic intrauterine insemination of ewes is undoubtedly the most significant development in sheep AI, for it circumvents many of the problems of the traditional methods (Haresign et al., 1986). The numbers of sperm required for insemination are lower and the volume of inseminate is proportionally greater, allowing more appropriate dilution rates and, therefore, better preservation of sperm. Hence, conception rates are closer than those of natural service, and embryonic mortality is reduced to an acceptable level. The method also allows for the possibility of genuine progeny testing of rams, as semen from an individual sire can be used in many flocks, over prolonged periods of time. Laparoscopic AI is therefore already the basis of several sire referencing schemes world-wide.

Finally, a method that attempts to achieve an intrauterine insemination via the cervical route has also been developed. In this method, transcervical intrauterine insemination, the cervix is fixed by being grasped with forceps, and an insemination needle is introduced as far as possible through its lumen. Although better conception rates can be achieved by this method than by conventional intracervical insemination (Souza et al., 1994), both the retraction of the cervix and its penetration of the cervical lumen are associated with significant levels of damage (Usboko, 1995; Campbell et al., 1996). It is probable, with the development of expertise in the method, that its use will become more widespread but, at present, it seems to have little to commend it over laparoscopic insemination.

AI OF GOATS

Artificial insemination of goats is generally very similar to that of sheep. However, it is much easier to achieve an intrauterine insemination via the cervix of the goat than in the ewe. Moreover, conception rates to frozen semen tend to be much higher in the goat than in the sheep (Corteel et al., 1984), since, in addition to the better site of insemination, caprine semen appears to survive cryopreservation better than ovine semen. As a consequence, in the substantial flocks of goats that are maintained for milk production and in the small flocks of goats maintained by many amateur farmers, AI has been making an increasing contribution to breeding. Thus, in France, for example, the numbers of goat inseminations increased 4-fold during the 1980s (Chemineau et al., 1991). In the Western world, AI of goats is based predominantly upon frozen semen, inseminated by an intracervical route (Haibel, 1986).

Cryopreservation of goat semen differs from that of the ram in one important aspect: namely, that the seminal plasma must be removed before dilution in media that contain egg yolk. A phospholipase that is secreted by the bulbourethral gland coagulates egg yolk media and hydrolyses lecithin to fatty acids and spermicidal lysolecithins (Iritani and Nishikawa, 1964). Therefore, diluents for goat semen have either been based upon skimmed milk, in which the sperm survive adequately, or the seminal plasma has been removed before using egg yolk-based diluents (Corteel, 1974). Where yolk-based diluents are to be used, washing the spermatozoa is achieved (Corteel et al., 1984) by dilution in Krebs–Ringer phosphate solution and centrifugation.

For direct insemination, caprine semen can be extended in a skimmed milk–glucose diluent or, after removal of seminal plasma, in an egg yolk–citrate or egg yolk–tris–fructose diluent (Haibel, 1986; Evans and Maxwell, 1987; Chemineau et al., 1991). For cryopreservation, egg-yolk-based diluents are more widely used than skimmed milk-based ones, although acceptable results can be achieved with skimmed milk (Corteel, 1974). Glycerolisation can be in one or two steps; Chemineau et al. (1991), for example, recommend a primary dilution in glycerol-free egg yolk–citrate diluent, followed by addition of diluent containing 14% glycerol once the semen has reached 4°C. However, when final concentrations of egg yolk are kept below 2.0% of the diluent (Ritar and Salamon, 1982), egg-yolk-based diluents can be used without removal of seminal plasma.

For intracervical insemination, there are similar problems of sperm numbers in relation to the volume of inseminate to those that apply in the sheep. Hence, Salamon (see Evans and Maxwell, 1987) also suggested variable composition extenders (which also contain a final concentration of egg yolk of less than 2.0%) for one-step dilution of goat semen for use in intracervical insemination at dilution rates of between 1:1 and 1:4. Semen can be frozen either by the pellet method (i.e. on the surface of solid carbon dioxide) or in paillettes suspended over the vapour of liquid nitrogen. Since highly acceptable post-thaw recovery rates are achieved with the latter method, the convenience of handling semen means that it is usually the preferred method of cryopreservation. Although much variation exists between individual animals, overall results with doses of 50 million motile frozen-thawed sperm, inseminated into the uterus, are comparable with those of natural service (Ritar and Salamon, 1983). Yet the relationship between numbers of spermatozoa upon fertility is not well known, since few data from large-scale surveys exist on the subject. Nevertheless, Haibel (1986) suggests that between 100 and 125 million spermatozoa are used per insemination, while Evans and Maxwell (1987) recommend similar numbers to those used in sheep.

Similar routes of insemination can be used in the doe to those employed in the ewe. Intravaginal insemination is not widely used, so intracervical insemination is the most widely practised method. In a significant number of does, an intrauterine insemination can be achieved via the intracervical route, since the caprine cervix is relatively easier to traverse than the ovine cervix. Interestingly, although intrauterine insemination can be achieved simply by direct pressure of the insemination catheter upon the cervix, many technicians prefer to deposit at least some of the semen into the cervical canal, in case the intrauterine insemination had been entirely into a single uterine horn (Haibel, 1986). Because the intracervical route is relatively successful, the impetus to the use of laparoscopic intrauterine insemination has been far less compelling in the doe than in the ewe.

AI OF PIGS

One of the main stimuli to the use of AI in pig breeding is the possibility it confers of maintaining a closed herd, by obviating the necessity of introducing purchased boars to the herd. Furthermore, because the traits of economic importance in the pig – carcass conformation, growth rate and feed conversion efficiency – have high heritabilities and can be evaluated in the sires themselves, genetic selection can be intense, so that the potential value of AI boars in enhancing the genetic base of a breeding herd is considerable.

Traditionally, the use of AI in pig breeding has been greatest in the large farms of Eastern Europe and in those countries of Western Europe such as Holland and Denmark with very high densities of pigs. In the UK, uptake of AI has traditionally been at quite a low level (about 9% of the national herd; Iritani, 1980), but in recent years a modest but sustained increase has occurred (Reed, 1985). In Eastern Europe, where AI is practised more widely, there is perhaps as much as 30% of national herds bred thus (Reed, 1982). Most inseminations in the UK are performed by farm staff, for the costs of a technician service are too great to be economically viable, although technician services are available in some other countries. An increasingly prevalent trend is for 'in-house' AI services to be maintained by major pig-breeding corporations. These organisations run large-scale breeding and selection programmes for the genetic development of their stock, and disseminate the genetic outcomes of their selection processes to the various herds that produce breeding stock and slaughter-generation animals. Hence, such organisations are well placed to develop commercial AI services and to use AI as an efficient means of genetic dissemination within their own breeding units.

Two main difficulties exist in pig AI. Firstly, the period of maximum fertility is not particularly easy to detect in sows (Evans and McKenna, 1986) and, secondly, the semen of boars has a short storage life, due to its inability to survive cryopreservation adequately. Hence, for optimum conception rate and litter size, multiple inseminations are required during the oestrus period, using semen that has been extended but not chilled (Reed, 1982). In consequence, only about 30 inseminations can be obtained from each ejaculate, due to the high number of sperm (1000–2000 million) required for each dose and the fact that double insemination is generally practised.

Collection, handling and storage of semen

Semen is manually collected from boars in the presence of a dummy sow. The frequency of collection depends upon the semen characteristics of individual boars, but it is somewhere between once every 2–3 days (Evans and McKenna, 1986), 3–4 days (Almond et al., 1998) or 4–5 days (Reed, 1982). The pre-ejaculatory fluid is generally discarded, while the post-sperm rich fraction is filtered to separate the gel fraction. Between 100 and 150 ml of sperm-rich semen are produced which, after evaluation for density, motility and sperm morphology, is diluted for insemination. Insemination doses are variously recommended to contain between 1000 million and 2000 million sperm (Evans and McKenna, 1986), or, for local use within a breeding establishment, between 2000 million and 4500 million sperm per dose (Almond et al., 1998). The volume of material that is inseminated is important, for low-volume insemination fails to achieve acceptable conception rates. Hence, a total insemination volume of 50–100 ml (typically 70 ml) is used. The necessity for using such a large volume is probably to stimulate uterine motility in the sow, thereby ensuring that adequate numbers of sperm reach the site of fertilisation.

Diluents, which are designed to be used at ambient temperature, were initially based upon the Illinois variable temperature (IVT) diluent throughout the 1960s and 1970s (Paquignon, 1984). These have more recently been superseded by diluents such as the Guelph (also called Kiev or EDTA diluent), BTS diluent (Beltsville thawing solution) and Zorlesco diluent (Haeger and Mackle, 1971; Pursel and Johnson, 1975; Gottardi et al., 1980). The Guelph diluent is simpler than the IVT diluent to prepare, but maintains the fertility of semen for a similar length of time. The more complicated Zorlesco diluent was claimed to maintain semen fertility for up to 12 days, although field trials indicated that it was of little greater value than the Kiev diluent. Newer diluents, such as the Zorpva (Cheng, 1988) and Reading (Revell and Glossop, 1989) diluents have reliably maintained sperm fertility over longer periods of up 5 days and are, consequently, in increasingly widespread use (Almond et al., 1998). Examples of short- and long-term diluents are given in Table 31.6.

The semen of boars does not generally respond well to cryopreservation. Recovery after thawing is poor and highly variable between individual sires,

Table 31.6 Examples of ambient temperature diluents used for long- (5 days) and short- (3 days) term preservation of porcine spermatozoa (derived from Haeger and Mackle, 1971, Evans and McKenna, 1986, Cheng, 1988 and Almond et al., 1998)

Constituent	*Short-term: Guelph diluent (g/litre)*	*Long-term: Zorpva diluent (g/litre)*
D glucose	60.00	11.50
Trisodium citrate	3.70	11.65
Di-sodium EDTA	3.70	2.35
Sodium hydrogen carbonate	1.20	1.75
Polyvinyl alcohol (type 11)		1.00
Tris		5.50
Citric acid		4.10
Cysteine		0.07
Penicillin	5×10^5 IU (sodium)	0.60 (benzyl)
Streptomycin	0.50	1.00

and fertility of semen is invariably substantially below that of extended semen. Typical results for frozen semen are 40–50% sows conceiving to a double insemination, with 6–8 piglets born per litter. This compares with 65–75% conception rate and 9–11 piglets per litter following double insemination with extended semen (Reed, 1982). A great deal of research into the factors affecting survival of boar semen during cryopreservation has failed to improve matters substantially (e.g. Johnson and Larson, 1987), for there appear to be two major limitations to success. Firstly, neither egg yolk nor skimmed milk provides anything like the degree of protection against cold shock that it provides to the sperm of most other species (Watson, 1979). Nor, indeed, do any other cheaply available or natural sources of phospholipids. Secondly, while glycerol is probably the best cryoprotectant for the sperm of boars, its toxic effects upon sperm are more pronounced in this species than in most others (Wilmut and Polge, 1974). In consequence, the numbers of sperm that are required to obtain even the mediocre conception rates that follow the use of cryopreserved semen are very high indeed: typically 5000–6000 million sperm per insemination dose (Paquignon, 1984). Hence, when frozen semen is used, only about five animals can be inseminated per ejaculate. This low dilution rate effectively limits the use of frozen semen to international traffic in pig genetics and to the preservation of gene stocks for future use.

Insemination services

Inseminator service

Technicians employed by AI centres take the semen from the centre to the farm, where they inseminate the sows. This was the main form of insemination service in Western Europe when pig AI first started, but it has become progressively disfavoured, apart from in regions of exceptionally high pig density. Inseminator services generally provide only a single insemination of the sow in a given oestrus period, resulting in significantly lower conception rates and lower numbers of piglets per litter than are achieved by natural service (Reed, 1982). Also, the cost of a technician service is high in relation to the value of the progeny, especially as litter sizes are lower than with natural service. Furthermore, itinerant inseminators are potential vectors for disease transmission between farms. The trends towards closed herds and ‘minimal disease’ precautions on most pig farms have therefore mitigated against the use of such technicians.

Semen delivery service

This system was introduced into the UK in the mid-1960s (Melrose et al., 1968), since when it has undergone a steady expansion. Processed semen is either collected from the AI centre, or is despatched by rail or post to farms, where the sows are inseminated by the farm staff. This system overcomes most of the disadvantages of technician services, in that double inseminations can be performed, the risk of disease transmission is minimal and the cost is much reduced. It does require some technical proficiency on the part of the farm staff.

The use of AI with delivered semen has also received considerable impetus from increased use of batch management of pig herds. Thus, large groups of sows are weaned simultaneously, in the knowledge that most of them will be in oestrus at a predictable interval after weaning. Standing or advance orders are placed for the semen to inseminate these sows, with small numbers of boars kept to mate the small proportion of sows that return to oestrus outside the predicted time period, and to detect and mate animals that fail to conceive.

On-farm collection and insemination programmes

In the past this method was most widely used on the state farms and combines of Eastern Europe, where large numbers of sows had to be inseminated as cheaply as possible. In this situation, inseminations are performed with raw semen immediately after collection. However, in a number of countries, notably Spain and the USA, AI centres have provided producers with semen diluent for on-farm insemination programmes (Carbo and Dial, 1992). This method minimises the risk of disease transmission, but limits each farm to using the boars it has available. Access to performance/progeny-tested sires is therefore limited. This trend is being augmented by the increased use of 'in-house' insemination services of the major pig breeding and production companies, as described above.

Insemination of sows

The optimum time for AI is during a 24-hour period in the middle of the 50–60 hours of oestrus, so that capacitated sperm are present at the time of ovulation. The highest fertility results are achieved when insemination is undertaken 10–12 hours prior to ovulation (Evans and McKenna, 1986). However, the interval between the onset of oestrus and ovulation is variable, whilst even twice-daily oestrus detection may not accurately pinpoint the onset of oestrous behaviour. Hence, it is recommended that optimal conception rates and litter sizes are achieved by delaying insemination until 12–18 hours after the first detection of oestrus, with a second insemination 12 hours (Evans and McKenna, 1986) or 18–24 hours (Almond et al., 1998) later. In practice, this means that, if oestrus is first detected during the morning session, the sow should be inseminated for the first time that afternoon, or if oestrus is first observed in the afternoon, she should be inseminated the next morning.

A positive reaction to the 'back-pressure' test is the best indication of the correct time for AI (Madden, 1959), although up to 30% of gilts might pass through oestrus without giving a positive reaction. Oestrous behaviour is manifested most strongly in the presence of a boar, but if a boar is not available, oestrous behaviour can be elicited by spraying an aerosol containing the androgenic steroid present in the saliva of boars, which is responsible for boar odour (Reed et al., 1974). Oestrus detection can be difficult where sows are tethered in stalls, and the management of AI is also difficult in herds of pigs that are kept out of doors.

Insemination is achieved (Rowson, 1962; Melrose and O'Hagen, 1969) by passing a spiral rubber catheter into the cervix of the sow. The catheter is rotated into the cervix, until its spiral groove becomes locked into the cervical canal. The cervical lock prevents the catheter from becoming dislodged if the sow moves during insemination, and prevents loss of the large volume of fluid that has to be inseminated. The patience, gentleness and quietness of the inseminator greatly affect the success of insemination.

Insemination with the semen of a single boar is now the exception, rather than the rule, on most units other than studs. Instead, semen from two different boars may be used at the two successive inseminations, or the sperm from two boars can be mixed within an insemination dose. The reasons for this are twofold. Firstly, there is the pragmatic reason that, if one boar is infertile, it is unlikely that both will be affected. More interestingly, there is some evidence of increases in fertility when heterospermic inseminations are performed (Almond et al., 1998). When AI is first introduced to a production unit, there is usually a drop in fertility as staff become familiar with the procedure. One method that is recommended to avoid this drop is to use a combination of AI and natural service during the transition period. The rate of genetic progress will be reduced, but the decrease in fertility, which is likely to be of far greater economic significance, will be avoided (Almond et al., 1998).

Control of infectious diseases

A large number of pathogens can be transmitted through boars' semen. These include *Brucella suis*, *Staphylococcus aureus* and *Streptococcus spp.*, which are transmitted by the venereal route and have significant effects upon sow fertility. *Leptospira* serovars and *Erysipelothrix rhusiopathia*, whilst not directly spread through semen, are spread as urinary contaminants, so contamination of the semen through contact with urine or the presence of the organisms within the prepuce is likely (Cutler, 1986). More seriously, classical swine fever (hog cholera), African swine fever, porcine parvovirus and Aujeszky's disease can also be spread through semen, as, potentially, can foot-and-mouth virus (Cutler, 1986; Mengeling, 1986; Almond et al., 1998). Surprisingly, given the close regulation of bovine semen practised by most agricultural authorities, there is very little statutory regulation of the health status of AI boars. However, most AI studs undertake serological testing of boars to ensure that they carry neither venereally transmitted diseases nor other conditions that might jeopardise the health status of the herd. Antibiotics are added to semen to remove non-specific and pathogenic bacteria. However, the most valuable means of controlling disease transmission is to quarantine incoming boars for a month, during which time observations for signs of clinical disease and/or further serological testing are undertaken.

AI OF HORSES

AI of horses has been practised for many years; indeed, legend holds that, in medieval times, semen would be stolen from the freshly served mare of one's neighbour in order to inseminate one's own mare! Today, AI of horses is practised widely in Eastern Europe, the former USSR (Tischner, 1992) and China, while in the West it is used in most classes of horse other than the thoroughbred. The lack of use of AI in this breed is due to the refusal of the Thoroughbred Breeders' Association to allow registration of foals conceived by AI. Some other breed societies similarly restrict the use of AI, with the result that the method has failed generally to achieve its potential in Western horse breeding. Even in the face of venereal disease, such as the outbreak of contagious equine metritis, the restriction on the use of AI in thoroughbreds was not relaxed, thereby precluding the substantial benefits offered by the technique in the control of such a disease. In other breeds, AI, after appropriate treatment of the semen with antibiotics, has proved a most useful method of achieving pregnancies in mares that regularly have post-covering endometritis.

The technical demands of AI of horses differ somewhat from those of cattle. Firstly, it might be considered that the collection of semen from stallions is more difficult than from bulls, for coitus is both more prolonged and more violent in horses than in cattle. Secondly, the stallion produces a fractionated ejaculate, from which the viscous, post-sperm-rich fraction has to be separated and discarded (Brinsko and Varner, 1993). Thirdly, stallion semen has proved more difficult to store than has that of bulls, with cryopreservation proving difficult to achieve consistently (Amann, 1984). Finally, the peculiarities of the oestrus period of the mare make pinpointing the moment of peak fertility more difficult than in the cow. Mares do not reliably display visible signs of oestrus in the absence of a stallion and, furthermore, the oestrous period is prolonged, so that unless the ovaries of the mare are palpated to determine the time of ovulation, repeated insemination is likely to be required to ensure that the fertile period is covered. However, insemination of the mare is much easier than insemination of the cow, for the equine cervix is soft and does not have a convoluted luminal canal.

Handling, dilution and storage of semen

After collection, equine semen requires careful temperature control to prevent damage to the sperm by cold shock (Brinsko and Varner, 1993). If the mares that are to be inseminated are close by, insemination can be performed directly, using raw semen. Alternatively, the semen can be diluted (at a ratio of about 1:3) in a simple diluent (e.g. skimmed milk plus antibiotics; Table 31.7) and stored at 4°C for 12–48 hours before insem-

Table 31.7 Diluents for short-term storage of equine semen at 4°C (from Tischner, 1992; Brisnko and Varner, 1993; Blanchard et al., 1998)

Component (g/litre)	*Skimmed milk diluent (Kenny)*	*Modified Kenny extender*	*Egg yolk–glucose*	*Egg yolk–milk*
Skimmed milk powder	24	24		
Cow's milk (pasteurised)				1000 ml
Glucose	49	26.5	70	
Sucrose		40		
Sodium hydrogen carbonate	20 ml of 8.4% solution			
Egg yolk			70 ml	70 ml
Penicillin	1×10^6 IU	1×10^6 IU		5×10^5 IU
Streptomycin	1.5 g	1.5 g		0.5 g
Gentamycin	1.5×10^6 IU (pen/strep alternative)			

ination. Most equine AI is performed using direct insemination of chilled semen that has been stored for a short period (Klug, 1992; Brinsko and Varner, 1993). However the use of more complex diluents, together with the removal of seminal plasma by centrifugation, may extend the storage life of semen for up to 72 hours (Martin et al., 1979). The actual length of time for which the semen of an individual stallion remains fertile depends primarily upon the quality of the initial sample and upon the idiosyncrasies of the individual animal.

Stallion semen has been successfully cryopreserved (Pickett and Amann, 1993), using low concentrations (4%) of glycerol, in diluents (Table 31.8) containing either glucose skimmed milk and egg yolk (Rajamannan, 1968), or lactose, EDTA and egg yolk (Nishikawa et al., 1972; Cochran et al., 1984). In all cases, the seminal plasma was removed before freezing. Freezing (Amann and Pickett, 1987) may be undertaken in controlled-rate freezers, using moderate –10°C/min between +20 and –15°C, –25°C/min between –15 and –20°C) freezing rates, or, using less accurately controlled conditions, in the vapour of liquid nitrogen or as pellets on the surface of blocks of solid carbon dioxide. The timing of insemination in relation to ovulation is critical when cryopreserved semen is used, with even less tolerance than for unfrozen semen.

Table 31.8 Cryopreservation diluent for equine spermatozoa (see Pickett and Amann, 1993; Blanchard et al., 1998)

Centrifugation medium (per litre)	
D-glucose	59.98 g
Di-sodium EDTA	3.70 g
Sodium hydrogen carbonate	1.20 g
Penicillin	10^6 IU
Amikacin SO_4	1.00 g
pH	6.9 (adjust with $NaHCO_3$)
Cryopreservation medium	
D-lactose (11% w/v)	50 ml
Centrifugation medium	25 ml
Egg yolk	25 ml
Glycerol	5.0 ml
Equex STM	0.8 ml

Centrifugation medium is for the removal of seminal plasma, after which the semen is resuspended in cryopreservation medium

Evaluating the fertility of cryopreserved equine semen is not a straightforward process. Post-thaw motility is a poor predictor of fertility in all species; it screens out the worst ejaculates, but fails to discriminate between levels of fertility. In the stallion, it is particularly unreliable (Pickett and Amann, 1993), given the minimal amount of reliable data on conception rates in mares. However, attempts have been made to find objective measurements that have high levels of correlation with fertility. Christensen et al. (1997) used in vitro induction of acrosome reactions as a predictor of fertility, while Samper (1992) and Hellander (1992) have used combinations of glass-wool/Sephadex filtration and motility estimates to predict fertility. Nevertheless, despite the progress that these methods represent, it remains difficult to predict the fertility of a stallion's semen after cryopreservation.

Insemination

Unless palpation of the ovaries of the mare is undertaken to determine the timing of ovulation, AI needs to be performed every other day throughout oestrus when chilled, extended semen is used, or daily if the semen has been cryopreserved. However, if the presence of an ovulable follicle is determined, and insemination of frozen-thawed semen performed within 6 hours of ovulation (Kloppe et al., 1988) acceptable success rates can be achieved. Rather more latitude exists for chilled semen; insemination with semen from stallions of high fertility within 48 hours of ovulation produces acceptable pregnancy rates, although inseminations of semen from less fertile stallions need to be within 12–24 hours (Brinsko and Varner, 1993). Thus, at best, similar success rates can be achieved with natural service and AI with chilled or cryopreserved (with adequate post-freezing motility) semen. In most field studies, however, pregnancy rates achieved with frozen-thawed semen are significantly lower than with other methods (Pickett and Amann, 1993).

Insemination is best performed with the mare restrained in stocks. After applying a tail bandage and cleaning the perineal area, a hand is inserted into the vagina and the cervix located. The index finger is inserted into the cervix and an insemination catheter passed through the vagina, then alongside the index finger and so into the uterus.

Debate exists over the numbers of sperm and volume of semen required for adequate fertility. Initially, inseminations with chilled, extended semen used $1–2 \times 10^9$ sperm in a volume of 10–50 ml (Arthur et al., 1989). With an insemination dose of 1×10^9 sperm, conception rates of 73–75% have been recorded for this method (Hellander, 1992b). Selection of donor stallions allowed insemination doses to be reduced to $250–500 \times 10^6$ sperm, without adversely affecting conception rates. For highly fertile stallions, whose semen is inseminated close to the time of ovulation, reduction of the dose of sperm to 100×10^6 is quite feasible (Blanchard et al., 1998). It is possible that the semen of fertile stallions could be further diluted (Pace and Sullivan, 1975; Pickett, 1980). However, there is very considerable variation between stallions, even in the survival of diluted and chilled semen. Thus, for routine insemination with chilled semen, around 250–500 million total sperm are used (Klug, 1992; Brinsko and Varner, 1993). Sperm numbers required for acceptable fertility with frozen-thawed semen are of the order of 100–200 million motile sperm.

Control of infectious diseases

A considerable number of pathogens are transmissible through equine semen. Most importantly, this includes equine viral arteritis, which can be shed into the semen of viraemic and recovered stallions. Many other viruses can be transmitted through semen, including EHV-III, equine infectious anaemia and, possibly, EHV-II and vesicular stomatitis. The case with EHV-I is less clear-cut, for, although its transmission through semen appears likely, no recorded cases have occurred (Klug and Sieme, 1992). Many non-specific bacterial contaminants of semen may cause infertility in inseminated mares. The presence of β-haemolytic streptococci in semen is associated with reduced fertility of mares (Klug et al., 1974), as are haemolytic *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Klebsiella spp.* are of more clear-cut significance as a cause of infertility, while *Taylorella equigenitalis*, the causal organism of contagious equine metritis, is an important venereal pathogen. Within its geographical area of distribution, *Trypanosoma equiperdum*, the protozoon responsible for dourine, is venereally transmitted. Legislative control exists in many countries to control the spread of equine viral arteritis, equine infectious anaemia, contagious equine metritis and dourine, at least in the blood-stock industries, but control of venereal diseases in ponies and riding horses is often minimal. The simple precautions of serological examination of stallions for the presence of viral infection and serial bacteriological examination of their external genitalia before using the animals as AI (or, indeed, natural service) sires are all that is required to control most venereal pathogens, yet, all too often, this does not occur until after the onset of a disease outbreak.

