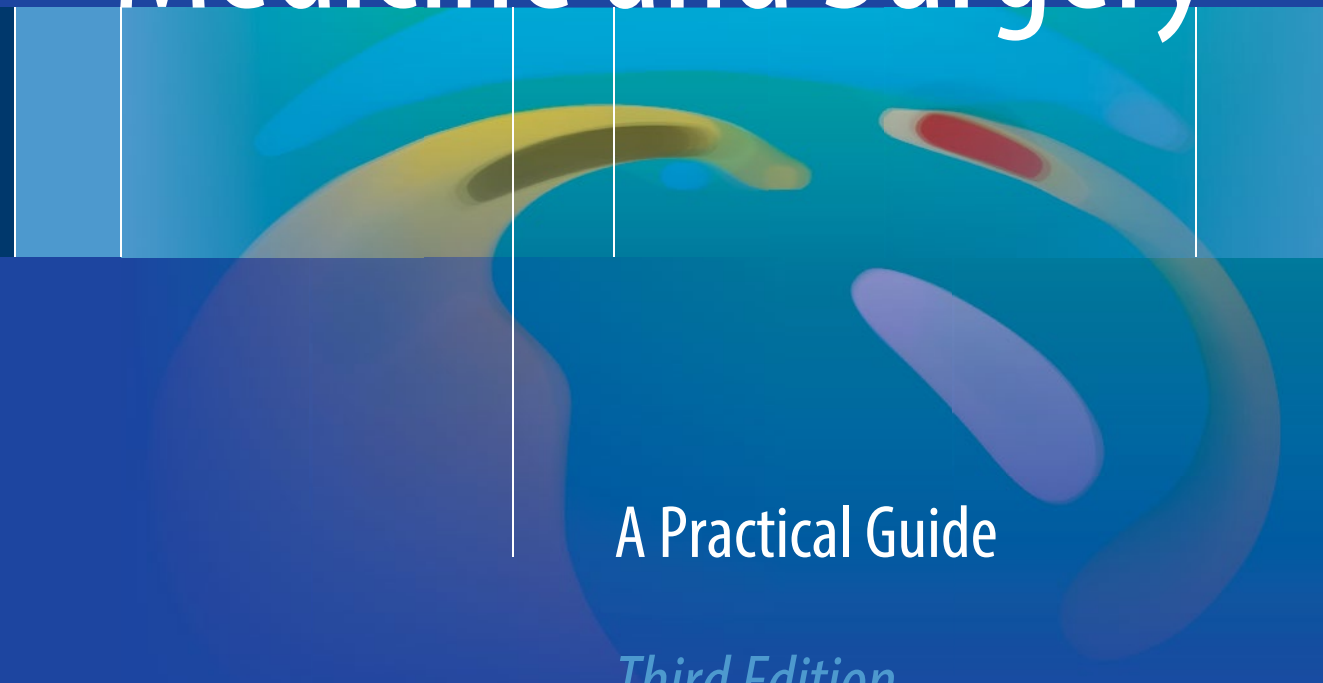
Tommaso Falcone
William W. Hurd
Editors

Clinical Reproductive Medicine and Surgery



A Practical Guide

Third Edition

Springer

Clinical Reproductive Medicine and Surgery

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Editors
Tommaso Falcone, MD
Department of Obstetrics and Gynecology
Cleveland Clinic
Cleveland, Ohio, USA

William W. Hurd, MD, MSc, MPH
Department of Obstetrics and Gynecology
Duke University Medical Center
Durham, North Carolina, USA

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The editors would like to dedicate the third edition of this textbook to all enthusiastic students of reproductive medicine, particularly the fellows, residents, and new clinicians for whom this book was created.

Preface to the Third Edition

We are very pleased to present the third edition of *Clinical Reproductive Medicine and Surgery*. With each successive edition, we have asked our authors to help us maintain a textbook that is comprehensive and easy to read and covers most aspects of clinical practice in reproductive medicine. Our target audience remains fellows, residents, and those who have recently begun independent practice for the first time. We have strived to keep the textbook manageable in terms of both cost and length.

For our third edition, we have made a few notable changes in an attempt to further improve upon the original textbook. In addition to asking the authors to update their chapters, we have asked them to start each chapter with a brief case presentation and a set of key words and phrases. We have also split some chapters that we had combined in the previous edition to allow for the addition of important details. We hope that these changes will further improve the readability and the usefulness of the book to clinicians. Our goal remains to confirm current clinical practices and introduce our readers to the newest approaches to both common and unusual clinical issues.

Tommaso Falcone, MD
Cleveland, OH, USA

William W. Hurd, MD, MSc, MPH
Durham, NC, USA

Contents

Contributors

Ashok Agarwal, PhD
Department of Urology, Cleveland Clinic, Cleveland, OH, USA

Paula Amato, MD, MCR
Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Oregon Health and Science University, Portland, OR, USA

Raj Kumar Anirudh, BA
University of Miami, College of Arts and Sciences, Coral Gables, FL, USA

Francisco Arredondo, MD, MPH
Department of Reproductive Endocrinology, RMA of Texas, San Antonio, TX, USA

Marjan Attaran, MD
Department of Obstetrics and Gynecology, Section of Pediatric and Adolescent Gynecology, Cleveland Clinic, Cleveland, OH, USA

Mohamed A. Bedaiwy, MD, PhD, FACOG
Division Head of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University of British Columbia, Vancouver, BC, Canada

Sarah L. Berga, MD
Department of Obstetrics and Gynecology, Reproductive Medicine Section, Wake Forest Baptist Medical Center, Medical Center Boulevard, Winston-Salem, NC, USA
Department of Obstetrics and Gynecology, Section of Reproductive Endocrinology and Infertility Wake Forest School of Medicine Executive Director, Women's Health Service Line Wake Forest Baptist Medical Center Obstetrics and Gynecology Medical Center Boulevard, Winston-Salem, NC, USA

Victor E. Beshay, MD
Dallas-FortWorth Center for Fertility and IVF, Allen, TX, USA

Charles L. Bormann, PhD
Vincent Reproductive Medicine and IVF, Vincent Department of Obstetrics and Gynecology, Massachusetts General Hospital, Boston, MA, USA
Department of Obstetrics, Gynecology, and Reproductive Biology, Harvard Medical School, Boston, MA, USA

Paul R. Brezina, MD, MBA
Department of Obstetrics and Gynecology, Vanderbilt University Medical Center, Nashville, TN, USA
Reproductive Genetics Center, Fertility Associates of Memphis, Memphis, TN, USA

Bruce R. Carr, MD
Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA

Gregory M. Christman, MD
Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Florida, Gainesville, FL, USA

Nina Desai, PhD, HCLD
IVF Laboratory, Department of Obstetrics and Gynecology, Cleveland Clinic Fertility Center, Beachwood, OH, USA

Sonia Elguero, MD
Boston IVF-The Albany Center, Albany, NY, USA

Tommaso Falcone, MD
Obstetrics, Gynecology and Women's Health Institute, Cleveland Clinic, Cleveland, OH, USA

Rebecca Flyckt, MD
Cleveland Clinic, Cleveland, OH, USA

Jason M. Franasiak, MD, TS (ABB)
Reproductive Medicine Associates of New Jersey, Basking Ridge, NJ, USA

Gary N. Frishman, MD
Woman and Infants Hospital, Hospital of Rhode Island, OB/GYN, Alpert Medical School of Brown University, Providence, RI, USA

Mark Gibson, MD
Department of Obstetrics and Gynecology—Reproductive Endocrinology, University of Utah Healthcare Hospital, Salt Lake City, UT, USA
Utah Center for Reproductive Medicine, Salt Lake City, UT, USA

Jeffrey M. Goldberg, MD
Department of Obstetrics and Gynecology, Reproductive Endocrinology and Infertility, Cleveland Clinic, Cleveland, OH, USA

Jaclyn M. Grentzer, MD
Department of Obstetrics and Gynecology, Washington University in St. Louis, St. Louis, MO, USA

Ahmad Hammoud, MD, MPH
Department of Obstetrics and Gynecology—Reproductive Endocrinology, University of Utah Healthcare Hospital, Salt Lake City, UT, USA
IVF Michigan Fertility Centers, Bloomfield Hills, MI, USA

Heather D. Hirsch, MD, MS, NCMP
Center for Specialized Women's Health, Cleveland Clinic, Cleveland, OH, USA

William W. Hurd, MD, MSc, MPH
Department of Obstetrics and Gynecology, Duke University Medical Center, Duke University School of Medicine, Durham, NC, USA

Keith B. Isaacson, MD
Newton Wellesley Hospital, Harvard Medical School, Newton, MA, USA

Erika B. Johnston-MacAnanny, MD
Department of Obstetrics and Gynecology, Reproductive Medicine Section, Wake Forest Baptist Medical Center, Medical Center Boulevard, Winston-Salem, NC, USA
Department of Obstetrics and Gynecology, Section of Reproductive Endocrinology and Infertility Wake Forest School of Medicine
Executive Director, Women's Health Service Line Wake Forest Baptist Medical Center Obstetrics and Gynecology Medical Center Boulevard, Winston-Salem, NC, USA

Miriam S. Krause, MD
Fertility and Endocrine Associates, Louisville, KY, USA

William H. Kutteh, MD, PhD, HCLD
Department of Obstetrics and Gynecology, Vanderbilt University Medical Center, Nashville, TN, USA
Recurrent Pregnancy Loss Center, Fertility Associates of Memphis, Memphis, TN, USA

Dan I. Lebovic, MD
Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Wisconsin School of Medicine, Middleton, WI, USA

James H. Liu, MD
Department of Reproductive Biology and Obstetrics and Gynecology, University Hospitals MacDonald Women's Hospital, Cleveland, OH, USA