AI OF DOGS

It was upon the dog that the earliest recorded studies of AI were undertaken, by the Italian natural philosopher Spallanzani. Despite such an impeccable pedigree, relatively little demand exists for AI of dogs, with a significant degree of resistance to it by many breed societies. Thus, AI of dogs is limited to two main circumstances. Firstly, it is used where copulation is not possible. Secondly, AI is employed as a means of using sires that are geographically remote from the bitch, particularly where these reside in a different country. For many breed societies, this latter circumstance is the only one under which they will allow registration of puppies conceived by AI.

Collection handling and storage of semen

Semen is collected from the dog by digital manipulation or by artificial vagina. The pre-ejaculatory fluid, sperm-rich fraction and a little of the post-ejaculatory (prostatic) fluid are collected. The whole ejaculate may be immediately inseminated into the bitch's vagina, but it is more common to dilute the semen so that multiple inseminations can be performed.

Dog semen can be stored in a chilled condition for 24–72 hours at 4°C, after dilution in simple diluents (e.g. skimmed milk) (Harrop, 1962). This is generally long enough for at least two inseminations to be performed on the bitch, or for air freight transport to most international destinations. Hence, this method is very widely used in dog AI.

Cryopreservation of dog semen is more difficult than for many species, but success has been improving over recent years. Many different diluents have been tried, largely on an empirical basis or because they work for other species. Egg yolk appears to be an important component of most diluents. Likewise, many different glycerolisation regimens have been employed, in an attempt to achieve a satisfactory compromise between its toxic effects upon sperm and the concentrations needed to give effective cryoprotection (England, 1993). Early methods for freezing relied upon the well-tried routes of freezing in pellets on blocks of solid carbon dioxide, or in paillettes suspended in the vapour of liquid nitrogen. More recently, the use of programmable freezers has resulted in significant improvements in post-thaw recovery rates, with control of cooling rates over the range between +4°C and –35°C being most important.

Insemination

The bitch has a prolonged period of receptivity to the male, but a relatively short fertile period. When natural service is used, many breeders allow bitches only a single mating, which typically occurs 12 days after the onset of pre-oestrous bleeding. For successful AI, much closer attention to the time of the fertile period is needed, with the timing of ovulation predicted from vaginal cytology or the preovulatory rise in circulating progesterone concentrations. Timing of insemination in relation to ovulation is particularly crucial when cryopreserved semen is used, although more latitude exists where chilled semen is used (Jeffcoate and Lindsay, 1989; Linde-Forsberg and Forsberg, 1989, 1993; Morton and Bruce, 1989).

Where the reason for AI is failure of copulation, semen is collected from the dog by digital manipulation, and the whole ejaculate may be inseminated into the vagina of the bitch immediately after collection. It may, however, be preferable to dilute the semen, inseminating one portion immediately and the remainder 48 hours later. Similarly, where chilled semen has been transported from the sire to the bitch, insemination may be performed upon receipt of the semen and 48 hours later. It is therefore imperative to ensure that the recipient bitch is approaching the fertile period of oestrus before semen is collected.

When fresh or chilled semen is used, intravaginal insemination is undertaken, with the semen deposited as close as possible to the external os of the cervix (Burke, 1986). The semen may be deposited through a shortened bovine insemination catheter, which may require the use of a speculum to be guided into the correct site. Once inseminated, the hindquarters of the bitch should be raised for a few minutes, to prevent retrograde loss of semen. Some authors recommend inserting one or two fingers into the vulva after insemination, in order to promote the motility of the female genital tract that normally occurs during the copulatory tie.

The fertility achieved in canine AI primarily depends upon achieving a correct insemination timing in relation to that of ovulation in the bitch. The inherent fertility of the dog is also of importance, with the longevity of his sperm in the female tract being a critical determinant of fertility. Dogs with long-lived sperm can achieve pregnancies even if the timing of insemination is not optimal, whereas with dogs whose sperm have poor survival, sperm death is more likely to have occurred prior to ovulation under such circumstances.

Where frozen semen is used, timing of insemination is critical and, as with most species, there is very considerable sire-to-sire variation in the ability of sperm to survive cryopreservation. Linde-Forsberg and Forsberg (1993) reported pregnancy rates of 39.0% with frozen semen, compared to 54.7% with fresh (diluted or raw) semen, with litter sizes of 4.1 and 5.9 pups, respectively. The site of insemination is also important; intravaginal insemination with cryopreserved sperm gives poor conception rates, so an intracervical or intrauterine route is required. Intrauterine insemination was first described by Andersen (1975) and is sometimes recommended for use with cryopreserved semen. Non-surgical, transcervical insemination was used by Wilson (1993) and Fontbonne and Badinand (1993) to achieve good pregnancy rates (85% pregnancy rate, 7.8 pups per litter).

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32 Reproduction in the camel

The one-humped (dromedary) camel (*Camelus dromedarius*) is economically important in northern Africa, particularly Sudan and Somalia, as well as in the Arabian states and in the Indian subcontinent. The two-humped (Bactrian) camel (*Camelus bactrianus*) is bred mainly in Russia and Central Asia. Many camels are exported from the Sudan and Somalia to Egypt and Saudi Arabia.

Female camels reach puberty at 3 years of age, but are not usually mated until they are 4–5 years old. Male camels are sexually active at 3 years of age, but are not usually used at stud until they are 5–6 years old. Both sexes breed throughout their lives but it is customary to breed from the female in alternate years, the gestation period being 370–400 days.

MALE REPRODUCTION

When sexually mature, in most countries male camels show an annual rut associated with a decline in day length and, roughly, from November to July, after which they are sexually quiescent (Al-Eknah 2000). During the rut, the temperament changes towards an aggressive, less tractable nature, including a predisposition to fight other males and an inclination to bite other animals, as well as human beings. Rutting males are notoriously unpredictable and should be approached with due regard to their potential aggression. When urinating they stand with hindlegs spread apart and spray the urine around their hindquarters by vigorous movements of the tail. A prominent feature of rutting behaviour is frothing at the mouth and loud vocal gurgling, accompanied by the protrusion of the markedly oedematous and mobile soft palate. An additional peculiarity is a profuse secretion of fetid fluid from the poll glands. The rutting peculiarities of male camels are especially marked in the presence of an oestrous female, and when the male smells her urine he displays the characteristic flehmen response.

The testes are relatively small. They are descended at birth. The non-erect penis is directed backwards and its extremity ends in a hook; otherwise it resembles that of the bull, and extension of it at coitus depends mainly on the straightening of the sigmoid flexure of the organ.

FEMALE REPRODUCTION

The structure and function of the genitalia of the female camel show several noteworthy features. The uterus is bicornuate, with a well-developed uterine body, from which the two horns diverge and taper cranially to give a combined uterine shape intermediate between that of the letters Y and T. The left horn is longer than the right, even in the fetus. The endometrium shows irregularly raised, mainly longitudinal folds, which are more conspicuous in the right horn. The cervix somehow resembles that of the cow but has five annular mucosal folds. A few centimetres behind the cervix is a concentric fringe-like fold of the anterior vaginal mucosa, which tends to obscure the os uteri externum, and behind it are several progressively less prominent circular folds.

The uterine tubes are 22–24 cm long. Their width increases towards the ovarian end, where the tubes are obviously funnel-shaped. The mesosalpinx and the mesovarium together form a very well-developed bursa that closely invests the ovary. The size and shape of the ovaries vary with their content of follicles and corpora lutea. The anoestrous ovary is roughly oval and thin, measuring about $4 \times 2.5 \times 0.5$ cm. Its surface is uneven and shows many small follicles and, in mature animals, projections of old corpora lutea.

The latter, corpora albicantia, are cream-coloured and up to 0.6 cm in diameter.

Mature follicles and current corpora lutea of the breeding season project from the main contour of the ovary and give the latter an exaggerated, lobular form. Graafian follicles may grow to a size of 10 cm in diameter, but a commoner ovulating size is 1.5–3.0 cm. The mature follicle can easily be detached from the ovary as a discrete sphere by gentle pressure at its attachment. Follicles also develop, but do not ovulate, at the beginning and end of pregnancy. The wall of the mature follicle is vascular; the follicular fluid is at first yellowish and later red.

Because ovulation is a sequel to coitus, corpora lutea are to be expected only in pregnant camels; however, smaller, apparently functional corpora lutea occur and probably they are a legacy of early pregnancy failures. The young corpus luteum is soft and spherical, brownish on section, with a central blood clot. It can be detached from the ovary by digital pressure exerted at its base. The mature corpus luteum is a compact sphere of 2.6 cm diameter and is flesh-coloured, with a central area of grey connective tissue. Older corpora lutea have a greenish or bluish grey external appearance. Corpora lutea persist throughout pregnancy, and the established corpus luteum cannot be detached from the ovary by finger pressure.

Corpora lutea, presumably from previous pregnancies (corpora albicantia), appear as creamy bodies of up to 0.6 cm diameter in the substance of, or projecting from, the flat main body of the ovary.

By repeated rectal palpation of the genital organs of female camels during the breeding season, Musa (1975) found that a period of about 6 days is required for a developing follicle to reach its full size (of 1.5–3 cm, but occasionally up to 10 cm); full size is maintained for an average of 13 days and then follicular regression occurs during the following 8 days. Another follicle then begins to develop in the other ovary. During the breeding season, from December to June in the Sudan, there are six or seven of these follicular cycles in unmated animals. This breeding season is followed by an anoestrous period of 6 months' duration. However, camel breeders in Saudi Arabia say that well-fed camels will cycle and breed throughout the year, but in natural conditions food shortage probably decreases breeding activity. The author's experience in that country was that newborn camels could be seen throughout the whole period from September to May and that there was a concentration of births in December, January and February.

The left and right ovaries function equally and ovulate alternately. Because ovulation is induced by coitus the length of oestrus depends on whether and when mating occurs. In the absence of a male, oestrus may last about 2 weeks, whereas if copulation occurs on the first day of oestrus, receptivity may disappear after 3 days. Twin ovulation occurs in 14% of matings (Musa, 1975).

Studies in China on the ovulation mechanism of the Bactrian camel (Chen et al., 1985) revealed that ovulation is induced by a factor or factors in seminal plasma. Furthermore, ovulation could be stimulated by either intrauterine or intramuscular injection of seminal plasma, while bovine seminal plasma had the same effect. The intervals from the intramuscular injection to the subsequent luteinising hormone (LH) peak in peripheral blood, and to ovulation, were the same as those that follow natural mating. The authors concluded that there is a gonadotrophin-releasing (GnRH)-like hormone in seminal plasma.

The signs of oestrus are restlessness, bleating, vulval swelling and mucous vaginal discharge. The female camel urinates and moves its tail up and down in rapid succession on the approach of the male, or when hearing the gurgling voice of the rutting male. At range, the rutting male pursues the oestrous female and, on catching up with her, presses his head on her neck and induces her to sit down. The male then mounts in a squatting posture. Copulation lasts from 8 to 15 minutes; it is accompanied by much male oral frothing and gurgling, with intermittent protrusion of the soft palate, and by female bleating. During these mating activities, which are interspersed with several bouts of male pelvic thrusting and correspondingly louder vocal responses from the female, the rest of the herd becomes alerted and assembles in a circle round the copulating pair. [During the phase of follicular growth the uterus develops an increasing tone, and when the follicle is ripe the cervix will admit two fingers.]

Pregnancy

Despite the equal function of the right and left ovaries, 99% of pregnancies are in the left horn (and uterine body) and although the incidence of twin ovulation is 14%, twin births occur to an extent of only 0.4% (Musa, 1975). Embryonic migration from right horn to left is frequent and always seems to occur when the right ovary ovulates and the left ovary does not. When both ovaries ovulate at the same oestrus, embryos develop initially in both horns but the one in the right horn dies when it reaches a size of 2–3 cm. This embryonic death occurs despite the coalescence of the chorions of two embryos. Presumably, allantoic vascular anastomosis does not take place, as in the bovine, and there is no record of a freemartin among the small number of twins born. It is a unique biological curiosity that successful placentation of a right-horn embryo does not develop in the corresponding horn, whereas the placenta of a left-horn embryo regularly intrudes into the right horn and develops extensively throughout the right horn.

As in the cow, the allantois of the camel elongates quickly and soon protrudes from the left horn into the uterine body and the right horn. Because the uterine body is relatively large, the shape of the whole placenta resembles more closely that of the mare than the cow. Moreover, the camel placenta is diffuse, like the mare's, not cotyledonary.

The amount of allantoic fluid increases from about 1.5 litres at a fetal body length 0–10 cm to approximately 5–6 litres at a body length 11–20 cm. This volume is maintained fairly constantly until 90–100 cm, when it rises to 6 litres; finally, at a fetal body length of 101–107 cm, the allantoic volume is about 8 litres. The allantoic fluid is like pale urine and sometimes contains yellow–brown hippomanes. From the gestation stage when the fetal body length is 41 cm, Musa (1975) noticed an inner amnion that very closely invests all the fetus except its orifices.

The volume of amniotic fluid rises from 13 ml at a fetal body length of 0–10 cm to a final volume of nearly 1 litre, i.e. its amount is always small relative to the allantoic fluid. The amniotic fluid is usually watery but sometimes cloudy and brown with bits of meconium and hippomanes.

Fetal growth is of a linear pattern. Posterior presentations predominate (54–60%) from early pregnancy to a fetal body length of 41–50 cm, at which point the situation changes to an anterior presentation of 51%. Thenceforward anterior presentations increase sharply to 93% at a fetal body length of 61–70 cm and then to the final gestation presentation of nearly 100% anterior. By post-mortem manipulation, Musa and Abusineina (1976) found they could not alter the presentation beyond the stage of 61–70 cm fetal body length. There is no tendency in late pregnancy for the amnion to separate from the allantochorion as it may do in the cow.

Pregnancy diagnosis

An intriguing and commonly held belief among camel owners is that a female camel, when pregnant, will curl its tail dorsally on being approached by a rutting male. Until recently, veterinarians have regarded this sign as unreliable. However, Abdel Rahim and Al-Nazeer (1992), in a well-conducted study of housed camels, have stated that from 2 weeks after mating this is a constant reaction of pregnant camels to a male-camel approach.

Clinical methods

Transrectal palpation. Barmintsev (1951), working with the Bactrian camel, and Musa and Abusineina (1978), using the dromedary, have given accounts of the rectal method of diagnosis. The technique of palpation of the genital organs is the same as for the cow, but the female camel needs to be restrained in the sitting position. In connection with early diagnosis, it is important to remember four features of camel reproduction:

- Large corpora lutea are only present in pregnancy.
- 99% of pregnancies are in the left horn.
- The empty (or early pregnant) right horn is congenitally shorter than the left.
- The amount of fetal fluid at all stages of camel pregnancy is less than in the cow.

From the foregoing, it is clear that the presence of a palpable corpus luteum in one or both ovaries is a very strong indication of pregnancy. However, a corpus luteum would form after a sterile mating and would be present initially in the cases where embryonic death occurs; in both these instances, however, the corpus luteum would not persist.

The earliest palpable swelling of the pregnant (left) uterine horn is at 1 month in the Bactrian camel according to Barmintsev (1951). In the dromedary, however, Musa and Abusineina (1978) are emphatic that no swelling is palpable until the eighth week, when the whole of the left horn is uniformly enlarged. At this time both ovaries (one or both with corpora lutea), together with the uterus, are within the pelvis. It should be noted that because the camel placenta is non-cotyledonary, it is not possible to 'slip the fetal membrane' as in the cow. By the eighth week, vaginal palpation or inspection reveals a plug of adhesive mucus in the os uteri externum.

At the end of the third month the pregnant left horn is clearly larger and softer and in front of the non-pregnant right horn. It is at the pelvic brim and its corresponding ovary is in the abdomen. At the fourth month the uterus is just in front of the pelvic brim but most of it is palpable. A month later the limits of the uterus cannot be defined, although its dorsal surface is still palpable. During the sixth month, and for the remainder of pregnancy, the fetus can be palpated and the ovary on the non-pregnant (right) side can be felt until the tenth or eleventh month. From the seventh month individual parts of the fetus, namely head and legs, can be identified. External observation of the right flank reveals spontaneous fetal movements from the ninth month and the fetus can be ballotted from the 10th month.

In the 11th month the vulva is slightly swollen and hypertrophy of the udder is first noticed. In the following month there is abdominal enlargement and the camel is lethargic. The caudal part of the uterus now projects backwards and occupies the anterior two-thirds of the pelvis. The sacrosciatic ligaments begin to relax.

In the 13th month relaxation of the pelvic ligaments is pronounced, tumefaction of the vulva is marked and hypertrophy of the udder is more evident. The fetus can be ballotted from both flanks.

Incidentally, regarding the length of gestation in the camel, almost incredible variations of 308 to 440 days have been given. The mean duration is probably around 375 days.

Ultrasonography

Diagnosis of pregnancy by transrectal B-mode realtime ultrasound scanning can be made consistently at 17–18 days after mating (Tinson and MacKinnon, 1992). The conceptus then appears as a discrete, non-echogenic, fluid-filled structure, about 4–6 mm in diameter and 10–15 mm in length. The embryo is first visible at about 20 days, when its heart beat is also detectable, and the amnion can be recognised as a slightly echogenic band around the embryo at about day 35.

Laboratory methods

Progesterone test. Because persistent corpora lutea are said to be present only in pregnancy, a progesterone assay carried out after a suitable interval from copulation should be effective in distinguishing pregnancy from non-pregnancy. This has been confirmed by Elias et al. (1985) and Xu et al. (1985), and later by other investigators, as follows: unmated and anovulatory camels have basal progesterone levels; those that ovulate but do not conceive show a peak value at 6–10 days after mating, which declines to the baseline by day 12; pregnant camels show raised progesterone levels throughout gestation. Obviously, embryonic death could occur after a positive test result, and it remains to be determined in what percentage of instances this would negate the result. Presumably milk, in lactating animals, or blood could be used for this test.

Gonadotrophin test. El Azab and Musa (1976) have demonstrated the presence of follicle-stimulating hormone (FSH) in the blood of pregnant camels using immature female mice, as in the method devised by Cole and Saunders (1935) in the mare. The mouse ovaries showed marked follicular activity when injected with the blood of camels pregnant with fetuses of fetal body lengths between 11 and 58 cm. However, the authors did not mention any spate of follicular activity in the maternal camel ovaries, as occurs in the mare, when equine chorionic gonadotrophin (eCG) is present. The present author has seen no such activity in his study of the pregnant camel; nor has he found endometrial cups that are the source of eCG. The source of the gonadotrophic factor in the camel is therefore unknown but it is presumably of placental origin.

Oestrogen test. El Ghannam et al. (1974) found that the Cuboni test for the demonstration of oestrogenic substances in urine can be successfully applied for pregnancy diagnosis in camels. The technique is the same as that described for the mare. In this connection no inordinate growth of fetal gonads during the second half of pregnancy like that of the mare has been seen in the camel. In the mare the fetal gonads are believed to be the source of the large amount of oestrogen present in the blood and urine of the mare.

Parturition

The premonitory signs of approaching parturition are abdominal distension, formation of grooves on each side of the base of the tail (due to relaxation of the sacro-iliac ligament), mammary hypertrophy with presence of colostrum, liquefaction of the cervical seal and oedema of the vulva. Most camels calve in winter and spring.

First-stage labour, which lasts about 2–7 hours, commences with the intensification of restless behaviour, and ends with the complete dilatation of the cervix and the appearance of fetal extremities and membranes. Normally, the duration of the second stage of parturition is about half an hour. During this expulsive phase, the female camel assumes a variety of postures; these are staggering, rolling, standing or sitting. If the placenta is retained for more than a day, the mother is said to become ill due to the development of endometritis.

During the second stage the she-camel adopts a sitting posture. The allantochorion ruptures before reaching the vulva. There are bouts of straining at intervals of 30–60 seconds, and the fetal nose, covered by the amnion, appears first. Later one front foot and then the other are protruded alongside the head, and with further straining, they are both extended well beyond the head. Maximum straining effort then leads to complete emergence of the head, and the rest of the body quickly follows. In comparison with other domestic species, the impression of second-stage labour in the camel is of a rather effortless expulsion of a well-lubricated and beautifully streamlined fetal body. The umbilical cord ruptures as the offspring wriggles away from its mother or when the mother gets up, as she does immediately after the birth. She noses and nibbles at the offspring but does not lick it as do other animals.

During the third stage, the female camel shows intermittent restlessness and may get up and down several times. The afterbirth progressively emerges, and includes large retention sacs of allantochorion, containing up to 5 litres of allantoic fluid that presumably exert a gravitational pull on that part of the afterbirth still attached. The fetal membranes may be completely expelled soon after the fetus or, more commonly, within 1 hour of the end of the second stage; they are not eaten by the mother. The young camel can stand, after many unsuccessful attempts, within 30 minutes.

Dystocia

There is a scarcity of published information. However, conversations with Bedouin camel owners indicate their familiarity with the recognition and treatment of dystocias due to faulty disposition, such as carpal flexion, lateral deviation of the head and hock and hip flexion, although posterior presentation is uncommon. Fetopelvic disproportion, monstrosities and transverse presentations are rare and the frequency of twin births is of the order of 0.1–0.4%. In a recent reported study conducted over 10 years in Saudi Arabia, maternal and fetal causes were responsible for 43 and 57% of the cases, respectively. Of the maternal causes, uterine torsion was responsible for 50%, primary uterine inertia for 20%, incomplete cervical dilatation for 20%, and vaginal and vulval stenosis for 10% (Al-Eknah, 1999). In the same study, faulty fetal posture was responsible for 90% of fetal dystocias.

Under field conditions, the correction of limb and neck flexions, where substantial fetal retropulsion is required because of the length of the neck and the limbs, the female camel is placed, front end first, into a deep pit, excavated in the sand. Head and limb extension seems much more difficult to achieve than in the cow. Limited experience indicates that the camel fetus survives dystocia better than the equine fetus, and that the camel is a good subject for the caesarean operation in cases of irreducible malpresentation with a living fetus. When a malpositioned fetus is dead, fetotomy using Thygesen's embryotome seems feasible. For caesarean operations the camel is cast on its right side under xylazine sedation and the operation is performed under regional infiltration anaesthesia, along a vertical incision in the posterior aspect of the left sublumbar area. The writer and his colleagues have delivered by caesarean operation a live fetus, with irreducible hock flexion, 17 hours after rupture of the amniochorion.

Infertility

The fertility of camels is good. According to Bedouin breeders, for every 100 camels mated, 80–90 produce calves. About 1% are sterile. Poor nutrition in seasons of low rainfall and resultant poor grazing are causes of reduced sexual activity in both sexes. Abortion is rarely seen. Unthriftiness due to disease leads to infertility, and pleuropneumonia is a cause of abortion.

Their fertility is maintained throughout life; breeding in alternate years, which is the usual practice, a female can yield a total of 12 offspring, although an average of something less than eight seems more likely. One mating per oestrus is usually allowed and it is possible for a male to serve five or six females in a day. It is said that one male can sufice for 200 females, with controlled breeding, but a much smaller number is customary.

In abattoir genital tracts, endometritis, associated with a partially involuted uterus and a regressing corpus luteum, is sometimes seen. These are presumably post-parturient or post-abortion cases. The author has seen no cases of ovarobursal adhesions or of cystic ovaries and has not read of any in the literature. As mentioned in the discussion of the observed incidence of corpora albicantia, embryonic death after twin conception and fetal death after single or twin conception are probably common.

Wernery and Wernery (1992) studied the uterine bacterial flora of 80 barren camels in two herds bred for racing in Dubai – some with and some without endometritis. Their main findings were as follows:

- The range of organisms isolated was very similar to those obtained from equine and bovine uteri except that *Streptococcus zooepidemicus*, which is the commonest pathogen recovered from equine uteri, and the organism of contagious equine metritis (*Taylorella equigenitalis*) were not found.
- *Campylobacter fetus* and *Tritrichomonas fetus* were both isolated from infertile camels.

According to Bedouins, unsuccessful copulation due to incomplete intromission is not uncommon. In the belief that intromission is intracervical, they treat this condition by gentle and gradual digital dilatation of the cervix, using butter as a lubricant. After this treatment complete intromission is said to be possible and pregnancy ensues.

Prolapse of vagina

Several cases of vaginal prolapse have been seen in pregnant camels whose exercise was restricted and which were fed ad libitum on lucerne and barley. Bedouins control the condition by the application of a body truss of bandages, which exerts constant pressure on the perineum. One case, for which veterinary attention was requested, was successfully treated by the Bühner technique of vulval circumferential, subcutaneous suture (see Chapter 5).

ARTIFICIAL INSEMINATION

Progress in the application of artificial insemination (AI) to camel breeding had been slow because of two main factors. Firstly, the nomadic, pastoral nature of typical camel husbandry in arid regions militates against frequent herding, enclosure and restraint of animals. Secondly, she-camels in natural circumstances ovulate only in response to mating. However, despite these two constraints, it has been shown that AI is possible in camels kept intensively and by using (1) service by a vasectomised male, (2) an injection of seminal plasma, or (3) an injection of human chorionic gonadotrophin (hCG) or a GnRH analogue.

Chen et al. (1990), working with Bactrian camels in China, and Sieme et al. (1990), using dromedaries in Sudan, have reported pioneering studies on the collection, dilution and storage, including freezing, of camel semen and its use for insemination.

Semen collection

An artificial vagina (AV), as used for bulls, is prepared as usual and the final internal temperature brought to 40–50°C (camels are not as sensitive to this requirement as bulls). An oestrous female is restrained in the natural sitting posture and the well-controlled male is brought up to her side and allowed to nuzzle her head and then to sniff her perineum before mounting. The operators kneel on each side of the squatting male; one holds the AV and the other directs the penis into it by holding the sheath. Copulation lasts from 5 to 15 minutes; it is characterised by several bouts of male pelvic thrusting at intervals of a few minutes, and these coincide with ejaculatory squirts of semen into the collecting cup.

The semen sample is greyish white in colour and quite gelatinous, but sometimes it soon liquefies on standing at room temperature; volumes vary from 2 to 10 ml and average 3 ml. Normal sperm counts are from 200×10^6 to 400×10^6/ml. The fresh semen can be diluted three or four times with an egg yolk lactose extender, for use with inseminating doses of 2 ml. For freezing, Musa et al. (1992) recommended the technique used for boar semen, while Chen et al. (1990) used a diluent containing sucrose, egg yolk and glycerol, with the addition of penicillin and streptomycin for their frozen semen studies.

Insemination

The oestrous female camel must have a palpable follicle of about 1.2 cm in diameter. Of the methods available for promoting ovulation, Anouassi et al. (1992) obtained the best results after preliminary service by a vasectomised male 24 hours before the insemination. However, for general use this was considered an impracticable method. The Chinese workers prefer two inseminations, 24 hours apart: the first with 0.8 ml of whole undiluted semen, and the second with 2 ml of the diluted semen. The experience of workers experimenting with embryo transfer in camels indicates that the most convenient method of inducing ovulation is the injection of 20 μg of a GnRH analogue (buserelin) or 3000 IU of hCG (McKinnon and Tinson, 1992). Remarkable fertility from frozen semen inseminations was reported by Chen et al. (1990); for 31 animals inseminated the conception rate was 93.54%.

EMBRYO TRANSFER

The preliminary trials of embryo transfer in camels are quite recent and have been performed in Israel and in racing camel breeding studs in the United Arab Emirates (Anouassi et al., 1992; Yagil and van Creveld, 1990; McKinnon and Tinson, 1992; Skidmore et al., 1992; Bravo et al., 2000). Compared with cattle, the technique is more difficult in camels because the non-pregnant camel does not have a cyclical corpus luteum and does not ovulate spontaneously; these factors make superovulation of the donors and preparation of the recipients less reliable. A substantial and meticulously conducted trial of embryo transfer was carried out in the United Arab Emirates and reported in 1992 by McKinnon and Tinson.

Donors were given daily injections of 100 mg of progesterone for 10–15 days. From the last day of that series, twice-daily injections of 1–3 mg of ovine FSH were given for 5 days. Then the ovaries were examined to assess follicular development for mating; those follicles of 1.6–1.8 cm were considered optimum and they were most commonly found 8–12 days after the gonadotrophic treatment was begun. Ovulation was then induced by one or two natural matings, 12 hours apart. Alternatively, ovulation could be induced by injection of 3000 IU of hCG or by 20 μg of GnRH (buserelin), and followed by AI.

Recipients were prepared like donors by daily injection for 10–15 days of 100 mg of progesterone. This schedule was designed to terminate on the day that gonadotrophin was first given to the donor. Five days after these progesterone injections ceased, the ovaries of potential recipients were examined and grouped according to follicular size. On the day the donor was expected to ovulate, the recipient was injected with either 2000–5000 IU of hCG or 20 μg of GnRH (75% of the recipients prepared in this way ovulated at the correct time). Immediately before embryo transfer all recipients were scanned to ensure ovulation had occurred and that the genital tract was normal.

The day of mating was ‘day 0’, and embryo recovery was attempted on days 6.5 or 7.5, when the donor was tranquillised and given epidural anaesthesia. One litre of flushing fluid was injected and recovered in 30–70 ml aliquots, which were filtered and scanned for embryos; the latter were assessed to estimate age and normality. The embryos were placed in straws and introduced by means of a special injection gun into the left uterine horn of the recipient.

Commenting on their experimental study in which an overall pregnancy rate of 22% was achieved from these non-surgical transfers of 121 embryos, the authors concluded that: (1) best responses to the hormonal treatments were obtained during the natural breeding season, (2) more embryos were recovered from donors over 11 years old, on day 7 or 7.5 after mating, and from matings by particular bulls, and (3) more favourable pregnancy rates occurred in recipients under 11 years old and which had ovulated 0.5–1.5 days after the donor.

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33 Reproduction in the buffalo

The buffalo (*Bubalus bubalis*) contributes significantly to the economies of many tropical and subtropical countries. These include Mediterranean and Middle Eastern countries, the whole of the Indo-Pakistan subcontinent, Southeast Asia including Southern China and, more recently, some South and Central American countries and Australia. There are two distinct types of domestic buffalo, which have been named according to whether they wallow in stagnant pools (swamp buffalo) or in running water (river buffalo). The swamp buffalo is stocky in stature with a rounded conformation; it has a chromosome configuration of 48, and is mainly used for draught purposes and meat. The river buffalo, which has a chromosome configuration of 50, provides milk for human consumption. River buffaloes are larger in stature than the swamp buffalo, angular in shape and of a shy disposition.

In order to combine the higher milk yield of the river buffalo with the better working capacity and meat output of the swamp buffalo, cross-breeding between the two types is practised. Despite their different karyotypes, the cross-breds are fertile (Fischer, 1987). The hybrid female buffalo produces viable calves, and the male hybrid, despite the presence of a high percentage of degenerating spermatocytes and abnormal spermatids in the testes (Bongso et al., 1983), is capable of siring calf crops comparable to purebred male swamp buffaloes.

FEMALE REPRODUCTION

Anatomy of the reproductive organs

The structure and location of the internal reproductive organs of the buffalo are similar to those of cattle. However, the vulval labia are less tightly opposed, and the clitoris is more developed. The cervix is less conspicuous, and comprises 4–5 rings of tissue. The uterine horns are smaller and more coiled, and the number of caruncles is lower than in cattle. The ovaries, which are ovoid in shape and smaller in size than in cattle, are located within the pelvic cavity, caudal and lateral to the uterine horns. According to Samad and Nasseri (1979), the number of primordial follicles in the buffalo heifer ovaries is much lower than in heifers of *Bos bovis* of the same age (an average of 20 000 versus 50 000 for both ovaries). Similarly, the number of Graafian follicles >1 mm in diameter is much less, and follicular atresia is greater in the buffalo. Factors controlling follicular atresia may include age, stage of reproductive cycle, pregnancy, lactation, extraovarian or intraovarian hormones, nutrition, season and genotype. The genital tract and ovaries, including the cyclical corpus luteum (CL), and developing and mature follicles (>10 mm), can be palpated and imaged ultrasonographically by the transrectal route.

Puberty

The buffalo attains puberty later than cattle. On recommended levels of nutrition, the average age at puberty (first oestrus) in the female is about 15–18 months for the river buffalo and 21–24 months for the swamp buffalo; most first pregnancies occur when the buffalo heifer weighs about 250–275 kg.

The oestrous cycle

Breeding season

A major factor causing low reproductive performance in the river buffalo is its seasonal pattern of breeding. Decreasing day length and cooler ambient temperatures favour normal cyclical ovarian activity, whereas long day length and high summer temperatures depress cyclical activity. Some animals breed throughout the year if fed and managed well. In the Indo-Pakistan subcontinent, maximum breeding activity occurs during September to January, with a peak during October–November; minimal breeding activity occurs during the hot summer months. Thus, most of the buffaloes calve during July to November.

Season affects the reproductive process directly through environmental temperature and photoperiod, and indirectly through the quality and quantity of feed, incidence of disease and managemental practices. The onset of the breeding season is associated with a higher intake of metabolisable energy and a lower intake of crude protein. Hypoglycaemia and high serum urea concentrations observed in summer are associated with a lower level of fertility (Qureshi et al., 1999). Fluctuations in milk progesterone concentrations are inversely related to the environmental temperature. Low blood thyroxine levels in the hot season depress feed intake and body metabolism.

Since swamp buffaloes are mainly distributed in parts of the world with a constant, very humid tropical climate and the permanent availability of green fodder, seasonal influences on reproduction are minimal.

Cyclic periodicity

The oestrous cycle averages 21 days in length, and 'standing' oestrus is usually less than 24 hours. Oestrus usually commences towards late evening, with peak sexual activity during the night and the early morning. The duration of the luteinising hormone (LH) surge is about 9 hours, and ovulation, which is spontaneous, usually occurs 24–29 hours after this LH surge, or 15–18 hours after the end of oestrus.

Factors like season of the year, nutrition, management and delayed ovulation can prolong the length of the oestrous cycle. An incidence of 15.5% of short oestrous cycles has been recorded in the river buffalo, ranging from 6 to 14 days (Chohan et al., 1992). Plasma progesterone profiles reveal that short oestrous cycles are associated with reduced secretory activity of the CL or premature luteolysis. Delayed ovulation and 'split oestrus' are said to occur.

Signs of oestrus

Overt signs of oestrus in the buffalo are not as pronounced as in cattle. Heterosexual behaviour, particularly standing to be mounted by a bull, is the most reliable sign of oestrus in the buffalo, whereas homosexual behaviour, such as standing to be mounted by other females, is observed only occasionally. Signs such as swelling of the vulva, a clear mucoid vulval discharge, spontaneous milk letdown, bellowing, restlessness, frequent urination and raised tail vary in occurrence and intensity from animal to animal, and in relation to standing oestrus.

Mating behaviour

Mating behaviour in many respects resembles that of cattle. During the restraint period, the bulls exhibit circling, snorting, vocalisation, tucking up of the sheath and intermittent urination. After approaching a female, bulls exhibit sniffing, licking the perineum and vulva, and a flehmen reaction. An oestrous female responds by standing immobile for the male to mount and perform intromission. The copulatory behaviour includes penile erection, grasping the female at the level of pelvis, muscular contractions at the base of the tail, penile movements to locate the vulva, intromission and ejaculatory thrust. During this process, the animal either rests its head on the back of the buffalo cow or heifer, or waves it in the air (Anzar et al., 1988). However, the intensity of these events varies from bull to bull. Mating lasts 20–30 seconds. The male dismounts and gradually retracts the penis into the sheath, while the female remains with her back arched and tail elevated for a few minutes.

Methods of oestrus detection

A male buffalo, fitted with a chinball mating device, may be used for routine oestrus detection. The male either is kept in a corral with females from late evening until the next morning, or is led behind them if they are in stanchions, twice daily. If no male is available, a buffalo cow can be androgenised for oestrus detection.

Oestrus detection aids such as pressure-sensitive indicators placed on the sacrum or painting the tail-head are unsatisfactory because wallowing interferes with their efficiency. Where routine oestrus detection is not practised, buffaloes are submitted for insemination on the basis of a vulvar discharge of clear mucus, a drop in milk yield or a change in temperament. In these situations, inseminators often palpate the uterus for the presence of tone and examine the mucus before inseminating an animal.

Cyclical changes of the internal genitalia

Ovaries. The rising level of oestrogens, particularly oestradiol-17β secreted by the Graafian follicle, combined with the declining level of progesterone secreted by the regressing CL, trigger a surge of LH. The LH surge induces final maturation of a follicle, followed by ovulation about 24–29 hours later (Kaker et al., 1980; Shimizu, 1987).

During oestrus, a mature follicle, 10–20 mm in diameter, can be palpated transrectally as a turgid area protruding slightly from the surface of the ovary. On the day of ovulation (days 1–2), the follicle softens and the site of ovulation is felt as a pit or depression on the surface of the ovary. Normally, one oocyte is shed per cycle.

The growth, maintenance and regression of the CL are closely correlated with changes in progesterone concentrations in peripheral plasma or milk (Jainudeen et al., 1983a, b). The developing CL (days 2–7) is soft and difficult to palpate per rectum, but the mature CL (days 8–16) is palpable as a firm projection on the surface of the ovary. The mature CL secretes progesterone, resulting in peripheral plasma concentrations of up to 3.5 ng/ml. With the regression of the CL (day 17), progesterone secretion rapidly declines, resulting in concentrations of below 0.2 ng/ml at the next oestrus. Old CLs appear as white scars on the surface of the ovary.

Buffaloes have lower maximum peripheral plasma progesterone concentrations than cattle, with the river breeds having higher concentrations than the swamp breeds. According to Batra and Pandey (1983), the concentrations of metabolites of prostaglandin $F_{2\alpha}$ (PGFM) during the last four days of the oestrous cycle increased from about 250 pg/ml to peak levels of about 1000 pg/ml during oestrus. Temporal relationships between plasma profiles of LH and ovarian steroids in cyclical buffaloes are the same as those in cattle.

Uterus, cervix and vagina. The uterine horns are turgid and coiled with maximum tone during oestrus, and become oedematous at the time of ovulation. They gradually lose their turgidity and tonicity after ovulation, to become almost flaccid during the luteal phase of the cycle. The cervix dilates sufficiently during oestrus to enable the passage of an insemination catheter into the uterus. The clear, copious mucus that is secreted during oestrus changes to an opaque, thick, scanty discharge after ovulation. Hyperaemia of the vaginal mucous membrane and some swelling of the vulva occur during oestrus. Blood in the vulval discharge or ‘metoestrus bleeding’, often seen in cattle, rarely occurs in the buffalo.

Pregnancy

Gestation length

The buffalo has a longer gestation than cattle, being 305–320 days for the river buffalo and 320–340 for the swamp buffalo; male calves are carried 1–2 days longer than female calves. River × swamp hybrids have an intermediate gestation length of 315 days. The incidence of right-horn pregnancy is higher than the left horn (67% versus 33%; Usmani, 1992), and the transuterine migration of the embryo is very rare.

Physiology of pregnancy

Placentation. The epitheliochorial placenta of the buffalo is of the cotyledonary type. The fetal membranes and fetus mostly develop in one uterine horn. Most of the 60–90 placentomes are distributed throughout the gravid uterine horn. As pregnancy advances, the placentomes enlarge to mushroom-like structures measuring 5–7 cm in diameter.

Endocrinology. Although cyclical ovarian activity ceases during pregnancy, a few buffaloes may show behavioural signs of oestrus that is anovulatory. The CL is maintained throughout gestation but its role in the maintenance of pregnancy is not known. As in cattle, plasma progesterone concentrations remain elevated throughout pregnancy.

Methods of pregnancy diagnosis

Clinical methods

Transrectal palpation. Pregnancy can be accurately diagnosed per rectum from about 45 days, although an experienced clinician can diagnose pregnancy as early as 30 days after breeding. Manual slipping of the allantochorion is possible from about 42 to 56 days of gestation. The uterus is suspended at the level of the pelvic floor up to the fourth month of gestation, thereafter descending to the abdominal floor. In most buffaloes, placentomes and the fetus may be palpated beyond the 70th day of pregnancy; however, in some deep-bellied river buffalo breeds the fetus may be difficult to palpate, particularly between the sixth and eighth months. In such cases, palpation of the hypertrophied middle uterine arteries, with fremitus, or recognition of the placentomes aids in the diagnosis.

Laboratory methods

Hormone assays. As in cattle, pregnancy can be diagnosed on the basis of persistent elevated progesterone concentrations in milk or plasma 22–24 days after breeding. This test is accurate for the early detection of non-pregnant animals, but it is not accurate for the detection of pregnant ones, for the same reasons as stated for cattle (see Chapter 3).

Ultrasonography

As in cattle, diagnostic ultrasound can be effectively used for early pregnancy diagnosis in the buffalo. Using a linear array transducer designed for transrectal use, pregnancy can be diagnosed accurately as early as 30 days after service.

Parturition and puerperium

Parturition

Signs of approaching parturition. Buffalos' behaviour as they approach calving is similar to that of cows. About 1–2 weeks before, the buffalo or heifer shows marked abdominal enlargement, udder development, and hypertrophy and oedema of the vulval lips. As the time of parturition approaches, she normally isolates herself from the rest of the herd. The relaxation and sinking of the pelvic ligaments and muscles lead to an elevation of the tailhead, while liquefaction of the cervical seal of pregnancy results in a string of clear mucus hanging from the vulva, particularly when the animal lies down.

Initiation of parturition. Plasma concentrations of progesterone remain elevated throughout gestation, but about 15 days before parturition, plasma levels of both oestrone and PGFM increase and reach peak values 3–5 days pre-partum (Perera et al., 1981; Arora and Pandey, 1982; Batra and Pandey, 1982). At parturition, the sharp decline in plasma concentrations of progesterone is associated with a significant increase in plasma concentrations of cortisol (Prakash and Madan, 1984); whether the cortisol originates from the mother or fetus, or both, has not been established.

Stages of labour. About 12–24 hours before parturition, uterine contractions increase in both frequency and amplitude, causing the animal some abdominal discomfort. The cervix takes about 1–2 hours to dilate fully (stage one of labour).

As the fetus enters the birth canal, the dam lies down in sternal or lateral recumbency and starts straining (stage two of labour) (Figure 33.1). The allantochorion mostly ruptures before it reaches the vulva, and is quickly followed by the fetus contained within the amnion, appearing at the vulva. Strong abdominal contractions lead to the rupture of the amniotic sac, and the delivery of the fetus, usually in anterior longitudinal presentation and dorsal position, with extended limbs; posterior presentation is uncommon. This stage of labour lasts 30–60 minutes, but it may extend up to 6 hours, particularly in primipara. As in the cow, the umbilical cord ruptures before the calf reaches the ground. After delivery, abdominal straining ceases and the fetal membranes are expelled within 4–6 hours (stage three of labour). Twinning is rare, and the incidence is less than 1 per 1000 births.

Obstetrical disorders. The incidence of reproductive disorders is higher in the river buffalo than in the swamp buffalo. In the river buffalo, the incidence of cervico-vaginal and uterine prolapse has been reported to be 42.0%, that of retained fetal membranes (RFM) as 23.7%, dystocia as 21.5%, and abortion as 12.8%

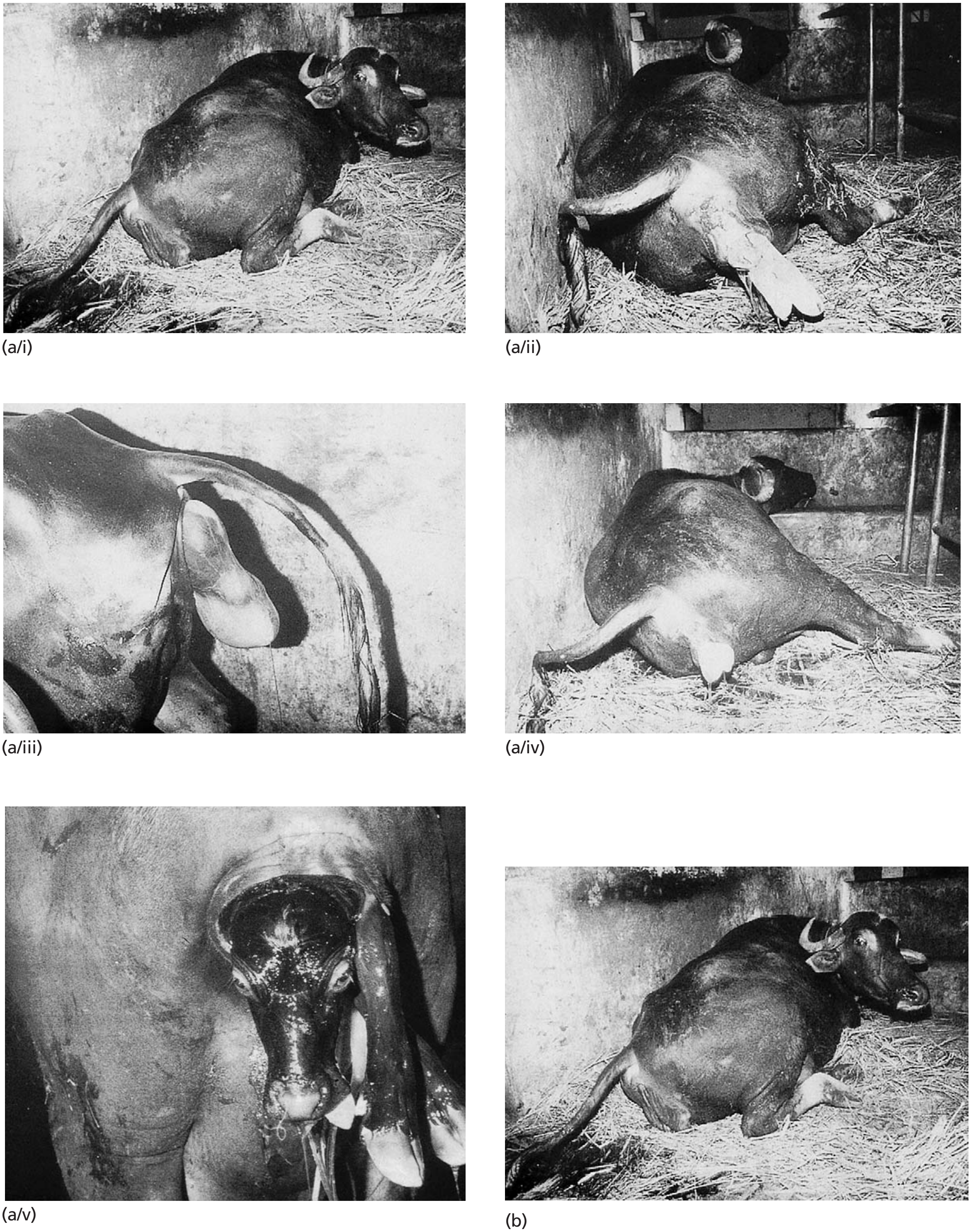

Fig. 33.1 (a) Parturition in the buffalo: progression of second-stage labour. (b) Parturition in the buffalo: end of second-stage labour.

Fig. 33.2 Prolapse of the uterus in a buffalo.

(Samad et al., 1984). About 64.8% of cases of prolapse were recorded prepartum (cervico-vaginal), while 35.2% were postpartum (uterine). Uterine prolapse mostly occurs within the first 6 hours after expulsion of the fetus, and involves a complete eversion of the gravid uterine horn (Figure 33.2). Infections, uterine inertia, dystocia and poor management practices have been implicated in the pathogenesis of RFM.

Dystocia. Dystocia is less common in the buffalo than cattle. Stabled river buffaloes are more prone to dystocia than the free-ranging swamp type. The commonest cause of dystocia is fetomaternal disproportion followed by a variety of faulty dispositions. The most frequent cause of maternal dystocia is uterine torsion, followed by incomplete dilatation of the cervix and uterine inertia. Occasional cases of hydroallantois and persistent hymen have also been reported.

Most cases of uterine torsion occur at the time of parturition or during the last month of pregnancy. The direction of torsion in the buffalo in more than 90% of cases is to the right (clockwise). Predisposing factors for both torsion of the uterus and prolapse of the vagina or uterus are anatomical in origin, related specifically to buffaloes. Such factors as relatively long uterine ligaments and low numbers of smooth muscle cells in the broad ligament may contribute. In addition management factors, such as constant confinement of buffaloes in a small and often sloping area with no facilities for exercise, are common under village conditions. Schaffer's method (see Chapter 10) has proved very useful for the replacement of uterine torsion in the buffalo.

Puerperium

Involution of the uterus. The uterus is palpable by the second week postpartum as a well-defined, completely palpable structure, cranial and slightly ventral to the pelvic brim. Involution is completed by about 30 days in the suckled swamp buffalo, and by about 45 days in the hand-milked river buffalo. Uterine involution is delayed in cases of dystocia and RFM. There are conflicting reports regarding the effects of age, season of year and parity on the rate of uterine involution.

Resumption of ovarian activity. The CL of the previous pregnancy is completely regressed by day 30 postpartum. Peripheral plasma progesterone concentrations decline rapidly following parturition to undetectable levels by day 3 or 4, and remain so till the first postpartum ovulation, which occurs at about 96 days in the swamp buffalo (Jainudeen et al., 1983a), and at 60 days in the river buffalo (Perera et al., 1981). However, in well fed and managed animals, follicular activity can commence earlier. The intervals from calving to resumption of follicular development and ovulation are shorter when the ovary contralateral to the previously gravid horn is involved (Usmani, 1992). Poor body condition, lactation, suckling and age can delay the onset of the first oestrus postpartum. Hand-milked river buffaloes have a lower incidence of postpartum anoestrus than suckled swamp buffaloes. Buffaloes calving during their normal calving season resume cyclical ovarian activity earlier than those calving in other seasons.

Progesterone-releasing intravaginal devices (PRIDs) (see Chapters 1 and 22) initiate ovulation and luteal activity in cyclical river buffaloes (Rajamahendran et al., 1980), but not in suckled acyclical swamp buffaloes (Jainudeen et al., 1984). GnRH does not initiate normal cyclical ovarian activity in suckled buffalo, but hCG triggers ovulation and the development of a normal CL. Although early weaning reduces the incidence of postpartum anoestrus, it has the disadvantage of increasing the cost of buffalo production for meat. As in cattle, temporary calf removal in suckled buffalo induces an anovulatory oestrus, which can be overcome by pretreatment with a PRID for 10–12 days. Improvement in body condition is necessary in conjunction with any method of reducing the calving to first postpartum oestrus interval (Jainudeen et al., 1984).

MALE REPRODUCTION

Normal sexual apparatus

Anatomy

The reproductive organs are similar to the bull of *Bos bovis*, but the testes and scrotum are smaller and the penile sheath is less pendulous. As in cattle, the testis and epididymis can be palpated through the scrotal wall, and the prostate, seminal vesicles and ampullae of the ductus deferens can be palpated per rectum.

Puberty

In river buffalo bulls, testis size shows a curvilinear increase in relation to age. It increases slowly between 5 and 15 months, rapidly between 15 and 25 months and again slowly between 25 and 38 months of age. The plasma testosterone concentrations are low up to 21 months of age, and increase thereafter. In these bulls, the prepubertal period seems to extend up to 15 months of age (Ahmad et al., 1984). Spermatogenesis commences at about 12–15 months in both buffalo types. However, sexual maturation, as indicated by the presence of motile spermatozoa in the ejaculate, is attained at about 24–25 months. The faster-growing F1 river × swamp cross-breds reach puberty earlier than the slower-growing swamp buffaloes.