Jennifer Ludgin, BA
OB/GYN/IVF Research Intern, Department of Obstetrics and Gynecology, Cleveland Clinic Fertility Center, Beachwood, OH, USA

Erica B. Mahany, MD
Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI, USA

Emanuela Molinari, PhD
Department of Obstetrics and Gynecology and Reproductive Sciences, Yale University Fertility Center, New Haven, CT, USA

Levent Mutlu, MD
Department of Obstetrics and Gynecology, Yale School of Medicine, New Haven, CT, USA

Steven T. Nakajima, MD
Stanford Medicine: Fertility and Repro Health, OB/GYN—Repro Endocrinology and Infertility, Sunnyvale, CA, USA

Michelle G. Park, MD
Minimally Invasive Gynecologic Surgery Unit, Newton Wellesley Hospital, Newton, MA, USA

Caitlin Parks, MD
Department of Obstetrics and Gynecology, Washington University in St. Louis, St. Louis, MO, USA

Bansari Patel, MD
Department of Obstetrics and Gynecology, Wake Forest School of Medicine, Winston Salem, NC, USA

Pasquale Patrizio, MD
Department of Obstetrics and Gynecology and Reproductive Sciences, Yale University Fertility Center, New Haven, CT, USA

Jeffrey F. Peipert, MD, PhD
Department of Obstetrics and Gynecology, Indiana University School of Medicine, Indianapolis, IN, USA

Robert W. Rebar, MD
Department of Obstetrics and Gynecology, Western Michigan University Homer Stryker M.D. School of Medicine, Kalamazoo, MI, USA

Joseph S. Sanfilippo, MD, MBA
Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Hospital, Pittsburgh, PA, USA
Department of Obstetrics and Gynecology, University of Pittsburgh, Pittsburgh, PA, USA

Richard T. Scott Jr, MD, HCLD/ALD (ABB)
Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, Thomas Jefferson University, Philadelphia, PA, USA

Rakesh Sharma, PhD
Department of Urology, Cleveland Clinic, Cleveland, OH, USA

Howard T. Sharp, MD
Department of Obstetrics and Gynecology, University of Utah Health Sciences Center, Salt Lake City, UT, USA

Andrea Sikon, MD
Center for Specialized Women's Health, Cleveland Clinic, Cleveland, OH, USA

Yolanda R. Smith, MD
Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI, USA

Meir Jonathon Solnik, MD
Head of Gynaecology and Minimally Invasive Surgery, Mt. Sinai Hospital, Toronto, ON, Canada
Department of Obstetrics and Gynaecology, Mt. Sinai Hospital, Toronto, ON, Canada

Hugh S. Taylor, MD
Department of Obstetrics and Gynecology, Yale School of Medicine, New Haven, CT, USA

Holly L. Thacker, MD
Center for Specialized Women's Health, Cleveland Clinic, Cleveland, OH, USA

Togas Tulandi, MD, MHCM
Department of Obstetrics and Gynecology, McGill University, Montréal, QC, Canada

Roxanne Vrees, MD
Woman and Infants Hospital, Hospital of Rhode Island, OB/GYN, Alpert Medical School of Brown University, Providence, RI, USA

Kaitlyn M. Weeber, BS
Vanderbilt University Medical Center, Nashville, TN, USA

Hélène S. Weibel, MD
Department of Obstetrics and Gynecology, McGill University, Montréal, QC, Canada

Melissa F. Wellons, MD
Medicine-Division of Diabetes, Endocrinology and Metabolism, Vanderbilt University Medical Center, Nashville, TN, USA

Lynn M. Westphal, MD
Department of Obstetrics and Gynecology, Stanford University, Stanford, CA, USA

Hope Y. Yu
Woman and Infants Hospital, Hospital of Rhode Island, OB/GYN, Alpert Medical School of Brown University, Providence, RI, USA

Hypothalamic–Pituitary–Ovarian Axis and Control of the Menstrual Cycle

Victor E. Beshay and Bruce R. Carr

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1

1.1 Introduction

The menstrual cycle is the result of an orchestra of hormones. It involves the interaction of many endocrine glands as well as a responsive uterus. The menstrual cycle remains a complex process where many aspects are still not well understood. In this chapter we will examine the control of the menstrual cycle through the interaction of the central nervous system, namely, the hypothalamus and pituitary, and the ovaries, resulting in the cyclic and ordered sloughing of the uterine endometrial lining. The first section of this chapter, *The Menstrual Cycle*, will review the phases of the menstrual cycle. In the second section, *Anatomy of the Menstrual Cycle*, the hypothalamic, pituitary, ovarian, and uterine activities will be reviewed. The key hormones that play a role in the control of the menstrual cycle include gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and progesterone (Table 1.1). In addition to these key hormones, there are other peptide and non-peptide hormones that play a role in the menstrual cycle that will also be discussed. These hormones will be discussed in the third section, *Endocrinology of the Menstrual Cycle*.

Clinical Case

A 25-year-old woman comes to the office with report of unpredictable cycles. She has always had irregular and unpredictable menstrual cycles since her first menses. She is planning to get pregnant in the near future. She inquires as to why she has these irregularities and would like to know what goes into controlling and ultimately regulating her cycle.

1.2 The Menstrual Cycle

The menstrual cycle can be divided into three phases: proliferative (follicular), ovulation, and secretory (luteal). The menstrual cycle is also described based on its length (number of days between onset of menstrual bleeding in one cycle and the onset of bleeding of the next cycle). The median duration of a menstrual cycle is 28 days [1–3]. Most individuals will describe a cycle length between 25 and 30 days [1–3]. The variability in length of a menstrual cycle is based on the variable length of the follicular phase. The luteal phase is

Table 1.1 Major hormones of the hypothalamic–pituitary–ovarian axis[a]

Hormone	Structure	Gene location	Major site(s) of production	Half-life	Serum concentration
GnRH	Decapeptide	8p21–8p11.2	Arcuate nucleus of hypothalamus	2–4 min	N/A
FSH	Glycoprotein with α- and β-subunits	α: 6q12.21 β: 11p13	Gonadotrophs of anterior pituitary	1.5–4 h	5–25 mIU/mL
LH	Glycoprotein with α- and β-subunits	α: 6q12.21 β: 19q12.32	Gonadotrophs of anterior pituitary	20–30 min	5–25 mIU/mL
Estradiol	18 carbon steroid	NA	Granulosa cells	2–3 h	20–400 pg/mL
Progesterone	21 carbon steroid	NA	Theca-lutein cells	5 min	0.1–30 ng/mL
Inhibin	Peptide with α- and β-subunits Inhibin A = α + βA Inhibin B = α + βB	α: 2q33 βA: 2q13 βB: 7p15	Granulosa cells	30–60 min	A: 10–60 B: 10–150 pg/mL

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constant in most women and is 14 days in length. Polymenorrhea is described as menstrual cycles that occur at intervals less than 21 days. Conversely, oligomenorrhea is described as menstrual cycles that occur at intervals more than 35 days. During menstruation, blood loss is typically 30 mL [4], and amounts greater than 80 mL (menorrhagia) are considered abnormal [4].

The *proliferative phase* begins at the onset of menses until ovulation takes place. Folliculogenesis takes place during this phase of the menstrual cycle. A dominant follicle is selected from a pool of growing follicles that will be destined to ovulate. The growth of follicles in this stage will depend on pituitary hormones such as FSH. The growth of the follicle also leads to production of estradiol from the layers of granulosa cells surrounding it. Estradiol is responsible for the proliferation of the endometrial lining of the uterus.

Ovulation happens at the peak of follicular growth in response to an LH surge [5]. Prior to ovulation, follicles grow to sizes greater than 20 mm in average diameter [6]. LH is then released in a positive-feedback manner from the anterior pituitary due to prolonged exposure to estradiol. For this positive feedback to take place, levels of estradiol above 200 pg/mL for approximately 50 h are necessary [7] (Fig. 1.1). Approximately 12 h after the LH peak, the oocyte is released [8, 9]. In order for the oocyte to release from the follicle, several proteolytic enzymes and prostaglandins are activated, leading to the digestion of the follicle wall collagen [10]. Once an oocyte is released, the fallopian tube is responsible for picking it up where it will await fertilization.

The *secretory phase* starts after ovulation. During this phase, the remaining granulosa cells that are not released with the oocyte during the ovulation process enlarge and acquire lutein (carotenoids), which is yellow in color. These granulosa cells are now called the corpus luteum and predominantly secrete progesterone. Peak progesterone production is noted 1 week after ovulation takes place (see Fig. 1.1). Progesterone is required to convert the endometrial lining of the uterus from a proliferative one into a secretory endometrium in preparation for embryo implantation. The life span of the corpus luteum and, hence, progesterone production will depend on continued LH support from the anterior pituitary. If a pregnancy takes place, hCG (human chorionic gonadotropin) of pregnancy will maintain the corpus luteum. However, if a pregnancy fails to happen, luteolysis takes place and the corpus luteum is converted to a white scar called the corpus albicans. The loss of the corpus luteum and the subsequent loss of progesterone lead to the instability of the endometrium and the sloughing of the endometrium, signaling a new menstrual cycle.

1.3 Anatomy of the Menstrual Cycle

The initial signals for a menstrual cycle are initiated from the central nervous system. The pertinent endocrine portion of the central nervous system consists of the hypothalamus and the pituitary gland.

The *hypothalamus* consists of only 0.3% of the total brain, measuring 4 cm^3, and weighing approximately 10 g. Despite its small size, it contains many nuclei that are responsible for endocrine regulation, reproduction, metabolism, temperature regulation, emotional responses, and electrolyte balance [11] (Fig. 1.2). The hypothalamus lays beneath the thalamus, hence, the nomenclature. Laterally, it is bordered by the anterior part of the subthalamus, the internal capsule, and the optic tract [11]. The hypothalamus forms the lateral wall and floor of the third ventricle. The median eminence of the hypothalamus extends to the anterior pituitary and contains neurosecretory neurons that affect hormone production from the anterior pituitary. The hypothalamus is comprised of three zones: lateral, medial, and periventricular. Within each zone lie several nuclei, where the arcuate nucleus is pertinent to reproduction. The arcuate nucleus is responsible for the production of GnRH. GnRH is secreted into the portal pituitary circulation, reaching the anterior pituitary to affect FSH and LH release from the anterior pituitary. The hypothalamus also influences thyroid function via TRH (thyrotropin-releasing hormone), adrenal function via CRH (coricotropin-releasing hormone), and growth and metabolic homeostasis via GHRH (growth hormone-releasing hormone) [11].

The *pituitary* gland is a pea-sized gland, also known as the master endocrine gland. It measures 12 × 8 mm and weighs approximately 500 mg [11].

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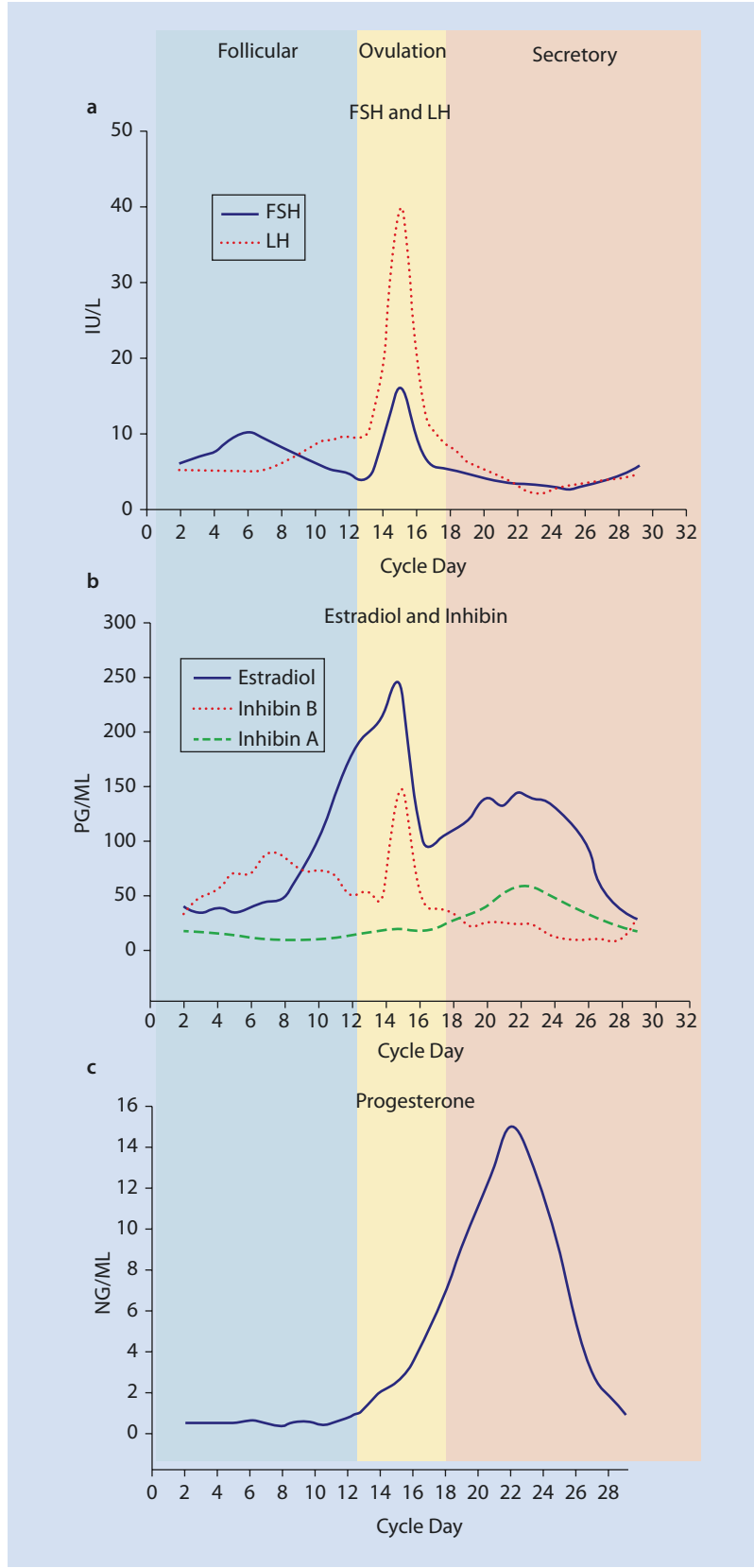

Fig. 1.1 Hormone fluctuations during the menstrual cycle. **a** Mean values of FSH and LH throughout the cycle. **b** Mean values of estradiol and inhibin. **c** Mean values of progesterone during the menstrual cycle. Reproduced with permission from Mahutte NG, Ouhilal S. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

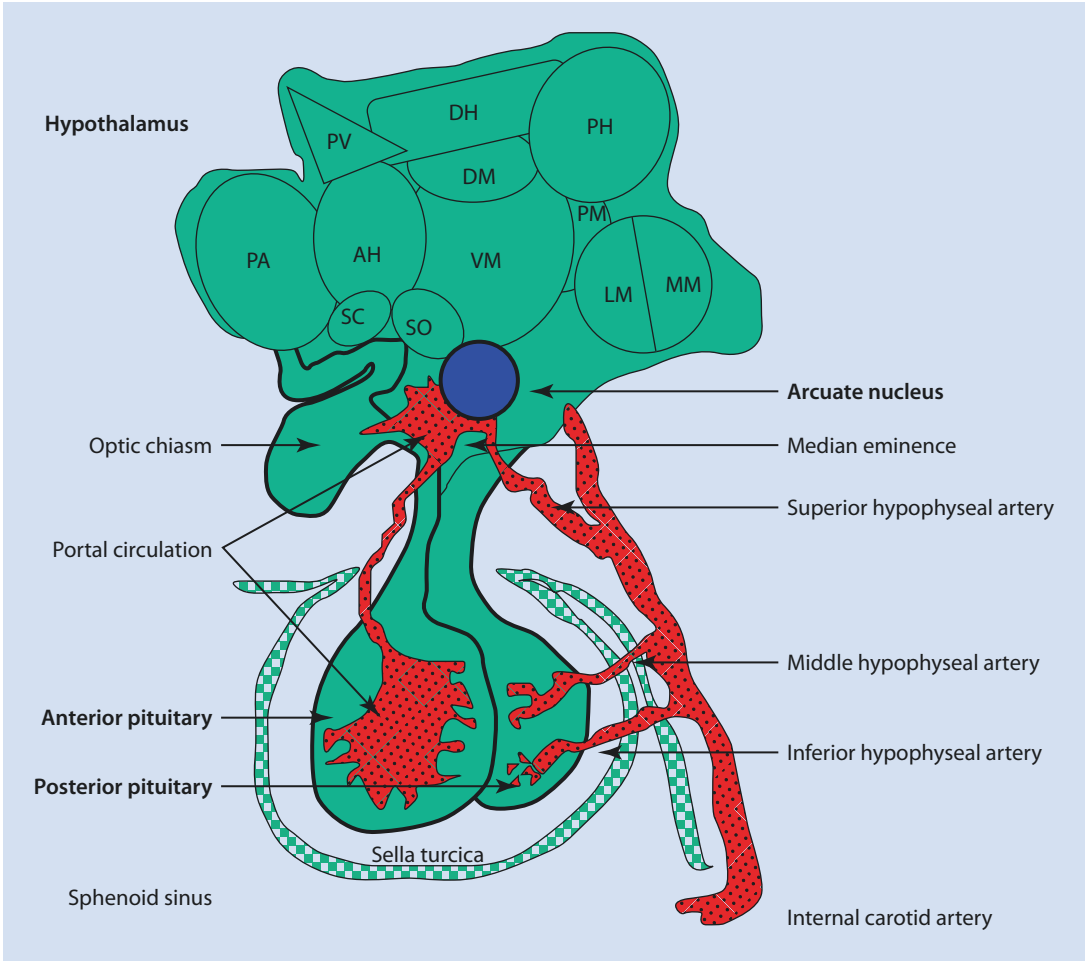

Fig. 1.2 Illustration of the hypothalamus, pituitary, sella turcica, and portal system. The arcuate nucleus is the primary site of GnRH-producing neurons. GnRH is released from the median eminence into the portal system. The blood supply of the pituitary gland derives from the internal carotid arteries. In addition to the arcuate nucleus, the other hypothalamic nuclei are *SO* supraoptic nucleus, *SC* suprachiasmatic nucleus, *PV* paraventricular nucleus; *DM* dorsalmedial nucleus, *VM* ventromedial nucleus, *PH* posterior hypothalamic nucleus, *PM* premammillary nucleus, *LM* lateral mammillary nucleus, *MM* medial mammillary nucleus. The three hypothalamic areas are *PA* preoptic area, *AH* anterior hypothalamic area, *DH* dorsal hypothalamic area. Reproduced with permission from Mahutte NG, Ouhilal S. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

It is located beneath the third ventricle and above the sphenoidal sinus in a bony cavity called the sella turcica (see Figs. 1.2 and 1.3). The adult pituitary gland contains two major parts: the adenohypophysis and the neurohypophysis. The neurohypophysis is a diencephalic downgrowth connected with the hypothalamus, while the adenohypophysis is an ectodermal derivative of the stomatodeum [11]. The pituitary gland can also be divided into two major lobes: anterior and posterior. The anterior lobe is equivalent to the adenohypophysis, while the posterior lobe is equivalent to the neurohypophysis. The difference is that the nomenclature of anterior and posterior lobes does not include the infundibulum, which extends from the hypothalamus to the pituitary gland, which contains neural hypophysial connections and is continuous with the median eminence [11]. The anterior pituitary contains several cell types: gonadotropes (responsible for secretion of FSH and LH), thyrotropes (responsible for the secretion of thyroid-stimulating hormone [TSH]), adrenocorticotropes (responsible for the secretion of ACTH), somatomammotropes (responsible for