Spermatogenesis

Among farm animals, the buffalo has one of the shortest spermatogenic cycles. The durations of the seminiferous epithelial cycle and spermatogenesis are 8.6 and 38 days, respectively (Sharma and Gupta, 1980). In general, the frequencies of the cell stages in buffalo and cattle are comparable.

The head of a normal buffalo spermatozoon has a specific rectangular shape with no resemblance to that of cattle. It measures about 8.3 μm in length and 4.5 μm in width. The average length of the midpiece is 12.2 μm while the tail is about 54.8 μm long (Saeed et al., 1989). The overall length of buffalo sperm is greater than that of cattle (75.4 vs. 69.3 μm).

Examination of semen

Semen is usually collected with a conventional bovine artificial vagina (AV) (see Chapter 30). Either a female or a castrated or intact male buffalo can be used as the teaser. The temperature of the water jacket of the AV should be about 40–42°C, and the pressure within the AV adjusted to suit individual bulls. Sperm concentration is increased by allowing 2–3 false mounts before the actual collection. The normal ejaculate collected with an AV is creamy to milky white in colour, varying from 1 to 6 ml in volume, although exceptional bulls can give up to 11 ml of semen; it has a sperm concentration of between 1 and 4 × 10^9 cells/ml. The values of ejaculatory volume and sperm concentration are higher in river than in swamp buffalo; the motility of spermatozoa is lower than in cattle. Semen can also be collected with cattle electroejaculators.

The parameters of semen quality are affected by precoital sexual excitement, number of false mounts, age, season of year, frequency of collection, diet and fitness of the bull. The temperature/humidity index adversely affects the volume of semen produced, depresses sperm concentration and initial motility, and increases the production of dead and abnormal spermatozoa. The decline in serum thyroxine during summer depresses feed intake and metabolism, and thus decreases sperm production. Similarly, increased body temperature has adverse effects on initial motility, and the number of dead spermatozoa in the ejaculate. Bulls with obvious testicular asymmetry yield considerably fewer spermatozoa per ejaculate. The presence of pathogenic bacteria, e.g. *Pseudomonas spp.* and *Escherichia coli*, can reduce sperm motility and increase the dead sperm percentage.

ARTIFICIAL INSEMINATION

Artificial insemination (AI) has been practised in the river buffalo for over 40 years in the Indo-Pakistan subcontinent, but has lagged behind its use in cattle largely because of the difficulty of detecting oestrus. In addition, lower fertility rates obtained with chilled or frozen semen are another constraint to the widespread use of AI in this species.

Buffalo semen differs from cattle semen in some of its metabolic and physiological properties: for example, sperm DNA–RNA, phospholipid and enzyme content. Due to these differences, the methods and particularly the composition of the extenders used for cattle are unsuitable for freezing buffalo spermatozoa. Thus, there remains a need to develop more effective extenders to preserve buffalo semen in the chilled or frozen form. Nevertheless, various extenders have been developed for freezing buffalo semen with varying results. These include, lactose–egg yolk–glycerol, lactose–fructose–egg yolk–glycerol and Tris–egg yolk–glycerol. A greater than 20% use of egg yolk does not enhance cryoprotection, but it can lessen sperm forward motility in the cervix due to increased viscosity. An equilibration period of 6–9 hours, and glycerol concentrations of 5–7% are most frequently used.

Extended semen is placed in 0.25 or 0.5 ml straws or paillettes, each containing 30 million spermatozoa. The straws are then exposed to nitrogen vapours at –120 to –140°C and stored in liquid nitrogen. Rapid thawing (at 37°C for 10 seconds) is preferred over slow thawing. The post-thaw progressive motility of buffalo semen varies from 35 to 60%. Inseminations are usually performed between 12 and 18 hours after the onset of oestrus.

FERTILITY AND INFERTILITY

Evaluation of fertility

Female fertility in the buffalo is commonly expressed in terms of the calving interval. A buffalo produces, on average, two calves every 3 years. Caution should be exercised in interpreting pregnancy rates based on non-return rates in the buffalo because of the inherent difficulty of detecting oestrus. Pregnancy rates based on rectal palpation in swamp buffaloes usually range from 20 to 75% during a 3–4-month breeding season, depending upon the nutritional and lactational status of the females at joining. The first-service pregnancy rate for the river buffalo varies between 50 and 75% for natural service, and 30 and 50% for AI with frozen semen.

Female infertility

The reproductive efficiency of the buffalo is lower than that of cattle. Delayed sexual maturity, seasonal effects on the reproductive cycle and extended calving intervals under traditional management systems provide few opportunities for a buffalo to calve during the most favourable months of 2 successive years. Both infectious and non-infectious factors contribute to the long calving interval, especially because of anoestrus, repeat breeding and abortion.

Anoestrus

As in cattle, two forms of anoestrus occur in the buffalo. In the first form, the animal possesses a palpable CL in one ovary, but has not been detected in oestrus due to suboestrus or silent oestrus, whereas in the second form, the animal has no palpable CL and does not exhibit oestrus because she is acyclical (true anoestrus). In a clinical survey among cases of reported anoestrus, 58.4% were true anoestrus, 33.3% silent oestrus and 8.3% of buffaloes had infantile genitalia (Samad et al., 1984).

A high incidence of true anoestrus occurs during the hot summer months. Clinical examination reveals that both ovaries are small and inactive, while the uterus is flaccid. Blood levels of calcium, phosphorus, glucose and total proteins are lower in anoestrus than in cyclical buffaloes. In most cases, the disorder resolves spontaneously with the arrival of more favourable climatic conditions and adequate feeding. The most effective treatment seems to be use of a PRID for 10–12 days, followed by eCG at the time of withdrawal.

In the past, silent oestrus was believed to be a major problem in buffalo breeding, but recent evidence suggests that it is due to the poor oestrus detection in these herds. The incidence has been drastically reduced in herds where routine oestrus detection practices have been implimented (Jainudeen, 1984). Treatment of silent oestrus in buffalo cows with $PGF_{2\alpha}$ resulted in 91% showing visible oestrus signs within 48–80 hours, and a 55% pregnancy rate to first insemination (Samad et al., 1981).

Cystic ovaries

The incidence of cystic ovaries is lower in buffaloes than in cattle. Among buffaloes, the condition is more common in the high-producing river buffalo than in the suckled swamp buffalo. In a survey, cystic ovaries accounted for 6% of reproductive failure in over 12 000 river buffaloes in India; most cases occurred before day 45 postpartum (Rao and Sreemannarayanan, 1982). The clinical findings and treatment are similar to those in the cow (see Chapter 22).

Repeat breeding and abortion

Repeat breeding is an important cause of low reproductive efficiency in the buffalo; the incidence varies from 15 to 32% and seems to be lower in animals kept individually on small holdings than in large herds. Similarly, the incidence is lower in heifers than adult buffaloes up to the third parity; thereafter, the incidence decreases, probably due to culling of affected animals from the breeding stock.

The incidence of specific infections that cause repeat breeding and abortion in cattle, such as brucellosis, leptospirosis, campylobacteriosis, trichomoniasis and infectious bovine rhinotracheitis (IBR), is very low in the buffalo. Non-specific uterine infections, leading to clinical or subclinical endometritis, are amongst the major causes of repeat breeding. Poor quality of semen, luteal dysfunction, delayed ovulation or anovulation can also be responsible. Nutritional deficiencies resulting in, for example, low serum calcium and phosphorus concentrations and hypoglycemia have also been implicated.

Significantly higher antisperm antibody titre in the serum of repeat breeder buffaloes than in the normal cyclical, pregnant or virgin heifers suggests that this may be responsible for pregnancy failure in some of these animals (Saeed et al., 1995). Abortion caused by *Brucella abortus* occurs during the latter half of gestation.

Endometritis

A high incidence of endometritis has been reported in infertile river buffaloes, being responsible for 46% of various reproductive disorders (Samad et al., 1984). Among cases of non-specific uterine infection in this study, first-degree endometritis, second-degree endometritis and postpartum metritis were recorded in 56.2, 16.0 and 24.2% of buffaloes, respectively. The common organisms isolated include *Escherichia coli*, *A. pyogenes* and *Staphylococcus aureus*. The high incidence has been attributed to natural mating by the infected bulls, unhygienic calving management, persistence of infection from the puerperal period, mid-cycle inseminations and malpractice of stimulating milk letdown through the introduction of instruments, the tail of the animal or the hand into the vagina. Furthermore in buffaloes, because the vulval labia are not closely opposed, there may well be a greater chance of an ascending infection. The methods of treatment are the same as for those cattle (see Chapter 22).

Male infertility

Genetic infertility in buffalo bulls is characterised by testicular hypoplasia and endocrine abnormalities, resulting in underdevelopment of testis and seminiferous tubules with arrested spermatogenesis. Acquired infertility is most likely to be due to infections that produce inflammatory changes including orchitis, epididymitis, seminal vesiculitis and testicular degeneration. The inhibitory factor for sperm motility in the seminal plasma is higher in buffalo than in cattle semen (Rao, 1984; Ahmad et al., 1988). High environmental temperatures during the summer months exert a deleterious effect on libido, as well as semen quality. Several sperm defects have been reported, but their relationship to fertility has not been ascertained.

Improving fertility

In the past, attention was given mainly to the control of infectious diseases and pathological conditions affecting fertility. However, with the recent development of sensitive methods for measuring reproductive hormones such as LH and progesterone, veterinarians are now paying greater attention to the non-infectious factors contributing to infertility in the buffalo.

Selective breeding among river breeds, crossbreeding between river and swamp breeds and improvements in nutrition can advance the onset of puberty. Similarly, management practices such as early weaning, a high plane of feeding and proper protection during the hot summer months can advance the restoration of postpartum cyclical ovarian activity and reduce the length of calving intervals. Pregnancy rates in repeat breeding buffaloes can be improved through double insemination at an interval of 6–8 hours during the same oestrus period, the use of GnRH at the time of insemination or intrauterine antibiotic infusion 24 hours after insemination.

The difficulty of detecting oestrus can be overcome by two methods of oestrus induction at a predetermined time: (1) premature luteolysis of the CL with $PGF_{2\alpha}$ or a synthetic analogue; and (2) the creation of an artificial luteal phase by the use of a PRID. The first method is of limited value in lactating or suckled buffaloes because of the high incidence of true anoestrus. Since $PGF_{2\alpha}$ causes abortion, buffaloes should be examined for pregnancy before treatment.

SUPEROVULATION AND EMBRYO TRANSFER

The first buffalo calf born following embryo transfer was in the USA after the non-surgical collection of a 7-day blastocyst and non-surgical transfer to an unrelated river buffalo (Drost et al., 1983). Four days' superovulatory treatment with FSH (5 mg, twice daily), beginning on day 10–12 of the oestrous cycle, gives good results. Similarly, 3000 IU of eCG given once on day 10–12 of the oestrous cycle has also been tried. In both these treatments, 500 μg of $PGF_{2\alpha}$ or a synthetic analogue is given as a luteolytic agent on the third day of treatment. However, FSH treatment produces better results in terms of a superovulatory response than eCG.

According to Anwar and Ullah (1998), embryos are in the oviduct around 85 hours and in the uterus about 108 hours after oestrus. They are at the 8–16-cell stage at 85 hours, and form a morula at 108 hours, a compact morula at 125 hours, and early blastocysts at 141 hours post-oestrus; blastocysts are predominant at 156–176 hours after oestrus. Thus, embryo recovery at day 6–7 was recommended.

Animals with high peripheral plasma progesterone concentrations at the start of the superovulatory treatment produce better results than those with low progesterone concentrations. The relatively lower ovarian response to superovulation treatment in buffaloes compared with similar treatments in cattle might be due to poor ovarian follicular populations and comparatively greater follicular atresia.

IN VITRO MATURATION/FERTILISATION

The poor recovery of usable oocytes from buffalo ovaries is a major constraint in the development of a successful in vitro system for this species. Scarifying the ovarian surface with a surgical blade, followed by instant rinsing and tapping of the ovary to release oocytes into the culture medium, results in a better recovery of good-quality follicular oocytes than the aspiration or the puncture methods. Buffalo ovaries with CLs yield a lower number of good-quality oocytes than ovaries without a functional CL, probably due to the inhibitory effects of the corpus luteum on follicular growth (Samad et al., 1998; Samad, 1999).

Culture media including tissue culture medium (TCM-199), bovine synthetic follicular fluid and

Ham's F-10 are equally good for in vitro maturation of buffalo follicular oocytes. Supplementation of TCM-199 with oestrus cow serum, oestrus buffalo serum or pro-oestrus buffalo serum improves in vitro maturation and fertilisation rates. Improved development of IVM–IVF-derived two-cell embryos to the morula stage can be achieved through conditioning the culture media with buffalo oviductal epithelial cells (Samad, 1999).

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34 Normal reproduction, and reproductive diseases and infertility in rabbits and rodents

This chapter outlines the basic and important features of reproduction in rabbits (*Oryctolagus cuniculus*), rats (*Rattus norvegicus*), mice (*Mus musculus*), Syrian hamsters (*Mesocricetus auratus*) and guinea pigs (*Cavia porcelus*). Space does not allow detailed descriptions of comparative anatomy and physiology, or to consider the variations exhibited between different breeds or strains.

Summaries of the important features of the reproductive physiology of the species covered in this section can be found in Tables 34.1 and 34.2.

Figures 34.1–34.8 provide pictorial guidance to sexing each of the species considered.

NORMAL REPRODUCTION

Rabbits

All domestic rabbits are descended from the European wild rabbit (*Oryctolagus cuniculus*). Originally classified as rodents, they were later given their own order, Lagomorpha, along with hares and pikas.

Under suitable husbandry conditions and provided with good nutrition rabbits are prolific breeders. As many as 50–60 offspring may be born to a farmed rabbit in a 1-year period.

Anatomy

Male rabbits are known as bucks. The scrotum presents as an inguinal pouch and the testes present no unusual features, though they are relatively large and flaccid in the sexually active animal. Although normally carried in the inguinal pouch, the testes may be easily withdrawn into the abdominal cavity through a large canal.

The penis, which does not have a glans penis, is posteriorly directed and lies within a prepuce that hangs within a fold of skin below the body wall. Glands immediately above and by the side of the penis produce secretions into the hairless inguinal spaces and are known as the white inguinal glands. They open by a single duct into folds of skin near the end of the penis. The white inguinal glands do not contribute to the seminal

Fig. 34.1 Young female rabbit. Note the proximity of the anus to the vagina.

Fig. 34.2 Young male rabbit. Note the protrusion of the penis achieved by applying slight pressure over the prepuce.

Table 34.1 Summary of the breeding of laboratory rabbits and rodents (from The Principles of Animal Technology I, 1988, with permission from the Institute of Animal Technology, 1988)

Species	*Type of Oestrous Cycle*				*Age at first Mating*
	Mating season in controlled conditions	*Duration of oestrus*	*Mechanism of ovulation*	*Time between oestruses in the unmated animal*	
	Polyoestrous (continuous)				
Syrian hamster	No definite season (more variable in winter)	Usually an evening	Spontaneous	4 days	6 weeks or paired at weaning
	Polyoestrous (continuous)				6 weeks
Mouse (outbred)	No seasonal variation	Usually an evening	Spontaneous	4 or 5 days	
	Polyoestrous (continuous)				
Rat (outbred)	No seasonal variation	Half a day	Spontaneous	5 days	**F** 10 weeks **M** 12 weeks
	Polyoestrous (continuous)				
Guinea pig (outbred)	No seasonal variation	1 day	Spontaneous	15 days	**F** 3 months **M** 4 months
	Governed by induced ovulation				
Rabbit	No definite season (more variable in winter)	Weeks if not mated	Induced by mating		Dutch **F** 6 months **M** 8 months New Zealand white **F** 8 months **M** 10 months

Table 34.2 Average litter intervals and sizes and expected productivity (from The Principles of Animal Technology I, 1988, with permission from the Institute of Animal Technology)

	Average litter interval or number of litters/year	*Average litter size (weaned)*	*Average expected productivity (per female)*
Outbred mouse	4.5 weeks	8	Nearly 2/week
Outbred rat	7 weeks	10	1.5–2/week
Syrian hamster	6–7 weeks	6	Nearly 1/week
Guinea pig	4 litters/year	3.5	14/year
Rabbit	4–5 litters/year	7	32/year

fluid but produce a sebaceous, odorous secretion that is associated with sexual attraction. Ampullary, vesicular, prostatic and bulbo-urethral glands are all present as ancillary sex glands.

The semen ejaculate volume is from 0.6 to 2.3 ml with an average density of 263 million sperm/ml. The gel plug comprises the greatest constituent of the ejaculate. The time required for sperm capacitation varies from 1 to 6 hours.

The reproductive tract of the doe presents no unusual or special features. The horns of the uterus are separate for their entire length, joining

Gestation period	Average litter size weaned	Age at weaning	Recurrence of oestrus	Average litter interval or no. of litters/year	Average expected productivity per female	Duration of economic breeding life
16 days	6	21 days	Postpartum then end of lactation	7 weeks	Nearly 1/week	6 litters 8 months
20 days	8	19–21 days	Postpartum then end of lactation	4–5 weeks	Nearly 2/week	6 litters 6 months
21 days	10	3 weeks	Postpartum then end of lactation	6–7 weeks	1.5–2/weeks	6 litters 8 months
About 9 weeks	3.5	2 weeks (180–200 g)	Postpartum then end of lactation	4 litters/year	14/year	8 litters 2–3 years
28 days 31 days	7	5–8 weeks	Postpartum then about fourth week of lactation	4–5 litters/year	32/year	10–12 litters 2–3 years

to form the cervix and vagina. There are normally 4 or 5 pairs of nipples.

Puberty and reproductive viability

Different breeds of rabbits reach puberty at different ages. Smaller breeds may be bred from 4 months of age, whilst the larger breeds may not become sexually active until around 5–6 months. Motile spermatozoa appear in the ejaculate of bucks from 4 months of age but maximum output is not reached until 7 or 8 months. Although some does may have an active reproductive life of 5–7 years, litter sizes and general fertility decline from around 3 years of age. Bucks may maintain an active reproductive life for 5 years or more with good husbandry and care.

Oestrous cycle

Rabbits do not have a regular oestrous cycle, although a rhythm will develop for sexual receptivity. Under favourable conditions does will remain in oestrus for long periods during which time ovarian follicles are continually developing and regressing at more or less the same rate.

In this way, the doe maintains a reasonable number of follicles available for ovulation. The active life of a follicle is around 12–16 days. Signs of oestrus are difficult to detect in the rabbit compared with other species. Full sexual receptivity is indicated by a congested purple and moist vulva (vent). Does may be restless and try to join neighbouring rabbits. Sexual receptivity is diminished during moulting and lactation, and it can also result from undernourishment. It is also highly variable between, and within, does. A doe may reject one buck but accept another. She may also accept a buck once but reject him a second time.

Copulation

When the doe is sexually receptive, she lies in the mating position, raising her hindquarters to allow copulation. The buck's movements are sudden and on mounting he rests his head on the doe's flanks or quarters. He performs 8–12 rapid movements to achieve intromission and copulation. The copulatory thrust can be so vigorous that the buck will fall backwards or sideways, emitting a cry.

Copulation can be repeated within a minute but if he is less vigorous, the buck may lose his balance or dismount from the doe without ejaculating. The vaginal plug formed from the gelatinous ejaculate is expelled within a few minutes of copulation.

The rabbit is a spontaneous ovulator with ovulation occurring 10 to 14 hours after copulation; it cannot usually be provoked by mechanical stimulation of the cervix. Some reports claim that 20–25% of does fail to ovulate post-copulation due to deficiency of luteinising hormone (LH). The cause of this hormonal failure is unknown and unproven as a reason for poor ovulatory rates.

The doe may accept the buck at any time during pregnancy, except for a brief period of 30–40 hours post-mating. Fertilisation can occur in such cases if a second ovulation takes place within 2–3 days of the first. This can produce litters of mixed buck parentage.

Fertilisation cannot occur in the presence of active corpora lutea, probably due to hormonal inhibition of sperm transport or capacitation. Eggs become covered with a layer of mucin within 6 hours of ovulation and cannot be fertilised after this period. Infertile matings can result in false pregnancy.

Gestation and parturition

The rabbit placenta is haemochorial (see Chapter 2).

Pregnancy in the rabbit usually lasts between 30 and 32 days. Many factors influence its exact duration, including breed, parity of doe, litter size and nutritional status. In larger litters, the duration of gestation is shorter. The source of progesterone to sustain pregnancy in the rabbit is the corpora lutea.

Until the middle of pregnancy (day 15), there is little increase in the size of the doe's reproductive tract or embryos. Uterine development and embryonic growth are accelerated throughout the second half, with litter viability and milk supply relying very much on the doe's feeding and nutritional status. Fetuses can be manually palpated from day 12 of gestation and this is the common method of pregnancy diagnosis, although transabdominal B-mode ultrasonography can be used in the same way as in the bitch and cat (see Chapter 3).

Onset of parturition (the term used by rabbit breeders is kindling) depends upon the withdrawal of the progesterone block on the myometrium due to regession of the corpora lutea; the process is very similar to that described in Chapter 6 for other domestic species. Parturition generally occurs in the night or early morning, with food consumption dropping 2–3 days before. Anterior and posterior presentations are normal (see Chapter 6). Dystocia in the rabbit is rare and usually associated with oversized pups or fetal monsters. Normal delivery is complete in around 30 minutes. Split parturition can occur, with intervals of a few hours to several days being recorded. This is most probably the result of accidental or intended double matings. All post-parturient does should be palpated or scanned 24 hours after the expulsion of the last pup to determine if there are retained fetuses. The normal litter size for a common farm breed, such as the New Zealand White or Californian, is 8–14 pups.

At birth, the rabbit pup is quite immature and totally dependent upon its mother. It has little hair, and hypothermia can be rapidly fatal. During week 1 of life, the pups grow rapidly, and they begin to emerge from the nest from week 3.

Lactation

It is frequently said by rabbit farmers that 'the quality of rabbits is made in the nest.' The first 3 weeks of life are very important and will affect the rabbit's future growth, development and ability to thrive. Mammary glands develop rapidly during the last week of pregnancy and milk letdown is usually delayed until parturition.

Does nurse their young for only a few minutes a day, usually in the night or early morning. The average daily yield of milk is about 160–200 g in primiparous does, rising to 170–220 g in subsequent litters.

Milk production reaches a maximum at around 2–3 weeks postpartum and begins to decline from about 4–5 weeks. Normal lactation lasts 6–8 weeks, depending upon nutritional status, parity of doe and litter size. Because there is a great variation in milk yield between does, this trait must be considered when selecting replacement breeders. The weight of litters at 21 days is considered a good indicator of milk yield but after 21 days the correlation declines. Rabbit milk contains about 15% protein, 10% fat and 2% carbohydrate. Pups are normally weaned from the doe between 6 and 8 weeks. Early weaning should only be undertaken by experienced rabbit breeders.

Finally, it should be noted that postpartum mating is commonly practised by rabbit farmers, with the result that the time interval between parturition and weaning may be as short as a few days.

Fig. 34.3 Young female guinea pig. Note the proximity of the anus to the vagina.

Fig. 34.4 Young male guinea pig. Note the protrusion of the penis achieved by applying slight pressure over the prepuce.

Guinea pigs

Guinea pigs are rodents, originating from South America where they inhabit open grassland, nesting in the taller vegetation. They live in small societies from a few to several dozen individuals, and feed in the open areas at dawn and dusk.

Anatomy

The male reproductive system comprises testes, epididymides, ductus deferens, urethra, vesicular glands, prostate, coagulating glands and bulbourethral glands. The vesicular glands are long (10 cm), coiled and tubular.

The semen ejaculate volume is around 0.5 ml, with an average density of around 40 million sperm/ml. The portion of male ejaculate secreted by the vesicular glands coagulates almost instantaneously during copulation to form the vaginal plug. The plug is rigid and fills the lumen of the vagina and cervix. Sperm capacitation takes 8–10 hours.

The female reproductive tract presents no unusual or special features. The mammary glands are inguinal in position with a single pair of glands and associated nipples.

Puberty and reproductive viability

Although female guinea pigs may exhibit their first oestrus around 30 days of age, they do not normally show sexual activity until around 70 days of age. Sexual maturity in male guinea pigs is around 70 days (fully developed spermatozoa), with androgen levels rising from around 30 days.

The expected breeding life of a sow (female) is around 2 years, over which time she may well have up to eight litters. Males have a similar productive life expectancy.

Oestrous cycle

The guinea pig oestrous cycle lasts approximately 16 days, with minor or inapparent seasonal fluctuations. A pro-oestrus of around 36 hours is characterised by vaginal swelling, increased sexual activity and rupture of the vaginal membrane. A vaginal smear reveals nucleated and cornified squamous epithelial cells.

The membrane remains open for 2–3 days, covering the period of ovulation. Oestrus lasts 8–12 hours and is characterised by the copulatory reflex (lordosis), an open vaginal membrane, vaginal congestion and cornified cells in the vaginal smear. Oestrus most commonly occurs at night.

There is no conclusive evidence for synchronisation of oestrus in the guinea pig. A fertile postpartum oestrus lasting around 4–5 hours occurs within 16 hours of parturition. Infertile matings do not result in an altered oestrous cycle length, suggesting that, if pseudopregnancy does occur, it does not interfere with cyclical activity.

Copulation

When the sow is in oestrus, the boar approaches, sniffs, circles, nibbles, licks and mounts. The female assumes lordosis with her rear quarters elevated. The boar makes one or two intromissions and then ejaculates. Coital completion is marked by grooming and perineal marking by the boar. A copulatory plug and sperm can be found in the vagina. Sperm capacitation occurs within 10 hours, with ova remaining viable for around 30 hours.