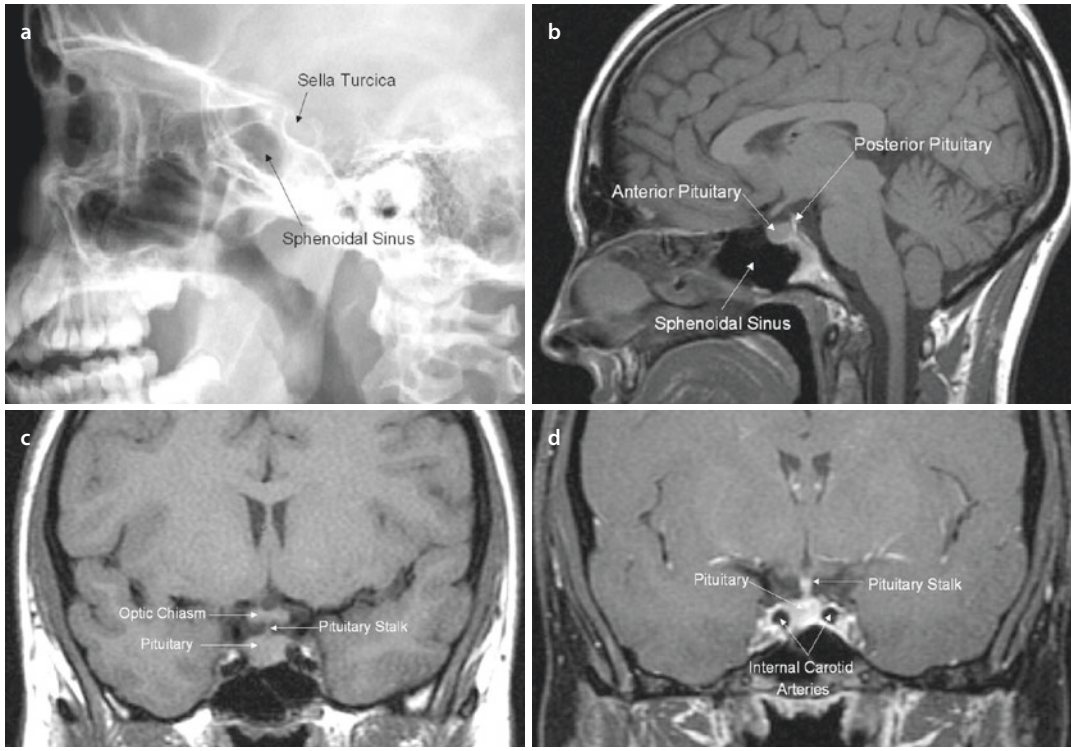

Fig. 1.3 X-ray and T1-weighted MRI images of the pituitary gland. **a** Lateral skull film with the sphenoidal sinus and sella turcica. **b** Sagittal section demonstrating the relationship between the sphenoidal sinus and the pituitary gland. The normal posterior pituitary is brighter on MRI compared to the anterior pituitary. The sella turcica is not well seen on MRI. **c** Coronal section demonstrating the relationship of the pituitary to the optic chiasm and the pituitary stalk. **d** Coronal section after gadolinium contrast, demonstrating the close proximity of the pituitary to the internal carotid arteries. Reproduced with permission from Mahutte NG, Ouhilal S. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

the secretion of GH), and lactotropes (responsible for the secretion of prolactin) (Table 1.2). In addition to these hormones, the anterior pituitary secretes activin, inhibin, and follistatin, which play a role in menstrual cycle regulation. The posterior pituitary lobe contains two cell types that secrete ADH (antidiuretic hormone) and oxytocin. The communication between the hypothalamus and the anterior pituitary is vascular; however, it is a neuronal connection between the hypothalamus and the posterior pituitary.

The gonads in the female consist of the bilateral *ovaries*. The ovaries are located in the pelvis along the sides of the uterus. In reproductive-age women, ovaries measure approximately 2.5 × 3 × 1.5 cm in size. Laterally, the ovary is attached to the pelvic sidewall by the infundibulopelvic ligament, which contains the vascular supply to the ovary (ovarian artery and vein). The ovary consists of an outer cortex and an inner medulla. The ovarian follicles are found in the cortex, while the medulla mainly contains fibromuscular tissue and vasculature. Each ovarian follicle consists of an oocyte surrounded by layers of granulosa and theca cells. These layers will vary depending on the maturation stage of the oocyte contained within the follicle. Within the ovarian cortex, follicles can be found in different stages of development. Earlier stages of follicular development are independent of central nervous system hormone production, while later stages of follicular development will depend on reproductive hormones produced by the central nervous system. The growing ovarian follicle will produce estradiol from the granulose cells (Table 1.3). After ovulation, the remnant cells of the follicle luteinize and start secreting progesterone. The granulosa cells are also responsible for the secretion of inhibin as well as anti-Müllerian hormone (AMH).

Table 1.2 Major cell types of the anterior pituitary gland[a]

Cell type	Appearance on light microscopy	Cellular frequency (%)	Hormone products
Somatotrophs	Acidophilic	50	Growth hormone
Lactotrophs	Acidophilic	20	Prolactin
Corticotrophs	Basophilic	20	Adrenocorticotrophic hormone (ACTH)
Thyrotrophs	Basophilic	5	Thyroid-stimulating hormone (TSH) and free α-subunit
Gonadotrophs	Basophilic	5	Follicle-stimulating hormone (FSH), luteinizing hormone (LH), and free α-subunit

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Table 1.3 Site of synthesis of major steroidogenic products of the ovary

Cell type	Major steroid hormone products
Theca cells	Androgens (androstenedione, DHEA, testosterone)
Granulosa cells	Estrogens (estradiol, estrone, inhibin, AMH)
Theca-lutein cells	Progestogens (progesterone, 17-hydroxyprogesterone)
Granulosa-lutein cells	Estrogens (estradiol, estrone)

The *uterus* is largely a receptive organ to all the steroid hormones that emanate from the endocrine glands. The uterus is a fibromuscular organ that is bordered anteriorly by the urinary bladder and posteriorly by the rectum. The uterus can be divided into two major portions: an upper body (corpus) and a lower cervix. The hollow portion of the uterus contains a mucosal lining called the endometrium. The endometrium contains several layers of cells: the basal layer and the superficial layer. The basal layer is responsible for the regeneration of the endometrial cells. The superficial layers undergo the cyclic changes of the menstrual cycle. The endometrium normally proliferates in response to the rising estradiol levels in the first half of the menstrual cycle and in the second half of the menstrual cycle it is converted to a secretory layer in response to progesterone produced by the corpus luteum. If the cycle does not result in a pregnancy, where there is lack of hCG, progesterone production is not maintained by the corpus luteum, and the endometrium becomes unstable and sloughs in preparation for a new cycle and another attempt for pregnancy.

1.4 Endocrinology of the Menstrual Cycle

GnRH is a decapeptide synthesized in the hypothalamus and first described in the 1970s by Schally [12–14] and Guillemin [15] for which they received the Nobel Prize [14–18] (Fig. 1.4). GnRH neurons can be detected in the fetal hypothalamus as early as 9–10 weeks of gestation [19]. GnRH neurons originate from the olfactory area [20], later migrating to the olfactory placode to rest in the arcuate nucleus of the hypothalamus [21]. The hypothalamic GnRH neurons then send projections to the pituitary. The association of GnRH neurons and the olfactory system can be demonstrated in a condition called Kallmann syndrome, where GnRH deficiency is coupled with anosmia [22]. Pheromones, small airborne molecules secreted by one individual and perceived by another individual, also suggest the common origin of GnRH molecules and the olfactory system. Pheromones may explain why women living or working in close proximity may develop synchrony in their menstrual cycles [23, 24].

1

pyro-Glu[1]-His[2]-Trp[3]-Ser[4]-Tyr[5]-Gly[6]-Leu[7]-Arg[8]-Pro[9]-Gly[10]-NH_2

Fig. 1.4 Structure of GnRH-1. Reproduced with permission from Mahutte NG, Ouhilal S. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

To date, three types of GnRH (GnRH-I, GnRH-II, and GnRH-III) have been detected in humans [25, 26]. Many other GnRH types have been described in fish, amphibians, and protochordates [27, 28]. GnRH-I is the classic hypothalamic hormone responsible for the regulation, synthesis, and secretion of the pituitary gonadotropins FSH and LH [29]. GnRH-II was first described in brain tissue and has since been found in many other peripheral tissues, such as the endometrium, breast, and ovaries [30–33]. GnRH-III was first identified in Lamprey in 1993 [34], and Yahalom et al. described the presence of GnRH-III in neurons from the hypothalamus [25]. The role of GnRH-III in humans is unclear. GnRH-III does not have strong LH and FSH releasing potency, but it has been shown to exert a direct antiproliferative effect on cancer cells and is being studied for use as an antitumor agent [35].

GnRH-I is synthesized from a larger, 92 amino acid precursor [36]. After synthesis, GnRH-I travels to the median eminence of the hypothalamus and is released in the portal circulation in a pulsatile fashion. The GnRH-I molecule lifespan is very short, as it is cleaved rapidly, with a half-life of 2–4 min. Because of this rapid cleavage, peripheral levels of GnRH-I are difficult and do not correlate well to pituitary action.

GnRH-I acts on the anterior pituitary leading to the synthesis and storage of gonadotropins, movement of the gonadotropins from the reserve pool to a readily released point, and finally the secretion of gonadotropins. For this action to take place appropriately, pulsatile GnRH release is necessary [37, 38]. Continuous GnRH secretion will lead to the suppression of FSH and LH release as well as suppression of FSH and LH gene transcription by the anterior pituitary [39, 40]. This is the basis of use of GnRH agonists such as Lupron for the suppression of gonadotropin secretion. The pulse frequency of GnRH will vary depending on the menstrual cycle phase. LH pulse frequency is used to indicate GnRH pulse secretion (Table 1.4).

Table 1.4 Menstrual cycle variation in LH pulse frequency and amplitude[a]

Cycle phase	Mean frequency (min)	Mean amplitude (mIU/mL)
Early follicular	90	6.5
Mid-follicular	50	5
Late follicular	60–70	7
Early luteal	100	15
Mid-luteal	150	12
Late luteal	200	8

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GnRH-II differs from GnRH-I by three amino acids at positions 5, 7, and 8 [26, 41]. Also, in contrast to GnRH-I, GnRH-II is mainly expressed outside the brain [26, 42, 43], including the human placenta [44]. Similar to GnRH-I release from the hypothalamus, GnRH-II is released from the placenta in a pulsatile fashion [44, 45].

Various factors are believed to play a role in GnRH secretion. Estrogen has been shown to have a positive as well as a negative effect on GnRH-I secretion. Estrogen suppresses GnRH-I secretion in a negative-feedback fashion [46]. In addition, estrogen has a differential regulation on GnRH-I and GnRH-II mRNA levels. Estrogen increased GnRH-II mRNA levels while it decreased GnRH-I mRNA levels [47]. Progesterone is also noted to play a stimulatory role on GnRH-1 mRNA, which was decreased by the progesterone receptor antagonist RU48 [48]. However, no difference in the expression level of GnRH-II was seen with progesterone or the anti-progestin mifepristone [48].

Two types of GnRH receptors have been described in humans: GnRH-I receptor (GnRH-IR)

Table 1.5 Properties of commercially available GnRH agonists[a]

	Structure and substitutions at positions 6 and 10	Half-life	Relative potency	Route of administration
GnRH	Native decapeptide	2–4 min	1	IV, SC
Nafarelin	Decapeptide 6: Nal for Gly	3–4 h	200	Intranasal
Triptorelin	Decapeptide 6: Trp for Gly	3–4 h	36–144	SC, IM depot
Leuprolide	Nonapeptide 6: Leu for Gly 10: NHEt for Gly	1.5 h	50–80	SC, IM depot
Buserelin	Nonapeptide 6: Ser(O^tBu) for Gly 10: NHEt for Gly	1.5 h	20–40	SC, intranasal
Goserelin	Decapeptide 6: Ser(O^tBu) for Gly 10: AzaGly for Gly	4.5 h	50–100	SC implant
Histrelin	Decapeptide 6: DHis for Gly 10: AzaGly for Gly	50 min	100	SC

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and the GnRH-IIR (GnRH-IIR). The GnRH-IR is a G protein-coupled transmembrane receptor (GPCR) [49, 50]. However, the mammalian GnRH-IR lacks the carboxyl-terminal tail [50, 51]. Activation of the GnRH-IR leads to the activation of phospholipase C, which in turn generates the second messengers inositol triphosphate and diacyl glycerol, stimulating protein kinase, cyclic adenosine monophosphate (cAMP), and release [52] of calcium ions. In addition to the brain, GnRH-IR can be found in the human placenta [53, 54], ovarian follicles [33, 55], in myometrium and leiomyomata [56, 57], as well as human pancreas, liver, heart, skeletal muscle, kidney, and peripheral blood [58–61]. GnRH-IIR is also a GPCR, but unlike the GnRH I-R, it has a C-terminal cytoplasmic tail [62]. GnRH-IIR can be found in the pituitary, placenta, ovary, uterus, prostate, mature sperm, pancreas, small and large intestines, kidney, and liver [26, 33, 63–65].

GnRH analogues have been developed by changes made to the amino acid sequence of the GnRH molecule. These changes result in the extension of the GnRH half-life as well as its biologic activity. There are two major groups of GnRH analogues: GnRH agonists and GnRH antagonists (Table 1.5) In the case of GnRH agonist use, the continuous activation of the GnRH receptor results in desensitization due to a conformational change of the receptor, uncoupling from G proteins, internalization of the receptor as well as reduced synthesis of the receptor [66, 67]. Prior to the desensitization by GnRH agonists, there is an initial flare where there is increased gonadotropin secretion. Desensitization then takes place 7–14 days later. Unlike GnRH agonists, GnRH antagonists do not cause a flare effect upon initial administration; instead, GnRH antagonists cause an immediate suppression of gonadotropin secretion that is rapid and is reversible [68]. Currently, GnRH analogues are available in injectable form in the treatment of many reproductive conditions, such as precocious puberty, endometriosis, and uterine leiomyomata; they are also being used in in vitro fertilization treatment cycles. Oral forms of GnRH analogues are under investigation. Elagolix is an orally active GnRH antagonist under investigation for use in reproductive conditions [69, 70].

GnRH acts on the anterior pituitary to secrete gonadotropins: FSH and LH. *FSH* is a glycoprotein

dimer consisting of two subunits: α (alpha)- and β (beta)-subunits. The α-subunit is common in FSH and LH as well as TSH and hCG. The β-subunit is distinct and hormone-specific, which allows the differential function of each hormone. The α-subunit consists of 92 amino acids, while the FSH β-subunit consists of 118 amino acids and five sialic acid residues. Sialic acid residues are responsible for the half-life of the hormone, where the higher the sialic acid content the longer the half-life of that molecule [71]. FSH has a half-life of several hours. The addition of sialic acid to urinary obtained or recombinant FSH products leads to their longer half-life. The rate-limiting step in gonadotropin production is the availability of β-subunits. In addition to GnRH stimulation of FSH β-subunit synthesis, FSH β-subunit synthesis is dependent on the presence of activin [72, 73].

FSH starts to rise a few days prior to the onset of menses and is responsible for the recruitment of a cohort of ovarian follicles as well as a selection of the dominant follicle (see Fig. 1.1). FSH induces granulosa cell growth and activates aromatase activity, which converts androgens into estrogens. FSH levels then start to decline owing to estrogen and inhibin B production by the growing follicular granulosa cells. Despite this drop in the FSH level, the dominant follicle continues to grow as it acquires the highest concentration of FSH receptors (secondary to increase in surrounding granulosa cell number), making it more resistant to the drop in FSH level [74]. In addition, the drop in FSH level causes a higher androgenic microenvironment in the non-dominant follicles. FSH then declines after ovulation of the dominant follicle.

LH is also a glycoprotein dimer consisting of two subunits: α (alpha)- and β (beta)-subunits. The β-subunit of LH consists of 121 amino acids and one to two sialic acid residues, giving it its shorter half-life of approximately 20 min. Because of this shorter half-life, LH needs to be rapidly synthesized and typically has pulses higher in amplitude than FSH. As with FSH, LH also starts to rise prior to the onset of menses. The LH increase throughout the follicular phase of the cycle is gradual. Immediately prior to ovulation, LH surges in response to estradiol production by the dominant follicle in a positive-feedback fashion. LH levels then decline in the secretory phase of the cycle (see Fig. 1.1). Little is known as to why LH initially responds negatively to estrogen, while later the feedback relationship becomes positive. Many researchers have looked into this, with some researchers describing possible synaptic transmission speed as the reason for the positive feedback [75], while others suggest that the continued estrogen presence causes an increase in Glutamate [76] and Gamma Amino Butyric Acid (GABA) transmission in GnRH neurons [77, 78]. However, these studies have been performed in animals and no equivalent studies have been shown in humans.

FSH and LH receptors both belong to the GPCR family. FSH receptors exist exclusively on the membrane of granulosa cells, while LH receptors are found on membranes of theca cells. In the presence of estradiol, FSH induces LH receptors on granulosa cells. LH receptor activity primarily stimulates androstenedione production from theca cells, which is transported to neighboring granulosa cells, aromatized to estrone, and eventually converted to estradiol. This is the basis of the two-cell theory of the ovary (Fig. 1.5).

Endogenous *opiates (opioids)* are naturally occurring narcotics produced by the brain. There are three classes of opiates: enkephalin, endorphin, and dynorphin. Endorphin levels increase throughout the menstrual cycle; they are at their lowest at the time of menses and at their highest in the luteal phase. Sex steroids appear to play a role in endorphin secretion. Estradiol has been shown to increase endorphin secretion, while the sequential addition of progesterone to estradiol showed a higher endorphin secretion in ovariectomized monkeys [79]. An increase in endorphin release has been shown to decrease LH pulse frequency [80], while opioid receptors blockers, such as naltrexone, have been shown to increase LH pulse frequency [81]. The suppression of gonadotropin secretion by endogenous opiates is secondary to suppression of hypothalamic GnRH release [82]; thus, opiates appear to play a role in hypothalamic amenorrhea (Table 1.6). Treatment of women with hypothalamic amenorrhea with opioid receptor antagonists appears to correct the problem, causing a return of ovulation and menstrual cyclicity [83, 84]. It is also believed that stress-related amenorrhea is the result of GnRH suppression by endogenous opiates. Women suffering from stress-related amenorrhea demonstrate higher hypothalamic corticotropin-releasing hormone. Proopiomelanocortin, the precursor to endorphins, is controlled mainly by corticotropin-releasing hormone [79]. In addition, hypothalamic amenorrhea that develops in athletes