Gestation and parturition

Embryos enter the uterus on day 3 of pregnancy and implant as blastocysts on day 6 or 7. Corpora lutea provide the source of progesterone for the first 20 days of gestation, after which pregnancy is maintained by the synthesis and secretion of the hormone from the fetoplacental units.

Gestation is around 68 days, with a quoted range of 58–72 days. Two to four viable embryos are the norm, with the total fetal mass increasing from around 100 g at 45 days to around 250 g at full term.

Pregnancy can be detected by gentle abdominal palpation at around day 15 of pregnancy. Firm, oval structures, around 5 mm in size, can be detected in the uterine horns. By day 25 they have grown to about 10–15 mm in size and they increase in size until term. In late pregnancy, abdominal distension is obvious and in the last week the pubic symphysis separates.

The guinea pig is not a nest builder, and onset of parturition is abrupt. It can occur at any time of the day or night, with no evidence of a time preference. Parturition is normally complete within 30 minutes, young being born every few minutes. The sow squats during delivery, cleaning the neonates and eating the placenta as they are delivered. Dystocia is rare but can be fatal in obese sows, very young sows and sows with oversized fetuses.

Guinea pigs are born mobile, fully haired, with teeth and with their eyes and ears open. These precocious characteristics are a result of the relatively long gestation period.

Newborn animals remain close to the sow but may not suckle for 12–24 hours. They will begin to take solid within a few days.

Lactation

Lactation peaks at 5–8 days postpartum and ceases by day 30 postpartum. A sow can produce up to 70 ml of milk daily. Pre-weanling animals will readily suck from any lactating female and may strip sows of milk intended for younger animals. Weaning usually takes place around 21 days of age when the animals will weigh about 175–200 g.

It should be noted that pregnancy/lactation alopecia is common in guinea pigs and may become progressive in the multiparous females.

Rats

Whilst wild rats are universally acknowledged as pests, responsible for carrying and transmitting zoonotic infections, they are also a very valuable animal in biomedical research and in recent years have gained increasing popularity as pets as well as show (fancy) animals.

All domestic (laboratory) strains of rat are descended from the wild rat, *Rattus norvegicus*, which originated in Asia and reached Europe in the early 1700s.

Anatomy

The male reproductive system has a number of highly developed accessory sex glands. These include large paired vesicular glands, a bulbo-urethral gland and a prostate gland. The inguinal gland remains open throughout life with the testes descending around 40 days of age.

The female rat has a bicornate uterus with the horns being separate for their entire length. There are paired ossa uteri and cervices. The female urethra does not communicate with the vagina or vulva but exits at the base of the clitoris. The female rat has six pairs of mammary glands and associated nipples.

Fig. 34.5 Female (reader's left) and male (reader's right) weanling rats. Note the increased anogenital distance in the male.

Puberty and reproductive viability

Sexual maturity occurs between 6 and 8 weeks of age in both sexes. The vagina opens between 35 and 110 days, and the testes descend around 15–51 days. Fertility wanes around 18 months – 2 years in the female, and male fertility is lost around the same time. Fertility in both sexes is regarded to be at its peak between 100 and 300 days of age.

Oestrous cycle

Although rats ovulate spontaneously, vaginal stimulation during mating is important in rat reproductive physiology. The more often intromission occurs before ejaculation, the greater the probability of a resulting pregnancy. Natural or artificial stimulation of the vagina within 15 minutes of a first mating will prevent any pregnancy from the first mating by inhibiting sperm transport. Oestrus, of 12 hours, duration, recurs every 4–5 days and postpartum without seasonal variation. Oestrus can be inhibited by group-housing females and synchronised in the presence of a male (Whitten effect); this latter effect is less pronounced than in the mouse.

Oestrus can be detected in a number of ways. Females are hyperactive and 'brace themselves' when touched. Their ears quiver when stroked and stroking of the pelvic region induces lordosis. The vulva becomes swollen and the vagina dry in contrast to the moist, pink wall seen during metoestrus and dioestrus. During pro-oestrus (approximately 12 hours), vaginal smears contain nucleated squamous epithelial cells, leucocytes and a few cornified cells. Oestrus begins when there are about 75% nucleated and 25% cornified cells. Cornified cells increase in number and eventually predominate as oestrus progresses. Metoestrus (approximately 20–24 hours) is characterised by the appearance of leucocytes which, together with the cornified cells, produce a vaginal detritus. Dioestrus last for about 55–60 hours. Breeding dates can be confirmed by examining vaginal smears for sperm or examining the distal vagina and cage floor for copulatory plugs.

The rat oestrous cycle is extremely sensitive to the influence of light, with as little as 3 days' constant lighting leading to persistent oestrus, hyperoestrogenism, polycystic ovaries and endometrial hyperplasia and metaplasia.

Copulation

Males mount the oestrous female many times, with one or two rapid ejaculations occurring in the course of 15–20 minutes. Ejaculated semen coagulates to form the copulatory plug, which remains in the vagina for a few hours before being dissolved or extruded. Normally, copulation occurs at night.

Gestation and parturition

Gestation can range from 19 to 23 days depending upon age, strain, nutritional status, litter size and parity. The average gestation length is 21–22 days, with primiparous females tending to have a slightly longer pregnancy than multiparous females. Abdominal enlargement becomes obvious at about 2 weeks. Pseudopregnancy is rare.

Parturition is signalled by pronounced postural stretching and rear leg extension. A vaginal discharge may be noted 1–4 hours before the onset. Normally, it is complete in 1–2 hours, depending upon litter size. Dystocia is extremely rare. Postpartum mating is the norm in pair-housed animals.

Litter sizes vary between 6 and 12, with the highest fecundity being seen in or around the sixth litter. Inbred strains will produce smaller litter sizes. Cannibalism is not uncommon, especially in primiparous females subjected to stress from any cause. Neonates weigh around 5–6 g, depending upon litter size and strain. Pups are born hairless and blind, with closed ears, undeveloped limbs and short tails. Ears open around day 2 or 3, incisors erupt at days 8–10 and eyes open around days 12–16. They are fully haired by day 10.

Lactation

Maternal antibody is transferred both transplacentally and via the colostrum for up to the first 18 days of suckling. Although pups can be weaned as early as 17 days, the normal weaning age is around 21 days.

Mice

All laboratory and pet (fancy) strains of mouse are originally derived from the house mouse of North America and Europe (*Mus musculus*). It was employed in comparative anatomical studies as early as the 17th century. Acceleration of biological research in the 19th century, a renewed interest in genetics and the requirement for a small, economic mammal that was easily housed and bred were instrumental in the development of the 'modern' laboratory mouse. In genetic terms, the laboratory mouse is probably the most thoroughly characterised mammal on earth.

Anatomy

There are no unusual or significant features of the male reproductive organs, there being all of the associated accessory sex glands present.

The female, in addition to the usual reproductive organs, has paired clitoral glands. These are analogous to the male preputial glands and secrete a sebaceous substance through ducts entering the lateral wall of the clitoral fossa. The female mouse usually has five pairs of mammary glands and associated nipples. Three pairs lie in the cervicothoracic region and two in the inguinoabdominal region.

Puberty and reproductive viability

Puberty in the female usually occurs around 24–28 days of age, with oestrogen-dependent changes,

Fig. 34.6 Female (reader's left) and male (reader's right) weanling mice. Note the increased anogenital distance in the male.

Fig. 34.7 Female (reader's left) and male (reader's right) day-old mice. Note the increased anogenital distance in the male.

such as cornification of vaginal epithelium, becoming evident. Sexual maturation in the male occurs slightly later (up to 14 days). Sexual maturation varies between strains and stocks and is subject to seasonal variation.

Whilst the theoretical breeding life of male and female mice may approach 2 years, in reality most breeders are retired between 6 months and 1 year.

Oestrous cycle

The mouse is polyoestrous and cycles every 4–5 days. During pro-oestrus and oestrus, active vaginal epithelial growth occurs in the genital tract and culminates in ovulation. The whole cycle can be followed by observing the changes in the exfoliative vaginal epithelial cytology, which are often used to determine the optimum receptivity of the female to mating. Oestrus can be detected by the patency of the vaginal opening and the swelling of the vulva.

Although oestrus occurs at around 14–24 hours postpartum, vaginal cornification is often incomplete, leading to an infertile mating. Mice are spontaneous ovulators, and ovulation may not accompany oestrus, and vice versa. The cycles of oestrus and ovulation are both controlled by the diurnal rhythm of the photoperiod. Oestrus, copulation and ovulation most frequently occur in the dark; thus by reversing the timing of the light/dark cycle, it is possible to reverse the timing of oestrus, copulation and ovulation.

Pheromones and social environment can also affect the oestrous cycle. Oestrus is suppressed in mice housed in large groups due to induced dioestrus, and can be countered by the olfactory stimulation from male pheromones (Whitten effect). This effect can be deployed to synchronise oestrus in group-housed females.

Copulation

Mating is usually detected by identifying the formation of the vaginal plug; however, its prevalence is highly strain-dependent. The plug normally fills the vagina from the cervix to the vulva.

Gestation and parturition

The corpora lutea are the main source of progesterone for about the first 13 days of pregnancy; thereafter the placenta takes over the main role. If the mating was sterile, pseudopregnancy occurs during which there is neither oestrus nor ovulation. Ova can be fertilised for up to 10–12 hours post-ovulation. Gestation is usually 19–21 days. If the female conceives at a postpartum mating, she may lactate at the same time as being pregnant. In certain strains of mice this extends pregnancy by a significant number of days.

Parturition normally occurs at night and dystocias are extremely rare. Litter sizes can vary in number up to about 14.

Lactation

Suckling the litter can account for up to 70% of the variation in body weight of neonatal mice. Nursing females usually lactate for about 3 weeks, with milk production increasing up to about day 12, and then declining steadily until weaning at around 3 weeks. Passive immunity is transferred via the milk and continues throughout the lactation.

Hamsters

Although there are four common types of hamster in the family *Cricetidae* (Syrian (golden), Chinese, Armenian and European), the Syrian or golden is by far and away the most popular, both as a pet and for use in biomedical research. This section

Table 34.3 Reproductive characteristics for different species of hamster (from The UFAW Handbook on the Care and Management of Laboratory Animals. Volume 1. 7th Edition 1999)

Character	*Syrian*	*Chinese*	*Djungarian*
Age at puberty	45–60 days	48–100 days	45–60 days
Min. breeding age	50 days	70–84 days	50 days
Breeding season	All year, maybe a decrease in winter	All year in laboratory conditions	All year in laboratory conditions
Oestrous cycle	Polyoestrus: all year	Polyoestrus: all year	Polyoestrus: all year
Duration of oestrus cycle	4 days	4 days	4 days
Duration of oestrus	4–23 hours	6–8 hours	–
Gestation	16 days	21 days	18 days
Average litter size	6	5	3.2
Ovulation time	Early oestrus	Shortly before oestrus	–
Copulation	About 1 hour after nightfall	2–4 hours after start of dark period	–
Implantation	5 or more days	5–6 days	–
Birth weight	2 g	1.5–2.5 g	1.5–2.0 g
Weaned	21 days	21 days	18 days
Chromosome no.	44	22	28
Return to oestrus postpartum	5–10 minutes	Postpartum mating does occur	Postpartum mating does occur
No. of mammae	14–22	8	8

will concentrate on the reproduction of the Syrian hamster only. However, it must be noted that there are distinct and significant differences in the reproductive physiology of the four breeds and these are summarised for three of them in Table 34.3.

Anatomy

Male hamsters are easily identified visually by the prominent glands located in the costovertebral area, in which coarse hair over darkly pigmented skin can be readily identified. These are sebaceous glands that produce secretions in response to androgen stimulation and they become excited when the hair over these glands becomes wet; the animal will scratch and rub itself as though there is irritation over the area. There is some evidence that the glands are used for territorial marking. In addition, there are visually prominent glands above and by the side of large testes, that make the body shape of the male pointed and protuberant.

In sexually mature male hamsters, the testes lie within a scrotal sac that has no mediastinum. As a result, the penis is retracted when the animals are not mating. There is a full complement of accessory sex glands that have no special features. The male hamster has an os penis consisting of two distal lateral prongs and a dorsal prong.

Fig. 34.8 Female (reader's left) and male (reader's right) hamsters. Note the greater anogenital distance in the male. Also note the 'pointed' rear end of the male in comparison to the female.

The female hamster has a duplex uterus, with an undivided section of about 8 mm, whilst each horn is some 20 mm long. There are seven pairs of mammary glands and associated nipples stretching from the thorax to the inguinal region.

Puberty and reproductive viability

Male hamsters reach sexual maturity at around 12 weeks of age and weighing about 90 g. Females also reach sexual maturity at around the same age and weight. The vagina opens at about 10 days of age, which is different from most other rodents where the vaginal opening is delayed until sexual maturity.

It should be noted, however, that precocious sexual activity is the hallmark of hamsters, since spermatozoa have been found on the glans penis of males from as early as 6 weeks of age. There are reports of hamsters mating from as early as 4 weeks of age. For this reason, it is advisable to wean hamsters into single sex groups to prevent accidental sibling matings.

The optimal reproductive life for hamsters is around 10–12 months, with a significant drop in reproductive capacity after this.

Oestrous cycle

It should be noted at the outset that the mature female hamster is a relatively solitary animal except when sexually receptive, and generally will not tolerate the presence of a male outside this short period of time. There is some evidence that unlike in other rodents, olfactory stimuli are not involved in determining the onset of sexual receptivity.

The oestrous cycle of the hamster is quite regular, lasting 4 days. The presence of a white, stringy, opaque vaginal discharge signals day 2 of the cycle. A waxy secretion appears on day 3. Establishing the correct day for mating is based on detecting the presence of the white opaque discharge, since this shows that the female was in oestrus the day before (day 1). From this, it can be reliably predicted that the female will achieve peak oestrus on the following day, viz. day 3. A 90% pregnancy rate has been recorded when mating large groups of hamsters in this way.

It should be noted that the hamster's vagina has two lateral pouches lined with cornified epithelium, and this can cause confusion if vaginal smears are used to detect oestrus.

Within the 4-day oestrous cycle, there is rapid development and regression of the corpora lutea, unlike in the rat and mouse where there is retention of several sets from previous cycles.

Copulation

At peak behavioural oestrus (which is approximately 8 hours in advance of ovulation), the female tolerates the presence of the male and almost immediately exhibits lordosis. Copulation takes place shortly afterwards and lasts about 30 minutes. Because of this aggressive behaviour on the part of the female, most matings are set up as a monogamous system, with the male and female occupying separate cages. In such a system, one male can usually maintain a harem of 12 females.

Gestation and parturition

Implantation of the embryos takes place on day 5–8 after fertilisation. Experience shows that this is a critical time and, to increase the likelihood of pregnancy occurring the female hamster, should be subjected to minimal handling.

The hamster has the shortest gestation period of any of the common laboratory animals. The period is 15–16 days, with a consistent mean of 15.5 days. There are variations of a few hours around this, dependent upon the prevailing ambient conditions. Gravid females should be given a clean cage and additional nesting material some 2–3 days before parturition. They should be given enough food to last 7–10 days so that there can be minimal disturbance over the periparturient period. Fresh water must, of course, be available at all times. Excessive disturbance over the period of parturition frequently results in cannibalism, particularly in primiparous females. Dystocia is extremely rare in the hamster.

Generally, the first litter is smaller in number than subsequent litters, with an average size of 11 (range 4–16).

The young are born with their eyes and ears closed, hairless but with teeth present. Ears open at day 4, they begin to eat solid food from day 7 onwards and their eyes open from day 14. It is important to remember that they will need fresh water consequent to them beginning to take solids. Hamsters are usually weaned around 3 weeks of age.

Although a postpartum oestrus is said to occur, it is rarely seen in practice and it is usual for the females to mate successfully for the first time 3 days post-weaning.

Other than the period of lactation (3–4 weeks), little has been published on the volumes or make-up of hamster milk.

Finally, it is interesting to note that, while suckling hamsters can be successfully cross-fostered on to surrogate lactating mothers, rederivation of a colony by caesarian operation and hand-rearing have yet to be accomplished and recorded in the literature. The failure to hand-rear successfully is thought to be connected with either the milk content and/or the suckling behaviour of the young animals.

REPRODUCTIVE DISEASES AND INFERTILITY

There are a number of non-infectious and infectious diseases of rabbits and rodents that have a specific effect on the reproductive system. In addition, there are other diseases that, whilst not specifically affecting reproduction, have a major impact. These diseases and disorders will be described in relation to their aetiology, rather than on a species-by-species basis.

Non-infectious factors

The following will be considered under the heading of non-infectious conditions:

- environment
- nutrition
- neoplasia.

Environment

Whilst it is commonly accepted that a reasonable environment coupled with good husbandry and hygiene are critical to any successful livestock enterprise, there are in addition some specific environmental conditions that will have a direct impact on the production of rabbits and/or rodents.

Heatstroke. Heatstroke in rabbits is a well-known condition, especially in the large breeds and during pregnancy. This sensitivity is thought to be linked to the relatively high ratio of surface area to body mass. In the summer months of hot climates losses due to heatstroke may exceed other causes of death.

Clinical signs include rapid respiration, cyanosis prostration, blood-tinged nasal exudate and finally death. Pregnant animals surviving the episode of heatstroke may go on to abort. High environmental temperature may also lower the libido of working bucks and some work suggests it may have a direct affect on the spermatozoa.

The best way to prevent heatstroke, where air conditioning (cooling) is not possible, is to provide the animals with cages sufficiently large to enable them to stretch out fully. Panting is the main mechanism rabbits have for losing heat, and they can only pant efficiently and effectively when fully outstretched.

Ultrasound. Whilst all noise within animal facilities must be controlled to reasonable volumes, it must be remembered that the frequencies to which the animals are sensitive are very different to those of man. It is certainly worthwhile considering the possibility that extraneous ultrasound may be a cause of non-specific poor productivity within a rodent breeding unit. Specific signs of the effect of ultrasound include poor mothering, cannibalism and poor conception rates. Sources of ultrasound in the modern animal facility are many, and include computers and visual display units, air conditioning units and even the effect of running water on to metal surfaces.

Lighting. There are three aspects of light quality that can affect the breeding and productivity of rodents:

- source (wavelength)
- intensity (brightness)
- duration (length of time the lights are on).

Whenever there is a fall in productivity within a rodent or rabbit breeding unit that has automatic lighting (duration), it is worth investigating whether the time switch is functioning correctly. This may mean actually being in attendance to observe the lights coming on and going off, rather than merely examining the electronics/mechanics of the timer.

Nutrition

As with the environment, it goes without saying that rodents and rabbits, like any other species, must have a balanced and adequate diet if they are to reproduce successfully and meet their full reproductive potential. There are a number of dietary conditions that directly or indirectly influence reproduction, and thus decrease production.

Pregnancy toxaemia. Pregnancy toxaemia occurs in both the guinea pig and rabbit. Its exact aetiology is unclear; however, it seems to be a combination of inappropriate diet, fetal load, inability to ingest sufficient feed and perhaps exacerbating stress factors. Clinical signs in both species vary from virtually none to sudden death. Clinical signs commonly observed are depression, dyspnoea, incoordination, 'star gazing', convulsions, coma, acetonaemia, decreased urine production and abortion.

The main sign at necropsy is fatty liver, which can be confirmed on histopathological examination. In addition, other common post-mortem findings in the rabbit and guinea pig include obesity, active mammary glands, large corpora lutea and a pale heart and kidneys.

Where more than the infrequent, sporadic case occurs in a rabbit or guinea pig breeding unit, a full investigation should be conducted to establish the nutritional status of the colony and any other possible contributory factors. In individual pet animals, standard treatments with oral ethylene glycol and parenteral corticosteroids can be tried, though any prognosis is very guarded. Economic constraints prevent treatment in commercial colonies, where preventative programmes are the more effective approach.

Vitamin C deficiency (scurvy). It is well known that, together with man and monkeys, guinea pigs are unable to synthesise their own vitamin C, and are totally reliant on a dietary source for all of their needs. Clinical scurvy in its many forms is a common disease of guinea pigs.

However, whenever a colony has suboptimal breeding performance, marginal vitamin C deficiency must be considered. The amount of actual available dietary vitamin C available must be investigated, and where it is suspected that there may be a deficiency, additional amounts must be supplied via the food or water. Vitamin C has a relatively short shelf life when mixed in rations; it is heat-labile and will react with metal water pipes because of its acidic nature. Therefore, guinea pig diets must never be stored for long periods, diets exposed to extreme heat (such as autoclaving) must have substantial additional vitamin C supplementation to survive the process, and they must never be placed in watering systems that have metal piping.

The actual daily requirement of vitamin C by guinea pigs is much disputed, and so the author prefers to take the pragmatic approach that if the clinical signs disappear, or if breeding performance improves after supplementation, then dietary intake was probably insufficient. Some studies indicate a requirement of up to 7 mg/100 g body weight/day; however, when extrapolated to the accepted requirement in man it is 10 times higher. Since such requirements are questioned by some authorities, the author believes that good clinical judgement supports the supplementary feeding of this vitamin.

Neoplasia

It is generally thought that neoplasia involving the genital system is rare in rabbits and rodents; this is probably because most laboratory animals are euthanased when comparatively young.

Uterine adenocarcinoma. Uterine adenocarcinoma is unquestionably the most common neoplasm of the rabbit. The tumour most frequently occurs in individuals that have a history of reduced reproductive performance due to lowered fertility, false pregnancy, fetal resorption and abortion. These signs usually precede diagnosis of the neoplasm by 6–10 months.

The rabbit is frequently presented to the clinician because of a swollen abdomen, or because the owner thinks it may be pregnant (even though it has not been exposed to a male). In animals presented at this stage, the neoplasia is usually well advanced, with secondary tumours and associated ascites, thus making palpation of the mass impossible. In these cases, radiography will help to confirm the diagnosis. In early cases, tumour masses of various sizes may be palpated within the uterus; if so, then pregnancy must be excluded when there is the possibility that the animal may have been mated.

Mammary adenoma (carcinoma). Mammary adenomas are common in certain strains of rat. They generally appear from 1 year of age, and whilst they are primarily hormone-dependent, there are studies to show that a high plane of nutrition (protein and carbohydrate levels) may contribute to, or exacerbate, the condition. Mammary tumours of mice are generally of a viral rather than hormonal aetiology. The virus causing the tumours is known as mouse mammary tumour virus (MMTV) and there are several strains.

Pituitary adenomas. Pituitary adenomas are relatively common in ageing rats of certain (laboratory) strains. They can appear in both sexes but are more common in females. Their frequency rises in animals over 18 months of age. Clinical signs vary immensely depending upon the size of the tumour, but in the later stages neurological signs develop due to compression of the brain, and general wasting of the animal becomes evident.

Infectious disease

There are few infectious diseases that affect only, or primarily, the reproductive tract of rabbits and rodents. However, there are a few diseases that are worthy of consideration. There are no specific infectious diseases affecting only the male reproductive system; however, males may experience lowered fertility and/or libido as a result of any number of febrile conditions.

Rabbit syphylis

Rabbit syphylis is a condition caused by the organism *Treponema cuniculi* and affects both sexes. The organism is a spirochaete and populates the vulva and prepuce of rabbits, reducing fertility mainly due to inhibition of copulation because of the associated pain. Transmission is both venereal and by contact of young rabbits with their dams.

The disease is characterised and recognised by dry, crusty exudative lesions around the vulva and prepuce. In addition, lesions around the mucous membranes of the face and mouth of the animal are common as a result of licking the affected perineal areas partly because of the irritation.

A positive diagnosis can be made by using dark field microscopy to identify the spirochaete organisms. Affected individuals and herds can be successfully treated using parenteral penicillin.

Pyometra

Pyometra can occur in all of the species under consideration in this chapter; however, it is rare and frequently only diagnosed at necropsy. Affected animals may be mistakenly diagnosed as pregnant. Whilst sporadic cases within a breeding colony are of no particular consequence, outbreaks should be investigated and the causative organism confirmed; Gram-negative bacteria are often implicated.

Pasteurellosis

Pasteurella multocida is a frequent bacterial pathogen of rabbits and whilst it primarily causes respiratory disease, it may in addition give rise to a number of associated clinical conditions. With regard to reproduction, *Pasteurella* has been associated with pyometra, abortion and general lowered fertility. Positive diagnosis is by culture of the organism.

Rodent parvoviruses

Rodent parvoviruses may infect rats, mice and hamsters. Some strains are host-specific (such as Kilham rat virus (KRV) and minute virus of mice (MVM), whilst others such as toolans may cross the species barrier. Their epidemiology and immunology are complex and cannot be covered in the context of this chapter.

Their effect on reproduction can be significant, ranging from reducing overall fertility to inducing teratogenic malformations of embryos in the uterus (KRV). In this regard they are very similar to the feline parvovirus (feline panleucopenia). Clinical manifestations in adults can be few to none and confirmation is by serology to detect antibodies and finally virus isolation.

Sendai virus

Sendai virus is a parainfluenza virus that can infect rats, mice, hamsters and guinea pigs, although there are few or no clinical signs of infection in the guinea pig.

Epizootic outbreaks in a naïve colony can have a devastating effect on the production. Overall fertility can be severely reduced, with or without accompanying signs of respiratory disease. Once the disease becomes endemic and breeding females develop maternal antibody, the consequences for production become less significant. In reality, due to the cyclical nature of the infection and subsequent passive antibody protection, the disease tends to appear in 'waves' of epizootics within a colony. Confirmation is via antibody detection and it must be noted that titre formation may lag behind clinical manifestations by 2–4 weeks.

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35 Embryo transfer in large domestic animals

The term 'embryo transfer', taken literally, refers solely to the collection of an embryo from a donor animal and its placement into the uterine tube or uterus of a recipient. However, by common usage, it has become accepted to cover a whole range of allied techniques, including superovulation of the donor, and storage and manipulation of embryos in vitro.