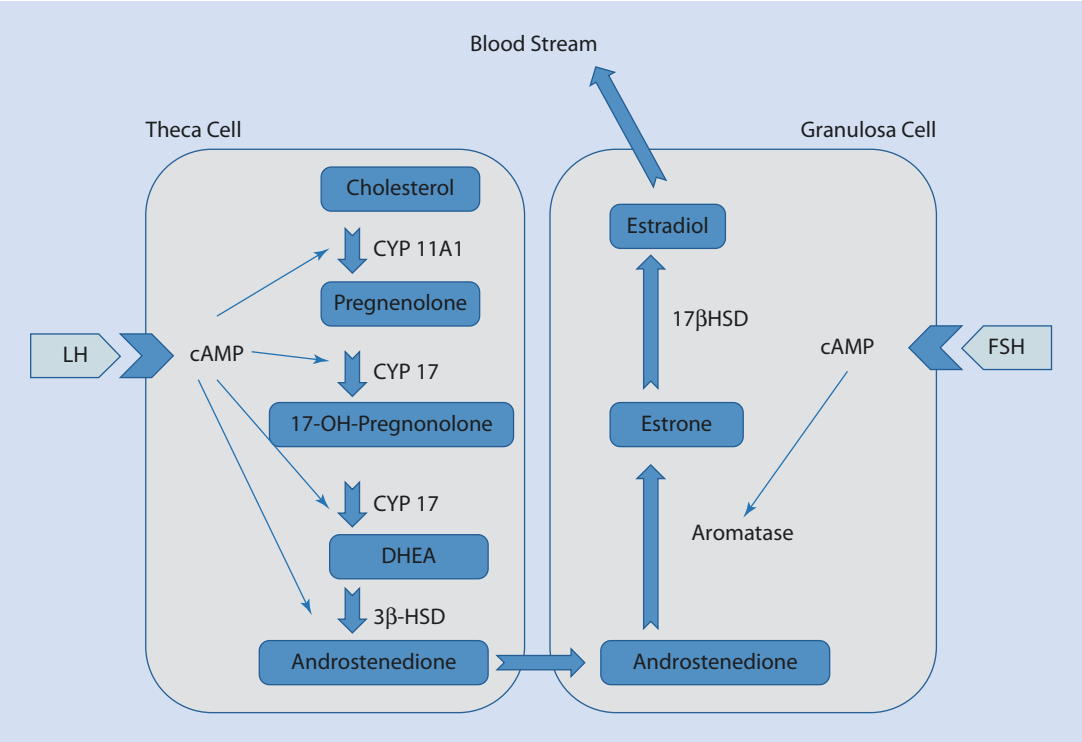

Fig. 1.5 The two-cell theory of ovarian steroidogenesis. Binding of luteinizing hormone (LH) to its receptor on ovarian theca cells stimulates the conversion of cholesterol to androstenedione. Binding of follicle-stimulating hormone (FSH) to its receptor on ovarian granulosa cells stimulates the aromatization of androgens to estrogens. *cAMP* cyclic adenosine monophosphate; *CYP11A1* side-chain cleavage enzyme; *CYP17* 17-hydroxylase; *HSD* hydroxysteroid dehydrogenase; *17-OH pregnenolone* 17-hydroxy pregnenolone

Table 1.6 Neurotransmitter effects on GnRH release[a]

Neurotransmitter	Effect
Dopamine	Inhibits GnRH release
Endorphin	Inhibits GnRH release
Serotonin	Inhibits GnRH release
Norepinephrine, epinephrine	Stimulates GnRH release

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may also be secondary to opioid rise during exercise [85, 86].

Ovarian peptide hormones such as inhibin, activin, and AMH also play a role in the menstrual cycle by modulating central nervous system hormone release. Inhibin, activin, and AMH all belong to the transforming growth factor-β (beta) superfamily (TGF-β) of ligands.

Inhibin is a polypeptide mainly secreted by granulosa cells, but has also been found in pituitary gonadotropes [87, 88]. Inhibin is comprised of a α (alpha)- and β (beta)-subunits. Two forms of inhibin have been identified: inhibin-A and inhibin-B, each containing an identical α-subunit but a unique β-subunit. Inhibin-A is predominantly secreted in the luteal phase of the menstrual cycle, while inhibin-B is predominantly secreted in the follicular phase of the menstrual cycle [89]. Inhibin is released by granulosa cells in response to FSH [90] and selectively inhibits FSH secretion from the anterior pituitary [91], thus creating a negative-feedback loop (see Fig. 1.1).

In contrast, *activin*, which is also secreted by the granulosa cells, augments the secretion of FSH by enhancing GnRH receptor formation [92, 93]. The effects of activin are blocked by inhibin and follistatin [94].

Follistatin is a peptide secreted by pituitary gonadotropes [95]. Follistatin inhibits FSH synthesis and secretion by sequestering activin [96, 97]. Inhibin inhibits follistatin production, while activin stimulates its production.

AMH is a product of the granulosa cells of small antral and pre-antral follicles and is reflective of their quantity [98]. It may be reflective of the ovarian reserve which is often a clinical term for the size of the primordial follicle pool. Although the role of AMH has been well described for causing Müllerian duct regression in the male fetus, its role in females in the post-fetal life period has not been well defined. It is believed that AMH, through a paracrine effect in the ovary, inhibits FSH-stimulated follicle growth, contributing to the emergence of the dominant follicle [79]. The relationship among AMH, the follicular pool, and recruitment throughout the reproductive life cycle is complex and is dependent on the stage of sexual development [99]. Clinically AMH has been used in the prediction of ovarian reserve in women undergoing fertility evaluation and treatment [100]. However, the dichotomy of poor reserve vs. normal reserve is not evident [100]. AMH levels are elevated in patients with polycystic ovary syndrome and decreased in women exposed to antineoplastic drugs.

Leptin is a protein cytokine secreted by adipocytes. It consists of 167 amino acids and is secreted by adipose tissue, reflecting amounts of body fat [101]. Leptin's most significant role is energy homeostasis. It is regulated by many factors, such as obesity, glucose, and insulin, which promote its secretion, whereas fasting, androgens, and thyroid hormone inhibit its secretion. Its role in reproduction is not well understood. As mentioned earlier, CRH is increased in stress-related amenorrhea and is also increased in weight-loss amenorrhea. It is not understood why CRH increases. The reduction in leptin level in these clinical scenarios may play a role in this CRH increase in the brain [102]. Leptin has also been shown to indirectly affect pituitary FSH and LH secretion in gonadotropin-stimulated fertility treatment cycles [103].

Estrogens are 18-carbon steroid hormones and include estrone (E1), estradiol (E2), and estriol (E3). The most potent estrogen is *estradiol* and is the product of the ovary. Estrone is mainly the product of peripheral androstenedione conversion. Estrone is also generated in the liver via 17 β (beta)-hydroxysteroid dehydrogenase conversion of estradiol. Estriol is the principal estrogen formed by the placenta during pregnancy. Serum estradiol levels rise during the follicular phase of the menstrual cycle and are in parallel to the growth of the follicle. Estradiol is mainly found bound in the bloodstream to carrier proteins. Albumin carries approximately 60% of estradiol, while sex hormone-binding globulin binds 38% of estradiol, with 2% remaining as free in the bloodstream. This free hormone is active and capable of entering target cells. In the early follicular phase, serum estradiol levels do not exceed 50 pg/mL. At peak follicular growth, the level rises to approximately 200–250 pg/mL. Estradiol levels drop with ovulation, but a second rise is seen in the mid-luteal phase, reflecting estrogen secretion from the corpus luteum (see Fig. 1.1). Circulating estrogens are conjugated in the liver to form sulfates and glucuronides; 80% are excreted in the urine and the remaining 20% in bile.

There are two known estrogen receptors: estrogen receptor-alpha (ER-α) and estrogen receptor-beta (ER-β) [104, 105]. Both receptors contain DNA-binding and hormone-binding domains, a hinge region, and a transcriptional activation function (TAF) domain. Estrogen will enter any cell, but only cells containing the estrogen receptor will respond. The receptor is typically nuclear in location, but can be shuttled to the cytoplasm via a process called nucleocytoplasmic shuttling [79]. Once estrogen binds to its receptor, activation of gene transcription then takes place.

It is also known that estradiol has a negative-feedback effect on FSH secretion. This negative-feedback effect is the direct effect of estradiol coupled to its receptor, causing repression of FSH-β (beta) subunit transcription [106].

Similar to estrogen, *progesterone* is a steroid hormone. Progesterone is a 21-carbon molecule and is the main steroid of the corpus luteum. In the follicular phase, progesterone levels are typically <2 ng/mL. Progesterone reaches its peak in the mid-luteal phase, with levels exceeding 5 ng/mL (see Fig. 1.1). The majority of progesterone in the bloodstream is bound to albumin (80%) and corticosteroid-binding globulin (18%). A very small amount of progesterone is bound to SHBG (0.5%). The remaining progesterone is free in the circulation. The liver is responsible for clearing progesterone from the circulation by converting progesterone to pregnanediol, which is conjugated to glucuronic acid and excreted in the urine.

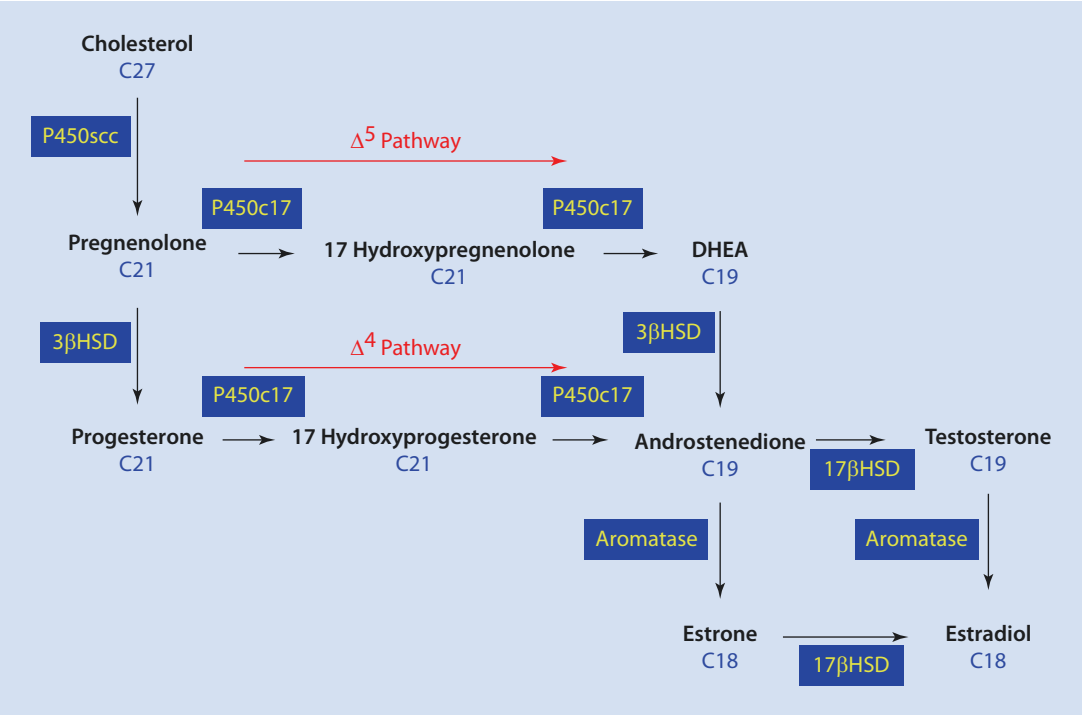

Fig. 1.6 The Δ(delta)^5 and Δ(delta)^4 pathways. The rate-limiting step in steroidogenesis is the conversion of cholesterol to pregnenolone via side-chain cleavage (P450scc). In the follicular phase, pregnenolone is preferentially converted to androstenedione via the Δ^5 pathway involving 17-hydroxypregnenlone and dehydroepiandrosterone (DHEA). In contrast, the corpus luteum preferentially converts pregnenolone to progesterone (Δ^4 pathway) via 3β(beta) hydroxysteroid dehydrogenase (3βHSD). Reproduced with permission from Mahutte NG, Ouhilal S. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

Similar to estrogen, there are several progesterone receptors: progesterone receptor-A (PR-A), progesterone receptor-B (PR-B), and progesterone receptor-C (PR-C). PR-B is the positive regulator of progesterone effects, while PR-A and PR-C antagonize PR-B.

At high concentrations, progesterone inhibits FSH and LH secretion through effects on both the hypothalamus and pituitary [107]. The presence of progesterone in the luteal phase also causes the decline in GnRH pulse frequency in the hypothalamus. At low concentrations, progesterone can stimulate LH release only after exposure to estrogen and progesterone [108]. Progesterone also causes a depletion of estrogen receptors, which is the mechanism of protection against endometrial hyperplasia by progesterone.

Androgens are the major products of theca cells. Androgens are 19-carbon steroids and include: androstenedione, testosterone, and dehydroepiandrosterone (DHEA). The principal secreted androgen by theca cells is androstenedione. Most of the testosterone is the product of peripheral conversion of androstenedione through the actions of 17 β-hydroxysteroid dehydrogenase. Under the effect of FSH, androstenedione and testosterone are then further aromatized in granulosa cells and converted to estrogens (Fig. 1.6).

The androgen receptor exists in a full-length B form and a shorter A form [109]. Androgens and progestins can cross-react to their receptor but only when present in high concentration.

In preovulatory follicles, the preferred steroid pathway for androgen and estrogen synthesis is the Δ (delta)^5 pathway, which involves the conversion of pregnenolone to 17-hydroxypregnenolone. In the theca cell, 17-hydroxypregnonolone is converted to androgens. Due to the lack of ability of theca cells to metabolize androgens, they are carried to the neighboring granulosa cells for aromatization (see Figs. 1.5 and 1.6). In contrast, in the corpus luteum the preferred pathway is the Δ(delta)^4 pathway of steroidogenesis, which deals with the conversion of pregnenolone to progesterone. The rate-limiting

step in steroidogenesis is the side-chain cleavage of cholesterol to pregnenolone. In the ovary, this step is regulated by LH. LH stimulation leads to increased cAMP production and increased low-density lipoprotein (LDL) receptor mRNA and subsequent increased LDL intake. LDL is the major form of cholesterol used for steroidogenesis. cAMP-activated steroidogenic acute regulatory protein (StAR) causes an increase in the transport of cholesterol across the mitochondrial membrane, where side-chain cleavage can take place [110]. From there, all the remaining ovarian hormones can be produced.

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Female and Male Gametogenesis

Nina Desai, Jennifer Ludgin, Rakesh Sharma, Raj Kumar Anirudh, and Ashok Agarwal

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2.1 Introduction

Oogenesis is an area that has long been of interest in medicine, as well as biology, economics, sociology, and public policy. Almost four centuries ago, the English physician William Harvey (1578–1657) wrote *ex ovo omnia*—"all that is alive comes from the egg."

During a women's reproductive life span only 300–400 of the nearly 1–2 million oocytes present in her ovaries at birth are ovulated. The process of oogenesis begins with migratory primordial germ cells (PGCs). It results in the production of meiotically competent oocytes containing the correct genetic material, proteins, mRNA transcripts, and organelles that are necessary to create a viable embryo. This is a tightly controlled process involving not only ovarian paracrine factors but also signaling from gonadotropins secreted by the pituitary.

The contribution of the male to the biology of reproduction is to produce a genetically intact spermatozoa that will fertilize an oocyte. The end product of male gametogenesis, the mature spermatozoa, is designed for one purpose: to deliver the male contribution of the genetic makeup to the embryo. The biology of gamete production is different in males compared to females. Gamete production in females is intimately part of the endocrine responsibility of the ovary. If there are no gametes, then hormone production is drastically curtailed. Depletion of oocytes implies depletion of the major hormones of the ovary. In the male this is not the case. Androgen production will proceed normally without a single spermatozoa in the testes.

This chapter presents basic aspects of human ovarian follicle growth, oogenesis, and some of the regulatory mechanisms involved [1], as well as some of the basic structural morphology of the testes and the process of development to obtain mature spermatozoa.

Clinical Case

A 41-year-old woman consult for infertility of 1 year duration. She was on birth control from age 16 until age 40. She enquires if suppression of ovulation during that period of time has preserved in oocyte quantity.

2.2 Structure of the Ovary

The ovary, which contains the germ cells, is the main reproductive organ in the female. It also functions as an endocrine organ, secreting estrogen and progesterone in response to gonadotropin and paracrine signaling. Ovaries exist as a pair of glands, approximately the size of almonds, on either side of the uterus. Within the abdominal cavity, ovaries are found closest to the lateral wall of the pelvis, attached to the back portion of the broad ligament of the uterus [2]. This area is known as the ovarian fossa and is surrounded by the external iliac vessels, the umbilical artery, and the ureter [2, 3].

The ovary comprises several different layers and types of tissues, shown in Fig. 2.1. The innermost layer is the medulla, which houses the blood vessels essential to supporting the ovary. To the outside of this is the ovarian cortex, which is made up of follicles and stromal tissue. The outermost layer of the ovary consists of a thin layer of epithelial cells. Known as *the germinal epithelium*, this layer produces thousands of primordial follicles during fetal growth [4]. Underlying the germinal epithelium is a strong connective tissue layer known as the tunica albuginea (TA). Ovum production and oocyte maturation occur within the cortex of the ovary [5]. As primordial follicles are recruited and develop, they move closer to the outer edge of the ovary, eventually bursting through the surface during ovulation [3].

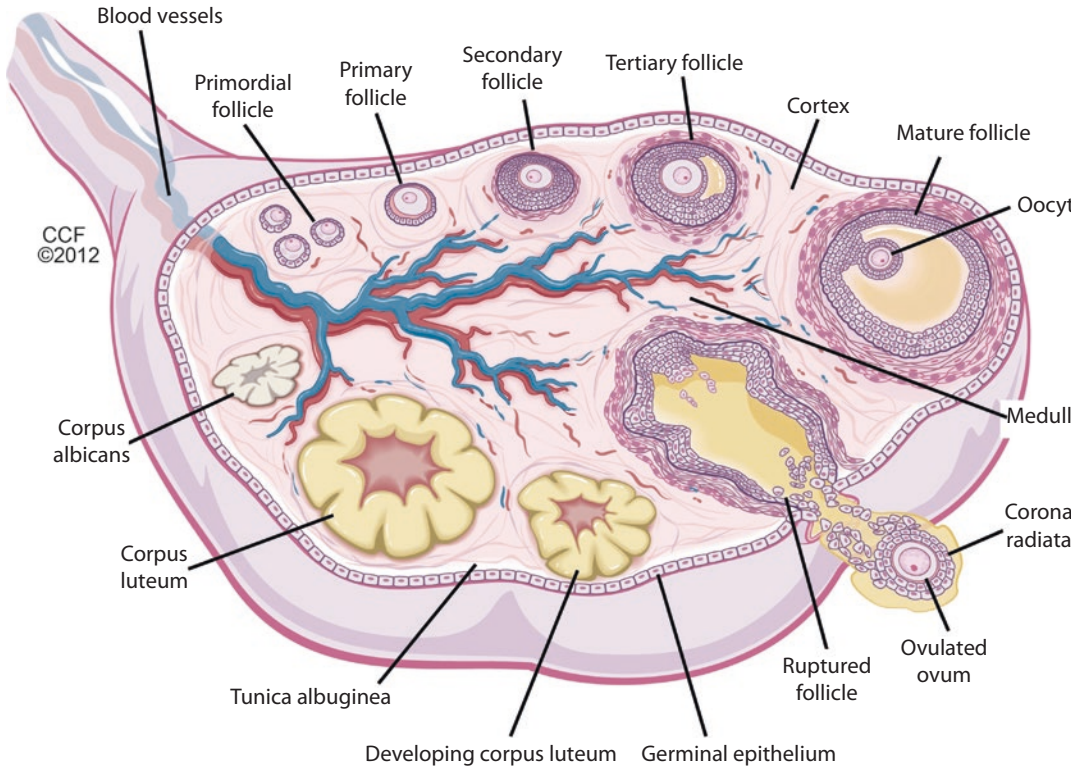


Fig. 2.1 This schematic of the ovary depicts the developing follicle and oocyte in the ovarian cortex. The outermost layer comprises a thin layer of epithelial cells known as the germinal epithelium, which gives rise to primordial oocytes during fetal growth. Just below there is a strong connective tissue layer known as the tunica albuginea. The medulla, located at the center of the ovary, houses blood vessels and ligaments that are vital to the survival and function of the ovary