The first successful embryo transfer was carried out over 100 years ago in rabbits (Heape, 1891) but it was some time before the technique was successfully applied to farm animals. Warwick and Berry (1949) produced the first lamb by embryo transfer, but despite intense research effort, the first calf was not born until 1951 (Willett et al., 1951). Even then, it was not until much later that the technique had advanced sufficiently to be of practical use in cattle breeding (Rowson et al., 1969). Since then, embryo transfer has been used successfully to increase the reproductive rate of cattle, horses, sheep, goats and pigs.

Embryo transfer has been applied most extensively in the cow; consequently the technology has advanced most rapidly in this species. In the early 1970s, general anaesthesia and laparotomy were necessary for both recovery and transfer of bovine embryos, and embryo transfer was used in the UK and North America mainly for the rapid multiplication of imported exotic beef breeds. With the advent of efficient non-surgical techniques for recovery of embryos, and effective methods for preserving embryos in liquid nitrogen in the latter part of the decade, demand for embryo transfer services increased dramatically in both the beef and dairy industries.

According to figures collected by the European Embryo Transfer Association, the total number of bovine embryos transferred in the EU in 1997 topped 118 000, and the trend continues upward. In excess of 23 000 of those transfers were carried out in the UK, although the current depression in UK agriculture is likely to result in a significant downturn in these figures over subsequent years. The majority of embryos are collected and transferred on the same farm, but national and international trade in frozen embryos contributes significantly to these figures.

The commercial application of embryo transfer has been much more restricted in the other domestic species. A reluctance on the part of the breed associations to register progeny produced by embryo transfer has partly been responsible for this in the horse, and economic factors, together with the need for surgery, have militated against widespread use in pigs, sheep and goats.

APPLICATIONS OF EMBRYO TRANSFER

Over the years embryo transfer has been, and continues to be, a valuable research tool (see review by Sreenan, 1983). It has been used exclusively in studies of uterine capacity, on the uterine environment, the maternal recognition of pregnancy, embryo–uterine relationships and endocrinology of pregnancy. Embryo transfer has also been used in disease transmission studies and to investigate the genetics of reproduction: for instance, litter size, gestation length, birth weight and postnatal production. The rapid development of new technologies is now expanding the scope of embryo transfer in research.

The production of identical twins and clones will accelerate progress in many fields of research, and the ability to manipulate fertilisation and modify the genome of the early embryo will advance the frontiers of knowledge. However, the most practical application of embryo transfer today depends on its capacity to increase the reproductive rate of female animals. The rapid uptake of embryo transfer by cattle breeders, in particular, has depended on the ability to increase the number of progeny from valuable brood cows, either as a means of rapid herd improvement or to produce surplus embryos, pregnancies or stock for sale.

Genetic improvement

The rate of genetic improvement within a breed depends on four variables: the amount of genetic variation for the traits under question, the accuracy with which the parents of the next generation can be selected, the selection intensity and the generation interval. Embryo transfer can be used to influence all four variables and improve rates of progress.

Genetic variation

This can be increased by introduction of a breed genetically superior for the desired traits. Embryo transfer can be used both to introduce the breed in question through the medium of frozen embryos, and to increase the reproductive rate of resulting females to facilitate its rapid distribution.

Selection of dams

The breeding value of females can be calculated from their own performance and conformation data, together with that of close relatives. The accuracy of the calculation depends on the numbers of relatives available for recording, and embryo transfer can be used to increase numbers in species with a low reproductive rate, allowing, for instance, sibling or progeny testing of cows (Nicholas and Smith, 1983).

Selection intensity

In dairy cattle, the majority of female offspring in a herd are needed as replacements to produce the next generation. Using embryo transfer to increase the reproductive rate of the best cows, selection can be restricted to the top 5–10% of females. Similarly, in a national bull selection programme the use of embryo transfer would allow the proportion of bull dams selected to be reduced from, say, 2 to 1%, by ensuring that a bull calf was produced from virtually every mating (Cunningham, 1976).

Generation interval

A method for dairy cattle improvement using embryo transfer intensively on selected individuals within one nucleus herd has been proposed by Nicholas and Smith (1983). Sets of full and half siblings, within this type of scheme, can be recorded for the traits in question in a uniform environment, and the selection of males and females to produce the next generation can then be made on the basis of sibling testing rather than progeny testing as in conventional improvement schemes. Breeding programmes based on this system have become known as MOET (multiple ovulation and embryo transfer) schemes, and have been applied in practice in dairy and beef cattle and sheep. The practical application of MOET in dairy cattle has been described by Christie et al. (1992).

This approach allows a dramatic reduction in the generation interval, and consequently allows the opportunity for more rapid genetic improvement than can be achieved with the application of a traditional progeny-testing system.

Genetic screening

Embryo transfer has also been used to expedite the screening of both dams and sires for genetic defects such as syndactyly in cattle (Baker et al., 1980).

Disease control

There is increasing evidence to suggest that embryos are unlikely to spread viral and bacterial diseases when transferred into recipients (see review by Wrathall, 1995). The zona pellucida would appear to be an effective barrier to infection of the embryonic cells from the uterine environment, and washing of embryos or treating with trypsin has been shown to remove viral contamination from the zona pellucida in vitro (Singh, 1984). Singh (1984) cites data from several authors. For example, 407 embryos transferred from enzootic bovine leucosis-seropositive donors have resulted in no seropositive recipients or calves. Similarly, 67 embryos transferred from blue tongue virus-infected donors and 62 embryos (trypsin-treated) transferred from infectious bovine rhinotracheitis virus-infected donors resulted in seronegative recipients and calves. Embryo transfer has also been effectively used for the introduction of new blood lines into specific pathogen-free pig herds (Wrathall, 1984). Sufficient transfers have been conducted with embryos from bovine leucosis, infectious bovine rhinotracheitis, blue tongue (cattle), *Brucella abortus* (cattle), foot-and-mouth disease (cattle) and pseudorabies (pigs)-infected donors to determine that these microorganisms will not be transmitted via embryos, provided they are washed properly (Stringfellow and Seidel, 1998). As the results of current and future experimental work become available, it is likely that similar conclusions will be drawn for other pathogens. However, more field trials will be necessary before the risk of disease transmission by embryos can be fully assessed. For instance Brownlie et al. (1997) demonstrated the presence of bovine viral diarrhoea (BVD) virus antigen within follicles and oocytes of persistently infected cattle, thereby throwing some doubt on the effectiveness of embryo washing for prevention of transmission of BVD via embryo transfer.

It is also likely that the risk of disease transmission through the transfer of in vitro-produced embryos is greater than in those produced in vivo (Stringfellow and Wrathall, 1995). The use of abattoir-derived cells for co-culture in some production systems increases the risk. There may also be differences between in vitro and in vivo-produced embryos that affect risk. For instance, Riddel et al. (1993) demonstrated differences in the zona pellucida between embryos derived from the two sources. It seems, therefore, that conclusions drawn from research involving in vivo-derived embryos cannot necessarily be extrapolated to those produced in vitro.

Import and export

The development of efficient methods for the cryopreservation of embryos of the cow, sheep and goat have stimulated a growing international trade in genetic material. Economy and convenience have been major considerations, but many governments have now made import regulations for embryos less stringent than those for live animals or semen, in recognition of the relatively lower risk of introduction of disease by embryo transfer. An additional advantage of embryo transfer in this situation lies in the fact that a calf resulting from an imported embryo transferred into an indigenous recipient acquires colostral immunity to local diseases, and consequently may thrive better than an animal imported on the hoof.

Circumvention of infertility

Embryo transfer techniques have proved valuable in the diagnosis, treatment and circumvention of certain types of infertility in cows (Elsden et al., 1979; Mapletoft et al., 1980; Figure 35.1). Careful screening of donors is necessary to ensure that the infertility is due to injury, disease or senility and is not of genetic origin; otherwise reproductive problems could be propagated.

Twinning in cattle

Studies have shown that the efficiency of beef production from suckler herds could be increased by twinning in intensively managed units (Sreenan, 1977).

Genetic selection for twinning has largely been unsuccessful (Sreenan, 1979), and gonadotrophin treatments to increase ovulation rates are not reliable (Gordon et al., 1962). Twinning by embryo transfer, by transfer of either two embryos or one embryo to a previously inseminated recipient, is a practical alternative (Sreenan and Diskin, 1982). The relatively high cost of embryo transfer has precluded practical application in the past. However, the technology of in vitro fertilisation applied to oocytes aspirated from abattoir ovaries dramatically reduced unit costs per embryo, opening up possibilities for commercial application of twinning to improve beef production (Lu and Polge, 1992). However, pregnancy rates achieved in the field have not been good enough for the technique to be economically viable.

Fig. 35.1 Embryo transfer can be used to circumvent certain types of infertility. This cow had ceased to breed because of senility but four young surrogates were able to carry her calves to term.

Conservation

Embryo transfer can be used to increase the population of rare or endangered breeds or species, provided there are recipients of a more plentiful breed or species that will accept the embryo.

EMBRYO TRANSFER IN THE COW

Superovulation

Single embryos can be recovered and transferred to other cows 6–8 days after service at natural oestrus, but because of the high costs involved, this is not usually an economic procedure in the practical situation. Consequently, a critical aspect of embryo transfer technology is the use of gonadotrophins to induce multiple ovulations in the ovaries of the donor cow (superovulation). For optimum response, gonadotrophin treatment is initiated on days 9–14 (oestrus = day 0) of a normal oestrous cycle, coinciding with the emergence of the second follicular wave. Prostaglandin is administered 48–72 hours later to cause regression of the mid-cycle corpus luteum and induce oestrus, which usually occurs 40–56 hours later. Behavioural manifestations of oestrus are usually normal, and it is common practice to inseminate donors on at least two occasions 12–18 hours apart when using frozen semen as ovulations may occur over a prolonged period of time (Maxwell et al., 1978). The superovulated donor would appear to be a sensitive indicator of the fertility of semen (Newcomb et al., 1978a), and only bulls of high fertility should be used.

Several different gonadotrophins have been used to superovulate cattle and these include equine chorionic gonadotrophin (eCG) (Betteridge, 1977), pituitary follicle-stimulating hormone (FSH) of porcine (Elsden et al., 1978), equine (Christie and Green, 1984) or ovine (Jordt and Lorenzini, 1990) origin, and human menopausal gonadotrophin (hMG) (Newcomb, 1980).

eCG has a longer biological half-life in the cow than either FSH or hMG; consequently, a single injection of 2000–3000 IU will induce superovulation. FSH and hMG require a multiple injection treatment regimen for optimum effect; for instance, porcine FSH is usually administered twice daily for 4–5 days. The long half-life of eCG can be a disadvantage, as its effect persists even after the induced oestrus, and in some cows embryo transport is adversely affected. This is manifest, over large numbers, by a poorer recovery rate of embryos after superovulation with eCG, compared with other gonadotrophins (Table 35.1). There is evidence that an eCG antiserum administered at oestrus will improve results (Saumande et al., 1984). The presence of substantial luteinising hormone (LH) activity in eCG, and in crude FSH preparations used for superovulation, can adversely affect the viability of some ovulated oocytes by causing premature maturation (Moor et al., 1984). Fertilisation failure has also been attributed to abnormalities of oocyte maturation (Moor et al., 1985), and to asynchrony between maturation of the oocyte and the follicle (Loos et al., 1991). The problems are compounded by deficiencies in sperm transport in superovulated animals, resulting in reduced numbers of sperm in the uterine tube at the time of fertilisation (Hawk, 1988). There is some evidence that the use of the more purified FSH preparations now available for superovulation will improve fertilisation rates and embryo quality (Donaldson and Ward, 1986).

Donor cows can be superovulated repeatedly at approximately 6–8-week intervals with no adverse effect on subsequent fertility (Christie et al., 1979a), but ovarian response to superovulation treatment is very variable, both between animals and between treatments of the same animal (Newcomb et al., 1979). With experience, variability can be reduced by adjusting dose rates, but this still remains one of the problem areas of embryo transfer, with some donors yielding no embryos and occasionally 30 or more being recovered. One of the sources of variation would appear to be the presence or absence of a dominant follicle, as the former has been shown to depress response to superovulation (Guilbault et al., 1991). Removal of the dominant follicle prior to the start of FSH treatment – for instance, by aspiration (Lindsay et al., 1994) – can improve response in some cases. Further progress in the understanding of ovarian follicular dynamics in cattle may be the best option for improving superovulation treatments in the future.

Collection of embryos

In the cow, the egg usually enters the uterus on day 4 after oestrus, at which time non-surgical embryo recovery becomes feasible by flushing the uterus through the cervix. Collection attempts are usually made on day 6, 7 or 8 after oestrus, but recovery and successful transfer are possible up to day 16 (Betteridge et al., 1976).

There are several methods of non-surgical embryo recovery in use, but the commonest fall broadly into the types depicted in Figures 35.2 and 35.3. The earliest reported method was the variable-distance three-way (Sugie et al., 1972)

Table 35.1 A survey of superovulatory response and egg recovery after treatment with each of three gonadotrophins in cows (from Christie and Green, 1984)

Gonadotrophin	*No. of cows*	*Mean ovulation rate*	*Mean no. of eggs recovered*	*Recovery rate (%)*	*Mean no. of viable eggs (%)*
eCG (2500–3500 IU)	149	10.6	7.54	71.1	6.4 (84.7)
Equine FSH (20–24 mg)	52	11.83	9.62	81.3	7.13 (74.2)
Porcine FSH (40–50 mg)	54	11.52	9.48	82.3	7.41 (78.1)

Fig. 35.2 Techniques for recovering bovine embryos non-surgically (a) Variable-distance three-way (continuous flow). (b) Two-way (ebb and flow) (from Newcomb et al., 1978b).

but the fixed-distance three-way (Newcomb et al., 1978b) is the most common technique in use in the UK. This method has the advantage of a continuous flow of medium within the distal third of the uterine horn, and a consequent efficient flush of the region of the horn where the majority of early-stage embryos are situated (Newcomb et al., 1976) (see also Figure 35.4). The ebb and flow two-way technique (Elsden et al., 1976; Greve et al., 1977) is simpler, but requires larger volumes of flushing medium and is more time-consuming. In non-superovulated cattle, a skilled operator using these techniques can recover an egg in six or seven out of 10 attempts. The results that can be expected from superovulated donors are summarised in Table 35.1.

Fig. 35.3 A fixed-distance three-way technique for recovering bovine embryos non-surgically (a) Speculum in the vagina; (b) Introducer passed through the speculum to the cervix (c) Introducer passed through the cervix into one uterine horn (d) PVC catheter passed through the introducer to the tip of the horn and the cuff inflated (from Newcomb et al., 1978b).

Embryos are located under a stereoscopic microscope after settling and siphoning or aspiration of the flushing medium (Newcomb et al., 1978b), or more commonly, after filtering through a commercially available embryo filter to concentrate the embryos in a small volume of medium. A modified phosphate-buffered saline (PBS) (Whittingham, 1971) is commonly used both for flushing the uterus and for storage. Embryos can be kept in PBS on the bench for at

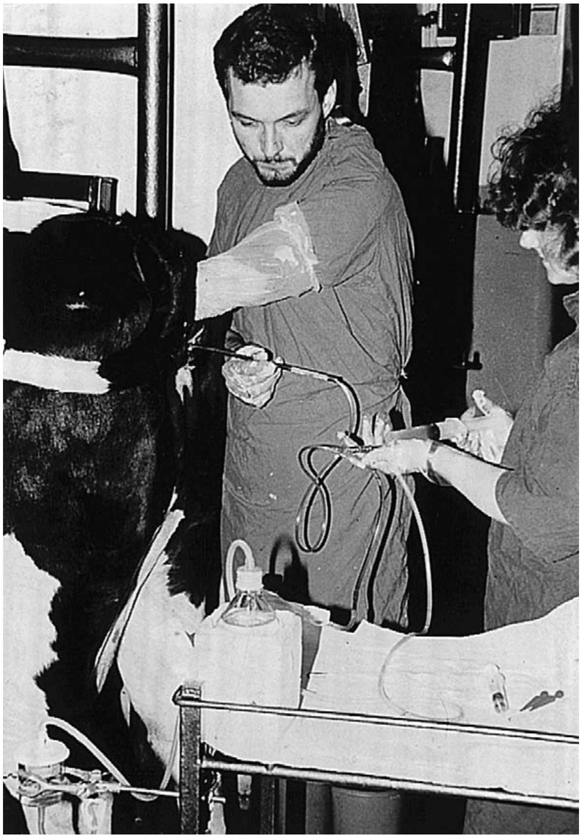


Fig. 35.4 A fixed-distance three-way uterine flush in progress.

least 8 hours with no loss of viability, and can be cultured for up to 48 hours with acceptable results on transfer (Trounson et al., 1976a). It is also possible to cool embryos to +4°C and maintain them in a state of suspended development for up to 3 days (Lindner et al., 1983), or store them long-term by deep-freezing (see later).

Day 7 bovine embryos are about 150–190 μm in diameter, and are still within the zona pellucida and at the late morula or blastocyst stage of development (Figure 35.5). They can be handled easily using a micropipette, and are evaluated under the microscope at 50–100× magnification. An assessment of viability can then be made by taking into account the stage of development relative to age, and the appearance of the cells. Embryos are usually classified as good, moderate or poor in quality, and this can be related to pregnancy rate on transfer (Table 35.2).

Transfer

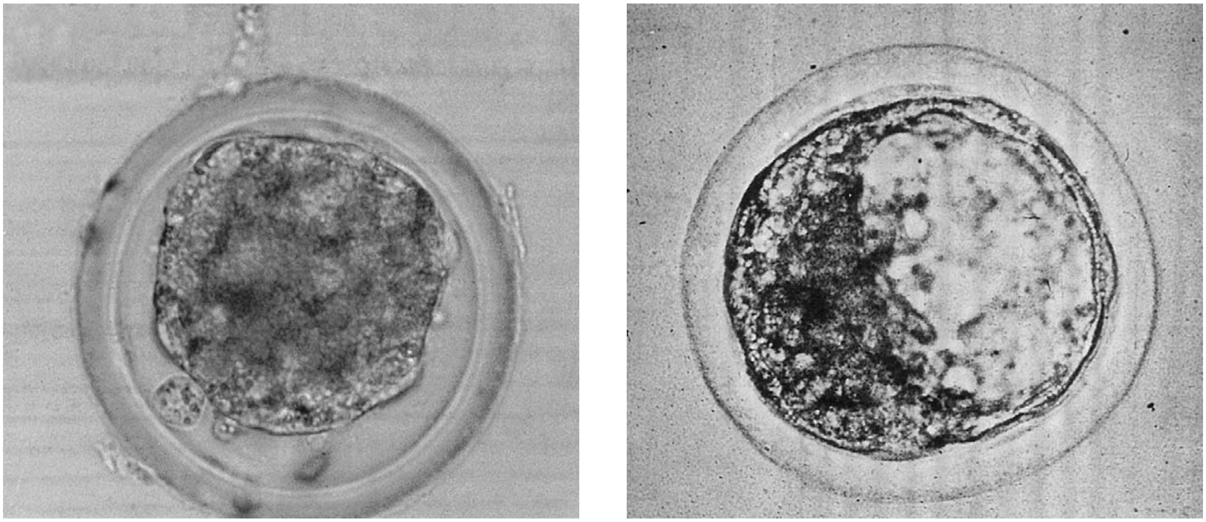
Many factors will affect the suitability of a recipient for embryo transfer. The animals used should be healthy, fertile heifers or young cows that are in good body condition, and can be reasonably expected to calve normally at term. Nutritional status should be good, and ideally the recipient should be on a rising plane for at least 6 weeks before and after transfer to achieve optimum results.

It has been shown conclusively that the oestrous cycle of the donor and recipient should be closely synchronised if transferred embryos are to survive (Rowson et al., 1972). An asynchrony of more than 24 hours results in a marked reduction in pregnancy rate; it is more economic to freeze and transfer later, when suitable recipients are available, than to step outside these limits. Of great importance too is the side of the transfer. Pregnancy rates are greatly reduced unless the embryo is placed in the lumen of the uterine horn on the same side as the corpus luteum (Christie et al., 1979b).

Embryos can be transferred either surgically or non-surgically. For surgical transfer, the uterus is exposed through a flank incision under local anaesthesia (Newcomb, 1979). A puncture is made with a blunt needle and the embryo transferred to the

Table 35.2 The quality of eggs/embryos recovered from superovulated cows and pregnancy rate achieved on transfer – a survey of 1437 eggs (from Christie, 1982)

	Quality of egg/embryo			
	Good	*Moderate*	*Poor*	*Unfertilised/degenerate*
Percentage in each category	50.4	13.2	10.7	25.7
Pregnancy rate (%)	79.0	63.8	38.6	Discarded



(a) (b)

Fig. 35.5 Day 7 bovine embryos. (a) Morula (b) Blastocyst.

lumen using a fine pipette or catheter (Rowson et al., 1969) (Figure 35.6). Under controlled conditions, a pregnancy rate of approximately 70% can be consistently achieved with embryos transferred on the same day as recovery.

Similar equipment to that used for artificial insemination can be used for non-surgical transfer, but stricter asepsis must be observed and the embryo is usually placed some distance into the appropriate uterine horn. Success appears to be skill-related, suggesting that trauma to the endometrium may be a limiting factor with this technique. However, experienced and dextrous individuals can achieve a pregnancy rate approaching that of surgical transfer, and as a consequence welfare considerations must mitigate against continued use of the surgical technique. In practice, non-surgical transfer has almost totally superseded the surgical method.

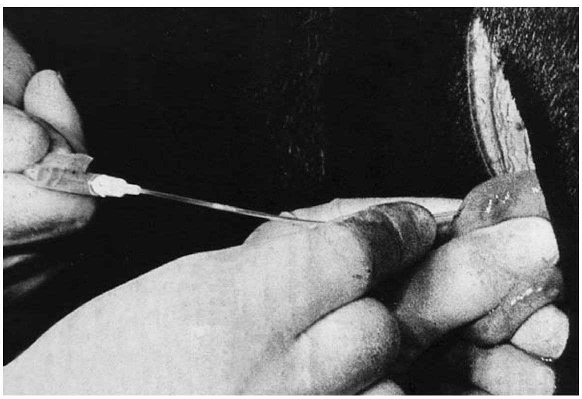


Fig. 35.6 Surgical transfer. A fine catheter is passed through a puncture into the uterine lumen to deliver the embryo.

EMBRYO TRANSFER IN SHEEP AND GOATS

Embryo transfer techniques are well established in sheep, mainly because the ewe has been used extensively in research as a low-cost model for the cow. Commercial use of the technique in sheep has not been widespread, with rapid multiplication of recently imported breeds and MOET schemes for breed improvement being the commonest applications. The use of embryo transfer in the goat rapidly expanded in the late 1980s, in line with the increased demand for valuable pure-bred Angora and Cashmere stock in the UK and Australasia, but has subsequently decreased to a low level.

Techniques for superovulation, embryo recovery and transfer are very similar for both species (Armstrong and Evans, 1983). The gonadotrophin preparations used are the same as those used in the cow, and they are administered in similar treatment regimens Gonadotrophin treatment is usually initiated mid- to late cycle and prostaglandin $F_{2\alpha}$ or analogues is administered 24–72 hours later, inducing oestrus within 24–36 hours. Oestrus and ovulation can also be controlled by progesterone or progestogen administration in the form of injections, implants or vaginal sponges. Sheep are treated for 12–14 days and goats for 14–18 days; using this method, superovulation can be induced outside the breeding season.

Insemination is commonly achieved by natural service or artificially, using freshly collected semen. However, fertilisation failure can occur commonly in the ewe, particularly when the ovarian response to gonadotrophin is high. This can be overcome by surgical insemination directly into the uterus, either by laparotomy (Trounson and Moore, 1974) or by a laparoscopic technique (Maxwell, 1984).

Surgical techniques for the recovery of embryos from the ewe and the doe have changed very little since the first reports (Hunter et al., 1955) and involve general anaesthesia, midline laparotomy and flushing of the catheterised uterus and uterine tube. Non-surgical recovery in the ewe has been reported (Coonrod et al., 1986), although the tortuous nature of the cervix makes catheter passage very difficult. Laparoscopy has been shown to be as effective as laparotomy (McKelvey et al., 1986) and is now widely used. The transcervical passage of catheters is much easier in the doe, and non-surgical techniques could be more successful in this species.

Collections are normally carried out 3–7 days after oestrus, and embryos can be evaluated and handled in the laboratory in a similar manner to the cow.

Most transfers are performed using general anaesthesia and midline laparotomy or laparoscopy. Embryos earlier than the eight-cell stage of development are best transferred to the uterine tube, and later-stage embryos to the uterus. Uterine transfer of day 6 and 7 embryos by laparoscope is as effective as laparotomy in the ewe (McKelvey et al., 1985), and has the advantage of not requiring exteriorisation of the tract. Recipients are synchronised using prostaglandin $F_{2\alpha}$ treatments or intravaginal progestogens, and oestrus is detected by the use of a harnessed, vasectomised ram or buck.

The requirements for synchrony of oestrus between donor and recipient are similar to the cow.

EMBRYO TRANSFER IN THE MARE

Embryo transfer in the mare is a relatively new procedure compared to the cow and many breed societies will not register the progeny (Figure 35.7). This, coupled with the difficulty in inducing superovulation, has limited the commercial application. The major uses, apart from the production of multiple offspring, are for the production of foals from subfertile mares, for the removal of the risks of gestation and parturition from older valuable brood mares, and for the production of foals from mares while they are in competition.

Limited success has been achieved with superovulation in mares using large doses of equine

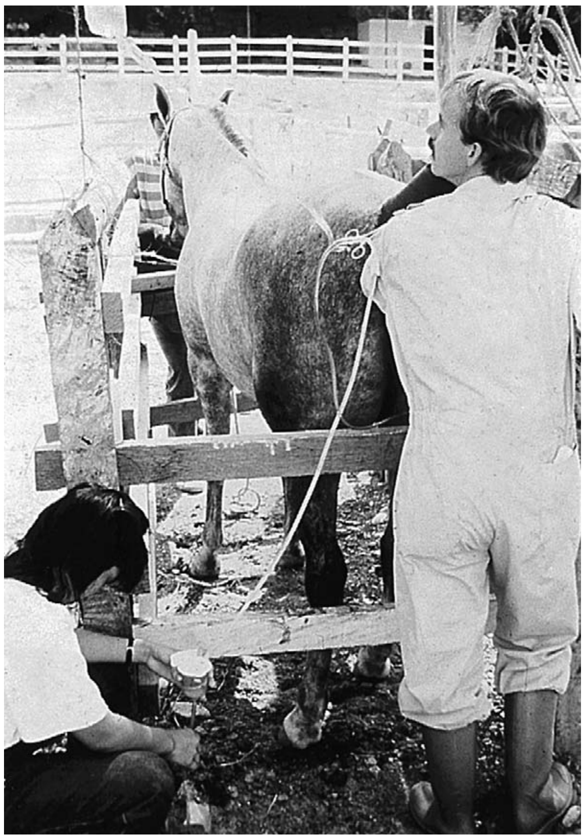


Fig. 35.7 A uterine flush in progress in a mare.

pituitary extract injected daily during dioestrus (Squires and McKinnon, 1986). In this study, two embryos were collected per mare compared with 0.65 embryos per untreated control. Porcine FSH was even less effective and eCG has been shown to have no effect at all on follicular development in the mare (Douglas, 1979). Consequently, it is routine for most groups involved in equine embryo transfer to collect single embryos.

Embryos are recovered non-surgically 6–8 days post-ovulation using a Foley-type catheter and an ebb and flow flush of the uterus with modified PBS. Day 9 embryos have been found to be less viable on transfer, possibly due to their relatively large size and consequent predisposition to handling damage (Squires et al., 1982). The use of prostaglandin on the day of recovery allows repeat collections to be made at approximately 17–18-day intervals without compromising embryo recovery.

The early equine embryo grows very rapidly and can usually be seen with the naked eye in flushing media, ranging from 0.1 to 4.5 mm in diameter from 6–9 days post-ovulation.

The degree of synchrony between donor and recipient is not so critical in the mare as in other large domestic species. Squires et al. (1985) found no difference in pregnancy rate between recipients ovulating 1 day before or up to 3 days after the donor, although those ovulating after tended to be best. Ovulation can be synchronised using prostaglandin $F_{2\alpha}$ or progesterone and human chorionic gonadotrophin (hCG) treatments. Transfer can be performed either non-surgically through the cervix or surgically through a flank incision. As with the cow, the results with non-surgical transfer are more variable than with surgical transfer, and are dependent on operator skill. Pregnancy rates of 50–70% can, however, be achieved by an experienced technician.

EMBRYO TRANSFER IN THE PIG

Embryo transfer has not been widely used in the pig, except as a research procedure. Potentially the major applications in the pig are international movement of genetic material and disease control, either to establish disease-free herds from infected donors (James et al., 1983) or for the introduction of new bloodlines into specific pathogen-free herds.

The basic procedures in pigs are well established (see review by Polge, 1982). When superovulation is required, gonadotrophins such as eCG are best administered during the early follicular phase of the cycle, 15 or 16 days after the onset of oestrus (Hunter, 1964). Oestrus then occurs $3\frac{1}{2}$–4 days later, and an average of 25–30 ovulations may be expected following a dose of 1000–1500 IU eCG. Synchronisation of oestrus of donors and recipients can be easily achieved by use of the oral progestogen altrenogest (Polge, 1982).

Embryo recovery in the pig is generally very successful and involves general anaesthesia and mid-ventral laparotomy 3–7 days after oestrus, although endoscopic procedures have been used successfully to recover porcine embryos (Besenfelder et al., 1997). Ovulation in pigs occurs 36–40 hours after the onset of oestrus, and embryos remain in the uterine tubes for less than 48 hours after ovulation. Consequently, embryo recovery from the uterine horns is the general practice. Modified PBS is flushed into the uterus from the fimbrial end of the uterine tube and is collected through a cannula in the uterine horn (Hancock and Hovell 1962). Donors can be used for collection two or three times if care is taken with the surgery.

Average recovery rates of over 90% can be achieved, and embryos can be stored for short periods in modified PBS before transfer. Embryos must be maintained at a temperature above 15°C in the laboratory, as they are extremely sensitive to cooling (Polge, 1977). Embryo survival after culture periods of more than 24 hours is low (Pope and Day, 1977).

Transfers are also performed using midline surgery, the usual method being to use a fine pipette that is passed through a puncture in the isthmus of the uterine tube and into the uterus. Embryos need only be transferred to one uterine horn, from which they will migrate throughout the uterus (Dzuik et al., 1964). About 14 embryos are routinely transferred to each recipient, but a minimum of four are required to establish pregnancy (Polge et al., 1966). Optimum pregnancy rates of 70% and embryo survival rates of 60–65% are achieved when day 3–7 embryos are transferred to recipients that were in oestrus on the same day or 1–2 days after the donor (Polge, 1982). Embryos can also be transferred endoscopically (Besenfelder et al., 1997), and recently successful non-surgical transfer has been reported (Hazeleger and Kemp, 1999).

CRYOPRESERVATION OF EMBRYOS

The earliest report of successful cryopreservation of mammalian embryos was by Whittingham et al. (1972). This group used the mouse as an experimental animal and demonstrated the importance of cooling rate, thawing rate and cryoprotectant on embryo survival. Initial attempts to apply the best method for the mouse to the cow resulted in the birth of a calf (Wilmut and Rowson, 1973), but the success rate was very low. The sheep was subsequently used experimentally as a model for the cow, and soon practical methods for both species were developed (Willadsen, 1977; Willadsen et al., 1978) and later extended to the goat and the horse. There are variations between species, however, in the stages of embryonic development that tolerate exposure to low temperatures.

The early experiments with mouse embryos (Whittingham et al., 1972, Wilmut, 1972) had demonstrated that embryos from one cell to the blastocyst stage could survive deep-freezing. However, Trounson et al. (1976b) showed that early bovine embryos were sensitive to cooling, but an increased tolerance developed once they had reached the compacted morula or blastocyst stage. Consequently, interest centred on day 6, 7 or 8 embryos in this species, particularly in view of the fact that these stages are readily recovered non-surgically, are easily handled and stored on the bench, and can be successfully transferred surgically or non-surgically into recipients.

In the mare, embryos do not enter the uterus from the uterine tube until day 6, at which stage they can be successfully recovered non-surgically. Day 6 embryos have been successfully frozen in the mare (Slade et al., 1985), but later-stage embryos do not withstand freezing so well using conventional protocols. However, day 7 and 8 equine embryos have been successfully frozen recently (Young et al., 1997) by equilibrating with a high concentration (4 molar) of glycerol, but stepping down to 2 M prior to freezing, suggesting that poor permeability to the cryoprotectant may be a problem with later-stage equine embryos.

In contrast, pig embryos at any stage of development appear to be extremely intolerant of cooling (Polge, 1977). There has, however, been a report of the birth of piglets after transfer of frozen/thawed expanded blastocysts (Hayashi et al., 1989), although with a low rate of success. More recently Dobrinsky et al. (1998) reported live births after transfer of vitrified hatched blastocysts, and development of this technology may be the way forward.

The principles of cryopreservation in the larger domestic animals are best discussed by referring to the cow, as techniques are well documented in this species (see review by Lehn-Jensen, 1984). The important features of successful bovine programmes are applicable to sheep, goats and mares, including the use of a modified PBS (Whittingham, 1971) as a freezing medium and glycerol as a cryoprotectant.

The cryoprotective effect of compounds such as glycerol depends on their presence intracellularly (Willadsen, 1980); consequently a period of equilibration is necessary. Embryos can be placed directly into 1.5 M glycerol in PBS at room temperature and equilibration will occur in 10–15 minutes without deleterious effect (Schneider and Mazur, 1984). In contrast, it is important that glycerol is removed slowly from the embryo after thawing, in order to avoid osmotic lysis of cells.

This can be achieved by serial dilution in four to six steps of 10 minutes each, and gradually decreasing concentrations of glycerol in PBS. Alternatively, a sucrose gradient can be used (Nieman et al., 1982). Sucrose does not permeate the embryonic cell membrane and when added to the medium during cryoprotectant removal, the resulting high extracellular osmotic pressure prevents the intermittent swelling of blastomeres that would otherwise occur during stepwise cryoprotectant removal. Several authors have reported an improvement in embryo survival after thawing in a sucrose gradient compared with the stepwise method (Nieman et al., 1982; Bielanski et al., 1986).

Glycerol can be removed in one step by transferring the thawed embryo directly into 0.25–1.0 M sucrose in PBS for 10–20 minutes before placing in PBS. The latter is the basis of a 'one-step' procedure for direct transfer of embryos after thawing. This technique requires that the embryo is placed in a plastic straw in a column of medium containing glycerol. The remainder of the straw is then filled with a column of 0.25–1.0 M sucrose in a medium separated from the embryo by air bubbles. The fluid columns are mixed after thawing by shaking the straw and the embryo is transferred non-surgically after an equilibration period (Renard et al., 1982; Leibo, 1983). More recently, ethylene glycol has been shown to be an effective cryoprotectant for bovine embryos. Ethylene glycol diffuses across the cell membrane much more rapidly than glycerol, allowing direct transfer of frozen–thawed embryos without cryoprotectant removal (Voelkel and Hu, 1992). Ethylene glycol is now rapidly superseding glycerol as the cryoprotectant of choice for field use where the practical benefits of 'one-step' thaw and direct transfer are appreciated.

The 6–8-day bovine embryo does not appear to be adversely affected by rapid temperature change above –7°C, and embryos suspended in freezing medium and sealed in plastic straws can be placed directly into the freezing machine at this temperature and left to equilibrate rapidly. Induction of ice formation, or 'seeding', in the freezing medium is necessary once the temperature has reached the true freezing point of the medium; otherwise supercooling, spontaneous freezing and intracellular ice formation will occur, with a consequent adverse effect on embryo viability (Bilton and Moore, 1976). Seeding is usually accomplished by pinching the straw gently with a pair of forceps cooled in liquid nitrogen, but some modern freezing machines include automatic seeding in the programme. Most laboratories are using programmable freezing machines to obtain the precise cooling rates necessary for optimal embryo survival but it is possible to use a relatively simple device with good success (Lehn-Jensen, 1984).

The damage incurred by cells during freezing and thawing is thought to be mainly caused by the formation of intracellular ice and the dehydration of the embryo. The cells dehydrate during cooling, as the water in the medium crystallises to form extracellular ice and the solute portion becomes increasingly hypertonic. The cryoprotectant helps protect the cells from the damaging effects of hypertonicity, and intracellular ice formation is minimised if the cells are allowed sufficient time to dehydrate before they reach the temperature at which they would freeze internally (Whittingham, 1980). It is evident, therefore, that the cooling rate, plunge temperature and thawing rate will be critical in balancing these effects for optimal survival.

Slow cooling from the seeding temperature (0.3–0.5° C/min, plunging between –30 and –40°C) and a rapid thaw (approximately 360°C/minute) are favoured by most laboratories. Using this type of technique, very acceptable results can be achieved in commercial embryo transfer programmes in the cow (Table 35.3).

A further cause of damage to the embryo during freezing is the formation of random fracture planes in the extracellular ice during rapid cooling to the storage temperature (Lehn-Jensen and Rall, 1983), and possibly also during rapid thaw. Fracture planes involving the embryo itself will cause varying degrees of cell damage and consequently affect viability. Damage restricted to the zona, in the form of cracks or holes, does not appear to be of any significance (Lehn-Jensen, 1984), although the presence of a zona, intact or otherwise, may well be beneficial in that it acts as a physical barrier to the growth of extracellular ice (Lehn-Jensen and Rall, 1983).

Table 35.3 The effect of quality of bovine embryos on pregnancy rate after transfer (direct or frozen/thawed) to recipients synchronised for oestrus within ± 12 hours of the donor (from Christie, 1986)

Quality of embryos	*Frozen transfers: no. pregnant/no. of embryos frozen (%)*	*Direct transfers: no. pregnant/no. transferred (%)*
Good	747/1224 (61.0)	338/545 (74.4)
Moderate	73/169 (43.2)	69/124 (55.6)
Poor	9/34 (26.5)	34/86 (39.5)
Total	829/1427 (58.1)	441/664 (66.4)

The optimum thawing rate depends on the method used for freezing. When embryos are frozen slowly and plunged into liquid nitrogen between –30 and –40°C, rapid thawing is essential to prevent residual water in the cells crystallising during warming (Willadsen, 1977). Current common practice is to thaw the straw for 10 seconds in air at ambient temperature and then to plunge into a water bath at 30°C when the cryoprotectant is glycerol, or in the case of ethylene glycol, to plunge direct into a 20°C water bath.

Although there are probably as many different techniques for freezing embryos as there are groups involved in bovine embryo transfer, the majority differ only in minor detail. Good results are more dependent on fastidious attention to detail in the laboratory and on good management and critical selection of suitable recipients, than to minor changes in equilibration times or cooling rates. Future research is needed to simplify techniques without prejudicing embryo survival. Field transfer of frozen/thawed embryos can now be carried out without the use of a laboratory set-up (Renard et al., 1982; Leibo, 1983; Massip and van der Zwalmen, 1984; Voelkel and Hu, 1992), but the protocols for freezing embryos are time-consuming and still require the use of sophisticated, programmable freezing machines.

Rall and Fahy (1985), however, showed that mouse embryos could be successfully cryopreserved by a simple, rapid vitrification technique requiring minimal equipment. This is a new approach dependent on the fact that concentrated solutions of cryoprotectants in PBS do not crystallise when cooled to low temperatures but become increasingly viscous and form a glass-like solid. Rall and Fahy used a mixture of four cryoprotectants in the mouse, but Massip et al. (1986) have reported successful vitrification of bovine embryos using a simplified technique that resulted in seven pregnancies from 13 transfers (53.8%). The embryos were equilibrated in two steps with 25% glycerol and 25%1,2-propanediol, and then immediately plunged into liquid nitrogen. A rapid thaw technique and one-step dilution of cryoprotectant in 1 M sucrose in PBS was used, and this suggests that a one-step transfer procedure could be applied successfully, making available an effective field technique, both for freezing and thawing, which requires minimal laboratory equipment. Vajta et al. (1998) have improved the vitrification technique by using an open pulled straw as an embryo container. This allows a much faster cooling and warming rate (over 20 000°C/min), and very encouraging pregnancy rates have been produced from in vitro-derived embryos vitrified at both the oocyte and blastocyst stage of development.

There is no doubt that simple field methods for freezing and thawing bovine embryos are becoming more widely used. Whether they are generally applied will depend on the comparative results. Bovine embryos are still expensive to recover, and most embryos collected commercially are potentially valuable. Consequently, even a few per cent advantage in pregnancy rate would mean continuous use of programmable freezing machines and laboratory thawing for a few years yet.

MANIPULATION OF EMBRYOS

The technologies associated with manipulation of oocytes and embryos are advancing at a very rapid rate. Successful cloning from somatic cells has been reported in the ewe (Wilmut et al., 1997) and the cow (Cibelli et al., 1998), and foreign genes have been injected into the nucleus and incorporated into the genome of single-cell, fertilised eggs of pigs (Hammer et al., 1985) sheep (Simons et al., 1988) and cows (Krimpenfort et al., 1991). Many other manipulations of the fertilisation process, such as androgenesis, gynogenesis and parthenogenesis, may also be possible in future (Seidel, 1982). Some of the simpler manipulations such as embryo-splitting, sexing and in vitro embryo production are currently being applied commercially in cattle breeding, but others may soon follow.

In vitro production of embryos

Preovulatory oocytes collected from ovaries and fertilised in vitro have resulted in live calves (Brackett et al., 1982), lambs (Crozet et al., 1987), goat kids (Keskintepe et al., 1994) and piglets (Cheng, 1985). The techniques for in vitro production of embryos have developed particularly rapidly in cattle, since Lenz et al. (1983) demonstrated that maturation and fertilisation proceeded most efficiently at 39°C and Parrish et al. (1986) described a method for capacitating sperm in vitro using heparin.

For experimental purposes the vast number of oocytes normally wasted in the abattoir can be recovered by harvesting the ovaries and releasing the oocytes from 2–5 mm follicles by aspiration. Maturation is achieved by culturing the oocytes for 20–24 hours in medium containing bovine serum and hormones. Most oocytes will reach the second metaphase stage of meiosis during culture, but not all have the full potential for development.

Matured oocytes are then cultured with sperm capacitated in vitro (Parrish et al., 1986), and up to 90% fertilisation can be achieved with some bulls.

Embryos must then be cultured for 6–9 days to ensure that they are at the morula or blastocyst stage of development before they can be frozen or transferred to the uterus of a recipient. By applying these techniques to abattoir ovaries, banks of cheap embryos have been made available for commercial transfer on a limited scale in the UK (Lu and Polge, 1992) (Figure 34.8).

Co-culture of early embryos with bovine uterine tube epithelial cells (Lu et al., 1988), or buffalo rat liver cells (Hasler et al., 1995) in medium supplemented with serum, has produced very acceptable rates of development in practice. Some groups, however, have reported an increase in abortion, dystocia, perinatal loss and anomolies in in vitro produced calves, and that their birth weight averaged significantly higher than in vivo-produced controls (Kruip and den Daas, 1997). (see Chapter 11). The use of serum and/or co-culture may be implicated and further research is necessary to reduce the incidence of these problems. It is not surprising, therefore, that culture of early embryos in chemically defined (serum-free) media without co-culture (Edwards et al., 1997) has now become the method of choice.

More recently, a technique for aspiration of oocytes from the ovaries of live cows has been described (Pieterse et al., 1991). This has opened up the prospect of a dramatic increase in embryo production from valuable pedigree cattle where slaughter is not an option. Van der Schans et al. (1991) were able to collect an average of 9.4 oocytes per aspiration from cows aspirated twice weekly over a 3-month period. Follicle aspiration was achieved using a transvaginal, ultrasound-guided puncture technique, and there appeared to be no significant detrimental effect of repeat sampling on the ovary or genital tract.

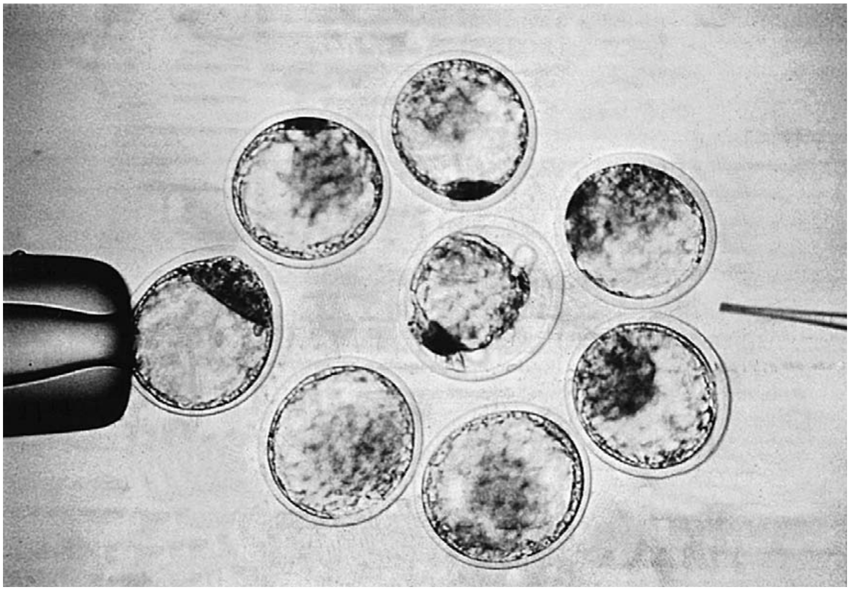


Fig. 35.8 A group of bovine embryos at the expanded blastocyst stage of development produced by in vitro maturation, fertilisation and culture.

Follicle aspiration and in vitro fertilisation in practice are particularly applicable to repeat breeders that are unsuitable for conventional embryo transfer, or normal cows that do not respond to superovulation treatments.

Micromanipulation

Division of embryos using microsurgical instruments is a practical method for creating identical siblings. Identical quadruplet sheep (Willadsen, 1981), triplet calves (Willadsen and Polge, 1981), and twin horses (Willadsen et al., 1980) and pigs (Polge, 1985) have been produced by separation of blastomeres of two-, four- and eight-cell embryos. Success rates are high when embryos are divided into two, but decline considerably when they are quartered. Identical quintuplets have been produced in sheep by mixing cells of four- and eight-cell embryos, when the more advanced cells apparently developed into the fetus and the less advanced contributed to the placenta (Willadsen and Fehilly, 1983).

A simpler and more practical method of producing identical twins (Figure 35.9) from morulae and early blastocysts involves microsurgical division of the embryo into two groups of cells, and immediate transfer into recipients (Willadsen and Godke, 1984; Williams et al., 1984). The half-embryos can be replaced in surrogate zonae pellucidae before transfer or transferred naked. Pregnancy rates of 50% or more per half-embryo have been reported,

Fig. 35.9 Identical twin calves produced by micromanipulation of a day 7 embryo.

Table 35.4 The results of embryo bisection in a commercial embryo transfer programme (from Christie and Green, 1984)

Number of embryos bisected	43
Number of demi-embryos transferred	86
Number of recipients pregnant (%)	52 (60.5)
Number of genetically identical pairs	16

resulting in a net pregnancy rate of over 100% per original embryo (Table 35.4).

Embryo division is being used commercially to increase the number of progeny produced in bovine embryo transfer programmes, and could also be a valuable method for producing identical twins for research.

Sex determination

The efficiency of livestock breeding enterprises would be considerably increased if it were possible to routinely predetermine the sex of offspring. In the past the vast majority of claims to alter the sex ratio significantly by the separation of X and Y chromosome-bearing spermatozoa have not been substantiated in practice. Recently, however, a method has been described for separation of spermatozoa on the basis of their DNA content, by flourescent labelling and cell sorting (Cran, 1992). Field trials in the USA (Seidel et al, 1999) have demostrated that acceptable pregnancy rates can be achieved after insemination of heifers with sexed, unfrozen sperm. Also, it was notable that in the majority of the most recent trials cited, the pregnancy rate with sexed frozen sperm was within 90% of unsexed frozen controls. Frozen sexed bull semen is now commercially available in the UK, but significant production limitations due to cost and the speed of sorting means that only a limited number of bulls will be available to breeders for a year or two.

Embryos have been sexed by cytological methods (see review by King, 1984). These involve chromosome analysis of cells in metaphase that have been sampled from the embryo using an embryo division technique. However, biopsy and karyotyping procedures are tedious, time-consuming and relatively inaccurate, making them impractical for routine commercial use.

An alternative approach is to use an antibody to the HY antigen, a protein present on the surface of male mammalian cells. The HY antibody binds to male embryos and can be detected by adding a fluorescently labelled antibody directed against the first, such that male embryos fluoresce in appropriate light. This procedure sexes mouse embryos with 80% accuracy (White et al., 1982) but has not been successfully applied to large domestic species.

A more accurate method for determination of sex has become available with the development of DNA technology and polymerase chain reaction (PCR), utilising primers that target DNA sequences specific to the Y chromosome (see review by Bredbacka, 1998). Only a few cells need to be sampled for testing, with a consequent minimal effect on embryo viability. Several versions have been developed for commercial use in combination with PCR technology, but the relatively high cost of the test has prevented widespread application until recently.

The manipulation of eggs and embryos of the large domestic species will have a major impact on the efficiency of animal production in the future. Although much of the research involved is in its infancy, it is growing fast and there are exciting prospects ahead for the geneticist and the animal breeder. It should be remembered, however, that it is through embryo transfer that new developments may be exploited and therefore the use of this breeding technique is likely to continue to expand.

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Hormones, related substances and vaccines used in reproduction

The preparations listed in this appendix are those that are available in the UK at the time of publication. The recommendations are not necessarily those of the manufacturers, since some have been modified by the authors in the light of their experience. Readers are warned of the importance of checking the current recommendations in case changes have been made since the publication of this book. In addition, they should be aware of the regulations and consequences of using of unlicensed preparations, and in species where the licence for use does not apply.

GONADOTROPHIN (LUTEINISING)-RELEASING HORMONE AND ANALOGUES (GnRH OR LHRH)

Naturally occurring hormone, produced by the hypothalamus and transferred to the anterior pituitary gland in the hypophyseal portal circulation. It is a peptide and stimulates the release of follicle-stimulating hormone (FSH) and luteinising hormone (LH).

Commercially available products

Fertirelin, synthetic GnRH peptide ('Ovalyse', Pharmacia and Upjohn Ltd, Corby, Northants).

Gonadorelin, synthetic GnRH peptide ('Fertagyl', Intervet Animal Health UK Ltd, Cambridge).

Buserelin, synthetic GnRH peptide analogue ('Receptal', Hoechst Roussel Vet Ltd, Milton Keynes).

Deslorelin, a synthetic GnRH analogue that is present as a slow-release implant (Ovuplant, Peptech Animal Health Pty Ltd, Dee Why, NSW, Australia). Not licensed for use in the UK

Pharmacological action

Stimulates a short surge of FSH and LH following a single bolus injection.

Indications

Cattle:

- follicular cysts
- delayed ovulation or anovulation
- acyclicity (doubtful if a single bolus is very effective)
- improved pregnancy rates, in cows with poor pregnancy rates, when used as 'holding injection' as a single bolus 12 days after insemination
- In oestrus–synchronisation regimens.

Horse:

- induce ovulation (preovulatory gonadotrophin surge lasts several days in mare); single bolus may not be effective, requires frequent repeated doses, or the use of a slow-release implant.

Dose rate

- Buserelin: cow, 10–20 μg; horse 40 μg preferably i.m. but can be given i.v. or s.c.
- Gonadorelin: cow, 0.5 mg i.m., s.c. or i.v.
- Fertirelin: cow, 100 μg i.m.

GONADOTROPHINS

1. *FSH and LH*. Both FSH and LH can be obtained in a semi-purified form, but are expensive. Porcine FSH and recombinant-derived FSH are used to induce superovulation in donor cows for embryo transfer.

2. *Equine chorionic gonadotrophin (eCG)*. Originally called pregnant mare's serum gonadotrophin (PMSG) but in order to use consistent nomenclature it is now called eCG. A protein hormone produced by the endometrial cups of the mare from about 40–120 days of pregnancy. It mainly has FSH-like activity but with a much longer biological half-life than FSH.

Commercially available products

eCG or serum gonadotrophin ('Folligon', Intervet UK Ltd, Cambridge; 'Fostim', Pharmacia and Upjohn Ltd, Corby, UK).

Pharmacological action

Mainly FSH-like in its action but has some LH activity.

Indications

Cattle:

- superovulation of donor cows for embryo transfer; over-stimulation can be a problem
- impaired spermatogenesis in bulls (doubtful value)
- at the time of withdrawal of intravaginal progesterone preparations when used to treat acyclicity.

Sheep and goats:

- in association with intravaginal progestogen sponges to advance the onset of the breeding season.

Pig:

- in association with hCG to stimulate onset of cyclical activity after farrowing.

Dog:

- induce oestrus during physiological anoestrus.

Dose rate

Cattle, 1500–3000 IU s.c. or i.m.

Sheep and goats, 500–800 IU s.c. or i.m. (depending on the breed and time interval to the onset of normal breeding season).

Pig, 1000 IU s.c. or i.m.

Dog, 50–200 IU.

3. *Human menopausal gonadotrophin (hMG)*. Extracted from the urine of menopausal women, this has primarily an FSH-like action. Used to a limited extent in superovulating donor cows for embryo transfer. It has a shorter biological half-life than eCG.

4. *Human chorionic gonadotrophin (hCG)*. A protein hormone extracted from the urine of pregnant women, this hormone has primarily an LH-like effect and hence is used as a substitute for the more expensive LH; it also has a longer half-life than LH.

Commercially available products

'Chorulon' injection (Intervet UK Ltd, Cambridge).

Pharmacological action

Stimulates androgen production by the thecal cells of the ovary and Leydig cells of the testis; stimulates follicular maturation and ovulation, corpus luteum formation and maintenance.

Indications

Cattle:

- delayed ovulation or anovulation
- ovarian cysts (especially follicular)
- luteal deficiency
- improve chances of pregnancy in cyclic non-breeders (repeat breeder cows), rationale is not always apparent
- improve libido in bull (doubtful value and may make temperament more aggressive).

Horse:

- induce or hasten ovulation
- 'rig test', stimulate rise in testosterone in peripheral blood of suspected cryptorchid.

Pig:

- with eCG to stimulate onset of cyclical activity after farrowing
- improve libido in boar (doubtful value).

Sheep and goat:

- improve libido in ram and male goat (doubtful value)
- cystic ovaries in female goat.

Dog:

- curtail prolonged or persistent pro-oestrus/ oestrus in bitches
- determination of abdominal cryptorchidism as in the 'rig' test in horses
- improve libido in male dog (doubtful value).

Cat:

- induce ovulation.

Dose rate

Cattle, 1500–3000 IU i.v. or i.m.

Horse, 1500–3000 IU i.v. or i.m.

Pig, 500–1000 IU i.m. or s.c.

Sheep and goat, 100–500 IU i.v. or i.m.

Dog, 100–500 IU i.m.

Cat, 100–200 IU i.m.

GONADOTROPHINS WITH OTHER HORMONES

A number of commercial preparations are available in which gonadotrophins are combined with other hormones as a single injectable substance. The rationale for their use is frequently doubtful since they are attempting to overcome complex hormone deficiencies too simplistically.

Commercially available products and manufacturers' indications for usage

hCG with progesterone ('Nymfalon', Intervet UK Ltd, Cambridge). Indications are essentially those listed above for hCG in the cow and mare.

eCG and hCG ('PG 600', Intervet UK Ltd, Cambridge). Indicated for the induction of oestrus in sows and gilts after weaning. There is evidence that this can be a useful method of overcoming postpartum anoestrus.

OXYTOCIN AND POSTERIOR PITUITARY EXTRACTS

Oxytocin is a peptide hormone produced by the neurones of the supraoptic nucleus and is transported to, and stored in, the posterior pituitary gland. Synthetic oxytocin is available commercially and is thus highly purified; however, aqueous extracts of mammalian pituitary glands are also available. These latter products will also contain other posterior pituitary hormones such as vasopressin and antidiuretic hormone (ADH).

Commercially available products

Oxytocin (Leo Laboratories Ltd, Princes Risborough, Bucks).

Oxytocin (Intervet UK Ltd, Cambridge).

'Hyposton', posterior pituitary extract (Pharmacia and Upjohn Ltd, Corby, Northants).

Pharmacological action

Causes milk letdown, myometrial contractions to facilitate gamete transport, myometrial contractions during parturition and postpartum.

Indications

Cattle:

- induce milk letdown
- hasten uterine involution following dystocia, caesarean operation, after replacement of uterine prolapse, uterine trauma or haemorrhage.

Horse:

- induce foaling
- cause expulsion of retained fetal membranes
- induce milk letdown.

Sheep

- As for cow.

Pig:

- induce milk letdown
- hasten second stage of parturition
- treatment of uterine inertia
- cause expulsion of retained fetal membranes
- hasten uterine involution.

Dog:

- treat uterine inertia
- expulsion of retained fetal membranes

- hasten uterine involution after dystocia or caesarean operation (perhaps treat subinvolution of placental sites)
- induce milk letdown.

Where there is trauma to the uterus, especially with haemorrhage, pituitary extracts should *not* be used.

Dose rate

Many recommended dose rates are too high. The myometrium is very sensitive to the effects of oxytocin and high dose rates can cause spasm rather than synchronised contractions. The myometrium will also become refractory to its effect, hence increasing incremental dose rates should be used. Most effective when used in an intravenous drip in saline.

Cattle, 10 IU i.m. or i.v.

Horse, 10 IU i.m. or i.v.

Pig, 5 IU i.m. or i.v.

Sheep and goat, 2–5 IU i.m. or i.v.

Dog and cat, 0.5–5 IU i.m. or i.v.

SPASMOLYTICS

These substances have a wide range of effects; some are specific for the myometrium, whilst others exert their action upon all smooth muscle. Assessment of their efficacy is frequently rather subjective during their clinical application.

Commercially available products

Hyoscine *N*-butylbromide and dipyrone ('Buscopan Compositum', Boehringer Ingelheim Ltd, Bracknell).

Vetrabutine hydrachloride ('Monzaldon', Boehringer Ingelheim Ltd, Bracknell).

Clenbuterol HCl ('Planipart', Boehringer Ingelheim Ltd, Bracknell). Clenbuterol HCl is a β-adrenergic stimulant.

Pharmacological action

Abolishes or reduces myometrial contractions and tone, thus causing relaxation of the uterus at caesarean operations and during embryo transfer, and enables easier repulsion of the fetus in obstetrical manipulations. Clenbuterol HCl can be used specifically to postpone parturition in cattle as a management aid, or to delay calving thereby allowing adequate softening and relaxation of the birth canal to occur.

Indications

Cattle:

- relaxation of myometrium to facilitate obstetrical manipulation to treat dystocia and during caesarean operations
- aid relaxation and softening of the birth canal
- in embryo transfer to facilitate manipulation of the uterus
- postpone parturition (clenbuterol HCl only).

Horse, sheep, pig and dog

- As for cattle except it cannot be used to postpone parturition.

Cat

- Some spasmolytics are contraindicated in this species and should be checked before use.

Dose rate

These should be checked for each product and species before use.

Clenbuterol HCl, when used to postpone calving during the night, should be given at a dose rate of 0.3 mg (10 ml) i.m. at about 18.00 hours followed by a second injection of 0.21 mg (7 ml) 4 hours later. This should postpone calving for 8 hours after the second injection. It must not be used if the cervix is fully dilated and second stage has commenced.

OESTROGENS

Oestrogens, which are steroids, play a wide role in the reproductive process. However, there are relatively few rational indications for oestrogen therapy in the treatment of reproductive disorders in domestic species. In recent years, several of the synthetic oestrogens have been withdrawn from use because of concern about residues in human food products.

Commercially available products

Oestradiol benzoate (Intervet UK Ltd, Cambridge). This contains 5 mg/ml of hormone in a sterile oily solution.

Oestradiol benzoate ('Mesalin', Intervet UK Ltd, Cambridge). This contains 200 μl/ml oestradiol benzoate in oil.

Diethylstilboestrol (non-proprietary). Tablets 1 mg and 5 mg.

Ethinyloestradiol (non-proprietary). Tablets 10, 50 μg and 1 mg.

Pharmacological action

Oestrogens are primarily responsible for oestrous behaviour in the female; they stimulate changes in the tubular genital tract which control gamete transport and, with progestogens, cause development of the mammary gland and increase the resistance of the genital tract to infection. They potentiate the ecbolic action of oxytocin and prostaglandins on the myometrium. They stimulate the preovulatory surge of gonadotrophins. They also reverse the effects of androgens on androgen-dependent tissue changes.

Indications

Horse:

- ripening of the cervix before oxytocin-induced foaling.

Cattle:

- treatment of endometritis (contraindicated in acute toxic metritis).

Dog:

- prevention of unplanned pregnancy
- urinary incontinence in the spayed bitch
- prostatic hyperplasia and anal adenoma in the male dog
- depress hypersexuality in the male dog.

Dose rate

Horse, 3–6 mg i.m.

Cattle, 3–5 mg i.m. (probably too high)

Dog, Oestradiol benzoate: following unplanned mating to prevent pregnancy 10 μg/kg, 3, 5 and possibly 7 days after mating s.c or i.m. Diethylstilboestrol: for urinary incontinence 1 mg daily for 3 days then 1 mg every third day; for prostatic hyperplasia 1 mg/day. Ethinyloestradiol: 50–100 μg/day orally. Oestrogens are not without risk in the bitch predisposing to cystic endometrial hyperplasia, and should not be used without warning.

PROGESTOGENS

These include the naturally occurring steroid progesterone, and a number of synthetic progestogens which are much more potent and have a longer half-life. Progestogens are used widely in all domestic species, mainly to control cyclical activity; this is because, as a group, they exert a powerful negative feedback effect upon the hypothalamus and anterior pituitary gland, thus inhibiting gonadotrophin release. The consequence of this effect is to suppress cyclical activity so that, following cessation of treatment in polyoestrous species, there is ovarian rebound within a few days.

1. Progesterone

Commercially available products

Progesterone-releasing intravaginal device ('PRID', Ceva Animal Health, Watford). Each device contains 1.55 g of progesterone (in addition to a 10 mg capsule of oestradiol ester). Used for synchronisation of oestrus/ovulation in cows and heifers, preferably in conjunction with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$); treatment of acyclicity (true anoestrus) in cows and heifers; treatment of non-observed oestrus in cows. The oestradiol ester is a weak luteolysin. One device should be inserted into the vagina and left in situ for up to 12 days, $PGF_{2\alpha}$ can be administered 24 hours before removal to improve the effectiveness of the synchronisation. Oestrus occurs 2–5 days after withdrawal.

Intravaginal progesterone release device (EASI-BREED 'CIDR', Animal Reproductive Technologies Ltd, Leominster). Each device contains 1.9 g of progesterone, which should be left in place for 7–12 days with $PGF_{2\alpha}$ treatment at the time of removal. Same indications as for PRID.

Progesterone in oil ('progesterone injection', Intervet UK Ltd, Cambridge). Contains progesterone at a concentration of 25 mg/ml in an oily solution. Used to suppress cyclical activity but requires injection i.m. daily, and to prevent pregnancy failure due to endogenous progesterone deficiency; the latter is of doubtful value.

Dose rate: bitch, 2–3 mg/kg per day; cat, 2.5–5 mg every 3 days.

2. Synthetic progestogens

Commercially available products

Altrenogest or allyltrenbolone ('Regumate Equine', Hoechst Roussel Vet Ltd, Milton Keynes). A liquid in-feed substance containing 2.2 mg of allyltrenbolone per 1 ml. Used to suppress cyclical activity where this may cause managemental or behavioural problems, to control timing of oestrus to meet the availability of the stallion, to induce cyclical activity in the breeding season. Dose rate of 27.5 or 33 mg in the feed as a single dose per day for 10 or 15 consecutive days. Oestrus occurs within 8 days of last dose and ovulation after 7–13 days.

Altrenogest or allyltrenbolone ('Regumate Porcine', Hoechst Roussel Vet Ltd, Milton Keynes). A liquid in-feed suspension that is placed on the food as a top dressing when gilts are eating, so that it is immediately consumed. It is used to synchronise oestrus in sexually mature and therefore cyclical gilts, by administering the suspension for 18 consecutive days. Oestrus occurs 2–3 days after cessation of treatment. Dose rate of 20 mg (5 ml) per day.

Norgestamet ('Crestar', Intervet UK Ltd, Cambridge). An implant, containing 3 mg of the synthetic progestogen norgestamet, which is inserted beneath the outer surface of the ear using a special applicator. In addition, for oestrus synchronisation, there is a fluid containing 3 mg of norgestamet and 5 mg of oestradiol benzoate per 2 ml for intramuscular injection. The implant can be removed either by withdrawing it along the injection tract or, preferably, by making a very small incision with a stylus over the distal end of the implant and expressing it by gentle squeezing with thumb and forefinger. The product must only be used in beef animals, in which milk is not used for human consumption, or dairy heifers. For the synchronisation of oestrus, the implant is inserted on day 0 and followed immediately with the intramuscular injection. On day 9, the implant is removed and, if the animals were acyclical at the time of the implant insertion, an injection of eCG should be given. The dose rate will vary from 400 to 700 IU. Animals can be inseminated at observed oestrus or at a fixed time. Beef heifers should be inseminated at 48 hours and nursing cows at 56 hours after implant removal; alternatively the latter group can be inseminated twice at 48 and 72 hours.

Fluorogestone acetate intravaginal sponges ('Chronogest', Intervet UK Ltd, Cambridge). Medroxyprogesterone acetate intravaginal sponges ('Veramix' and 'Veramix Plus', Pharmacia and Upjohn Ltd, Corby). Used to synchronise ewes and female goats or, in conjunction with eCG (PMSG) injections, to advance the time of onset of the breeding season by up to 6 weeks. Dose rate: each ewe receives a single sponge inserted into the anterior vagina where it should remain for 12–14 days before withdrawal; oestrus occurs 48–72 hours later. When the breeding season is being advanced, eCG is normally given at the time of sponge removal or just before. At least one ram per 10 ewes should be available.

Medroxyprogesterone acetate tablets ('Perlutex tablets', Leo Laboratories Ltd, Aylesbury). Used to interrupt oestrus in bitches and queen cats when pro-oestrus is observed and to postpone oestrus for a long period following treatment during anoestrus. Dose rate: bitch (interruption of oestrus), 10–20 mg daily for 4 days from the first sign of pro-oestral bleeding followed by 5–10 mg daily for 12 days; bitch (postponement of oestrus), 5–10 mg daily for as long as postponement is required; queen cat (interruption of oestrus), 2.5 mg per day for as long as is required; the same regimen and dose rate are recommended for postponement.

Medroxyprogesterone acetate injection ('Perlutex for Injection', Leo Laboratories Ltd, Aylesbury; 'Promone-E', Pharmacia and Upjohn Ltd, Corby). Used for prevention of oestrus in bitches and prostatic hyperplasia in dogs. Dose rate: bitches (prevention of oestrus), 50–150 mg s.c. in anoestrus; dog (prostatic hyperplasia), 50–100 mg s.c. every 3–6 months.

Megoestrol acetate tablets ('Ovarid', Schering-Plough Animal Health, Harefield). Used for the interruption of oestrus in bitches and queen cats when given at the first signs of pro-oestrus or the postponement of oestrus when given during anoestrus. Dose rates: bitch (interruption of oestrus), 2 mg/kg daily for 8 days; postponement of oestrus, 0.5 mg/kg daily for up to 40 days and then, if required, at a dose of 0.1–0.2 mg/kg twice weekly for not more than 4 months; queen cats (interruption of oestrus), 5 mg per day for 3 days commencing at the first signs of pro-oestrus/oestrus; postponement of oestrus, 2.5 mg per day.

Proligestone injection ('Covinan', Intervet UK Ltd, Cambridge; 'Delvosteron', Mycofarm UK Ltd, Cambridge). Used to interrupt and postpone oestrus in the bitch and queen cat. Dose rate: bitch (interruption of oestrus), 100–600 mg by s.c. injection at the first signs of pro-oestrus. The same dose rate can be given when the bitch is anoestrous to postpone oestrus temporarily, or at 3, 4 and then 5-monthly intervals to postpone oestrus for a longer period of time. Queen cat, 100 mg by s.c. injection at the first signs of pro-oestrus or oestrus; postponement involves a similar injection regimen to that described for the bitch.

Progestogens in bitches and queen cats are not without dangers, since they predispose to cystic endometrial hyperplasia (pyometra) and should be used with utmost caution in those individuals that are subsequently intended for breeding.

ANDROGENS

Testosterone is the principal circulating androgen in the male, being produced by the interstitial cells of the testis. As well as being responsible for the secondary sex characteristics, it is also involved in spermatogenesis. Androgens, either naturally occurring or synthetic analogues, have limited application in animal reproduction or disease.

Commercially available products

Methyltestosterone tablets ('Orandrone', Intervet UK Ltd, Cambridge). 5 mg tablets orally at a dose rate of 500 μg/kg per day.

Testosterone esters injection ('Durateston', Intervet UK Ltd, Cambridge). Contains testosterone decanoate 20 mg/ml, testosterone isocaprionate 12 mg, and testosterone proprionate 6 mg/ml. Dose rate of 0.05–0.1 ml/kg s.c. or i.m.

Pharmacological action

Since testosterone is involved in controlling libido in the male it is used to improve any deficiency that might be present, although it must be stressed that libido and sexual behaviour are complex and not just a reflection of endogenous androgens; therefore, the results of such therapy will usually be disappointing. Androgens have anabolic effects and can be used to treat debilitated animals. They have been used to postpone oestrus in bitches and overcome some of the behavioural problems associated with pseudopregnancy in bitches, and reverse feminisation associated with Sertoli cell tumours.

Dose rate

These should be checked for each product and each species.

COMBINED ANDROGENS AND OESTROGENS

Commercially available products

Ethinyloestradiol and methyltestosterone tablets ('Sesoral', Intervet UK Ltd, Cambridge). Used to control overt pseudopregnancy in bitches.

PROSTAGLANDINS AND PROSTAGLANDIN ANALOGUES

Only $PGF_{2\alpha}$ and synthetic analogues are available commercially for use in domestic species.

Commercially available products

Cloprostenol ('Estrumate' and 'Planate', Schering-Plough Animal Health, Harefield). For use in cattle, sheep, pigs, horses and goats.

Dinoprost ('Lutalyse', Pharmacia and Upjohn Ltd, Corby; 'Enzaprost', Sanofi Animal Health, Watford). For use in cattle, sheep, pigs, horses, goats and dogs.

Luprostiol ('Prosolvin', Intervet UK Ltd, Cambridge). For use in cattle, sheep, pigs, horses and goats.

Tiaprost ('Iliren', Hoechst Roussel Vet Ltd, Milton Keynes). For use in pigs.

Pharmacological action

$PGF_{2\alpha}$ and analogues are potent luteolytic agents, except in the bitch and cat. They play a role in ovulation, parturition and gamete transport, in the latter two by virtue of their effect on the smooth muscle of the genital tract. They have a short biological half-life because 90% of prostaglandins are metabolised at one passage through the pulmonary circulation.

Indications

Cattle:

- synchronisation of oestrus in cow and heifers
- treatment of non-observed oestrus
- induction of calving
- inducing abortion and expulsion of mummified calves
- treatment of pyometra
- treatment of endometritis
- treatment of luteal (luteinised) cysts.

Horse:

- inducing abortion before 35 days
- treatment of a persistent luteal phase
- induction of foaling
- hasten return to oestrus if service is missed
- hasten return to oestrus after the foal heat
- planning the time of oestrus for efficient use of stallion or AI.

Sheep and goat:

- synchronisation of oestrus
- inducing early abortion in sheep
- treating pseudopregnancy in goats.

Pig:

- induction of farrowing.

Dog:

- treatment of open pyometra in the bitch (dinoprost and cloprostenol, use with care).

Dose rate

Cloprostenol: Cattle, 500 g; horse, 12.5–500 g; sheep and goats, 125–250 g; pig, 350 g. All i.m.

Luprostiol: Cattle, 15 mg; horse, 7.5 mg; sheep and goats, 7.5 mg; pigs, 7.5 mg.

Dinoprost: Cattle, 25–35 mg; horse, 5 mg; pig, 10 mg; sheep, 6–8 mg; dog, 0.25–0.5 mg/kg. All i.m.

Tiaprost: Pig, 300–600 mg.

ANTI-ANDROGENS

These substances are progestogens and are used to counteract the behavioural actions of endogenous androgens.

Commercially available products

Delmadinone acetate ('Tardak', Pfizer Animal Health Ltd, Tadworth).

Indications

Dog:

- hypersexuality in the male dog
- prostatic hyperplasia and prostatitis.

Dose rate

1.0–2.0 mg/kg body weight, s.c. or i.m.

OTHER HORMONES AND RELATED SUBSTANCES

Melatonin

Melatonin, an indoleamine, is produced by the pineal gland. Its level of secretion is influenced by the photoperiod, with reducing day length stimulating, and increasing day length inhibiting, its secretion. Melatonin modulates, either directly or indirectly, the frequency of GnRH secretion from the hypothalamus, thus influencing the secretion of gonadotrophins and cyclical ovarian activity.

Commercially available products

Melatonin implant ('Regulin', Hoechst Roussel Vet Ltd, Milton Keynes).

Indications

Advancing the onset of normal cyclical ovarian activity in pure and cross-bred lowland breeds of sheep so that early lambing occurs.

Dose rate and treatment regimen

One implant (18 mg of melatonin) per ewe inserted subcutaneously on the outer aspect of the base of the ear. The earliest time of use of implants is determined by the breed of the ewe; details should be checked against the manufacturer's instructions. It can also be used in goats. It is critical to ensure that ewes (and does) are out of sight, sound and smell of rams (and bucks) for at least 7 days before and at least 30 days after the implant is inserted.

Prolactin inhibitors

Carbergoline ('Galastop', Boehringer Ingelheim Ltd, Bracknell). A viscous, non-aqueous solution containing 50 mg/ml carbergoline. It is a long-acting prolactin inhibitor which, because of the role of this hormone in initiating the signs of pregnancy and overt signs of pseudopregnancy, can cause their reversal in the bitch.

Indications

The treatment of overt pseudopregnancy in the bitch, orally at a dose rate of 0.1 ml/kg daily for 4–6 consecutive days. It is sometimes successful in suppressing lactation in goats.

VACCINES

Equine herpesvirus infections

'Duvaxyn EHV1,4' (Fort Dodge Animal Health, Southampton). An inactivated aqueous suspension of EHV-1 and EHV-4 for the vaccination of healthy pregnant mares to prevent infection which might result in abortion, or in contact mares. As an aid in the prevention of abortion due to EHV-1, pregnant mares should be vaccinated during the fifth, seventh and ninth months of gestation with a single injection together with in-contact maiden and barren mares.

Leptospira hardjo

'Leptavoid-H' (Schering-Plough Animal Health, Harefield). Formol-killed cultures of *L. hardjo* for vaccination against this organism. Primary course of immunisation involves two subcutaneous injections with an interval of at least 4 weeks before and not more than 6 weeks after the main season of the year for transmission of the disease. Thereafter, an annual booster can be given at about the same time of the year.

'Vaxall' (Fort Dodge Animal Health, Southampton). An adjuvanated vaccine containing inactivated cells of *L. hardjo*.

Bovine para-influenza virus (PI3) and infectious bovine rhinotracheitis (IBR)

'Imuresp' (Pfizer Animal Health Ltd, Sandwich). Freeze-dried live strain of P13 virus administered intranasally.

'Imuresp RP' (Pfizer Animal Health Ltd, Sandwich). The same freeze-dried live strain of PI3 as in 'Imuresp', and a live strain of IBR virus administered intranasally.

'Tracherine' (Pfizer Animal Health Ltd, Sandwich). Freeze-dried live strain of IBR virus for intranasal administration.

'Bovilis IBR' (Intervet UK Ltd, Cambridge). A living avirulent strain of IBR virus, preferably given intranasally, but can also be given by intramuscular injection.

'Bovilis IBR + PI3' (Intervet UK Ltd, Cambridge). The same living avirulent strain as in 'Bovilis IBR' together with an avirulent strain of PI3 administered intranasally.

Bovine viral diarrhoea virus (BVDV)

'Bovidec' (Vericore, Marlow). An inactivated non-cytopathogenic strain of BVDV, administered s.c.

Ovine enzootic abortion

'Enzovac' (Intervet UK Ltd, Cambridge). A live attenuated 1B strain of *Chlamydia psittaci*. Ewe lambs intended for breeding should be vaccinated from 5 months of age. Shearlings and older ewes should be vaccinated during the 4 months before tupping. May not prevent abortion in infected ewes.

Ovine toxoplasmosis

'Toxovax' (Intervet UK Ltd Cambridge). Live, concentrated aqueous vaccine containing tachyzoites of the S48 strain of *Toxoplasma gondii*.

Porcine parvovirus

'Porcilis Parvo' (Intervet UK Ltd, Cambridge). An inactivated vaccine of strain 014 against porcine parvovirus (PPV) infection. A combined vaccine with *Erysipelothrix rhusiopathiae* is also available.

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