2.3 Overview of Oogenesis

In humans, oogenesis begins approximately 3 weeks after fertilization. PGCs arise from the yolk sac and migrate via amoeboid movements, through the hindgut, to the genital ridge [6–9]. PGCs undergo rapid mitotic division during this migration. The genital ridge, formed at around 3.5–4.5 weeks gestation, is composed of mesenchymal cells overlaid with coelomic epithelial cells. Upon arrival, the PGCs give rise to oogonia or germ stem cells (GSCs) that continue to proliferate to further expand the germ cell pool. The number of oogonia increases from 600,000 by the eighth week of gestation to over ten times that number by 20 weeks. At around 7 weeks' gestation, these cells form the primitive medullary cords and the sex cords, respectively.

Follicle formation begins at around week 16–18 of fetal life. Oogonia are enveloped by somatic epithelial cells derived from genital ridge mesenchymal cells, forming primordial follicles. The oogonia then cease mitotic activity and enter meiosis [8, 10, 11].

Once meiosis has been initiated, the oogonial germ cell is termed a primary oocyte. The surrounding mesenchymal somatic cells secrete the follicle's basement membrane and give rise to the granulosa cell layer. By 4–5 months' gestation, the ovary has its maximum number of oocytes, between 5 and 8 million. This number decreases dramatically to 1–2 million at birth and less than 500,000 by puberty [12]. The primordial follicles containing these immature oocytes remain essentially dormant. Through the next 35–40 years of a women's reproductive life span, a small number of follicles are steadily released into the growing pool [13–15].

2.3.1 Oocyte Development and Meiosis

Oogenesis is the process by which the mature ovum is differentiated. It is not clear whether the signals controlling germline entry into meiosis are cell-autonomous or dependent on the mesonephric somatic cells. Meiosis is unique to germ cells. It results in the production of daughter cells with haploid DNA content as a result of a two-step division. During oogenesis, cell division is unequal. The result is a single ovum, with excess genetic material extruded as polar bodies [16]. This is quite unlike spermatogenesis, where meiosis results in four identical haploid daughter cells [17].

The four main stages of meiosis are prophase, metaphase, anaphase, and telophase (Fig. 2.2). DNA replication, chromosome pairing, and recombination, steps that are integral to sexual reproduction, all occur during prophase. Prophase can be subdivided into four stages, leptotene, zygotene, pachytene, and diplotene. DNA replication is finalized in preleptotene, while in leptotene, sister chromatids search for their homologous counterparts. Interaction between homologous chromosomes is facilitated by the formation of recombination nodules. In zygotene, homologous chromosomes pair and begin to synapse. The synapses are maintained by the synaptonemal complex. The crossing over and recombination of chromosomes occur in the pachytene stage, prior to the formation of ovarian follicles. Synapsis is completed in pachytene, and by the diplotene stage, homologous chromosomes are held together mainly at sites of chiasmata. Oocytes in primordial follicles are arrested at the diplotene stage of the first meiotic prophase [1, 18].

The nucleus in the prophase oocyte is called the germinal vesicle. Ooplasmic factors prevent the resumption of meiosis in the prophase oocyte until it reaches a specific diameter and stage [13, 19–22]. This stage, referred to as "meiotic competence," occurs in the antral follicle. Once meiosis resumes, there is rapid progression through the metaphase, anaphase, and telophase stages of the first meiotic division. The oocyte then arrests at metaphase 2 of the second meiosis until sperm entry. Oocyte morphology at different stages of maturity is shown in Fig. 2.3.

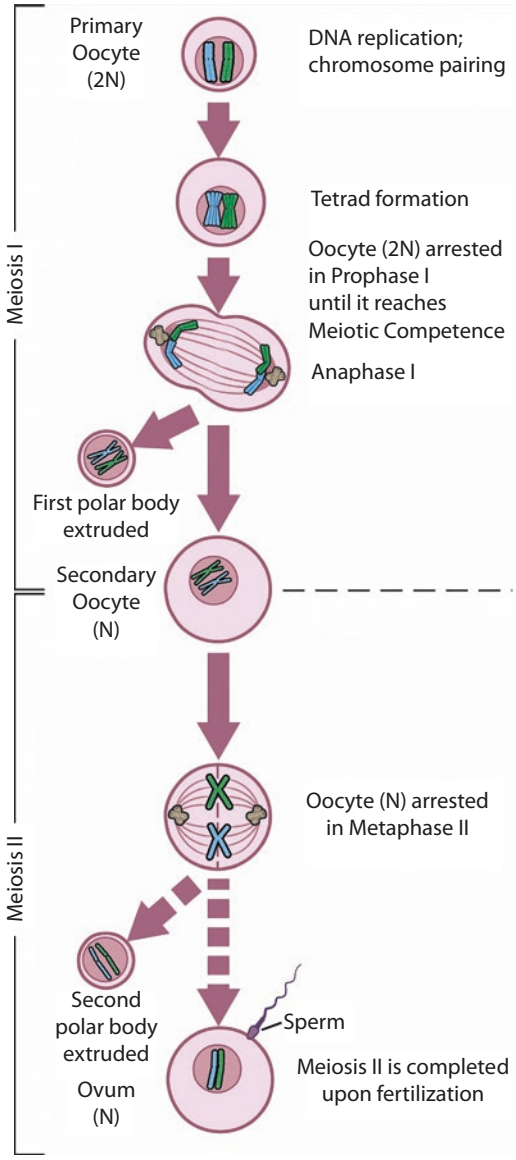


Fig. 2.2 The stages of meiosis I and II leading to the ovulation of a haploid ovum are depicted. Unlike with spermatogenesis, meiosis during oogenesis results in disproportionate cell division with the production of a single ovum with extraneous genetic material being extruded in the first and second polar bodies. During this division, the majority of the cytoplasm, containing important proteins, organelles, and growth factors, remains within the oocyte

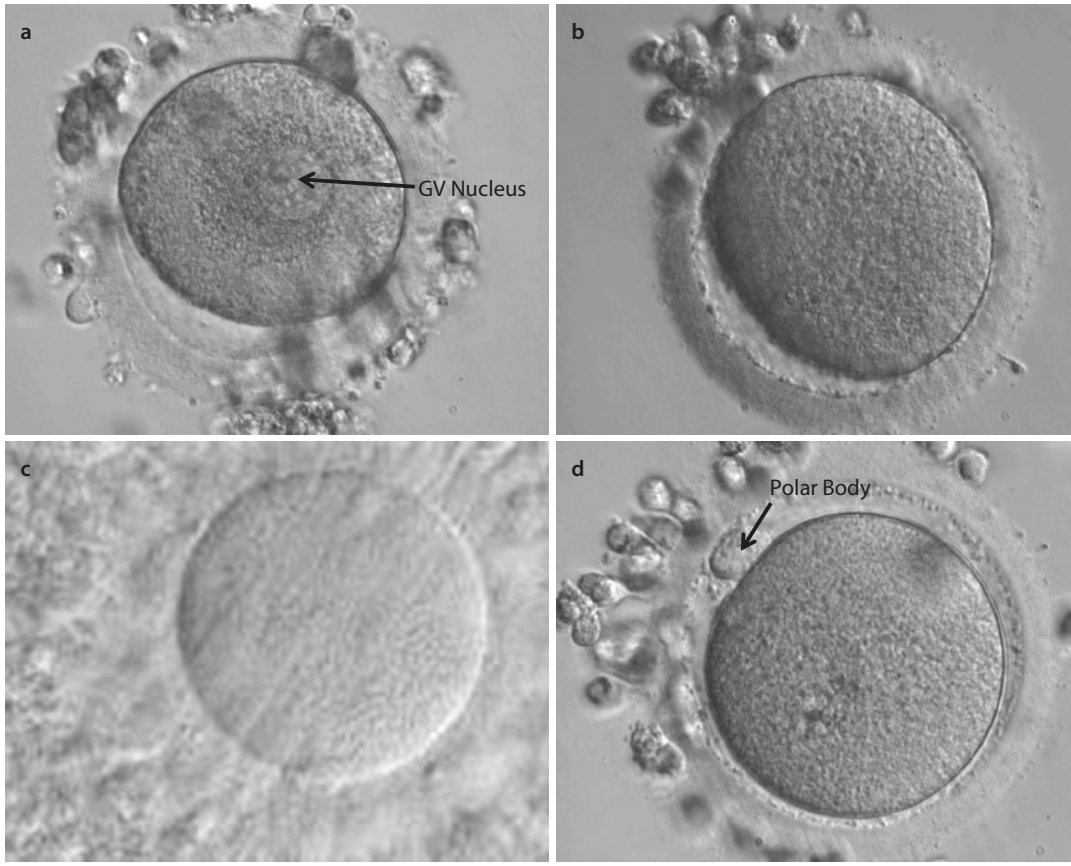


Fig. 2.3 Various stages of oocyte maturation: **a** immature oocyte in prophase I of meiosis I; **b** metaphase I oocyte; **c** cumulus:oocyte complex exhibiting morphology typically associated with mature oocytes at the time of ovulation; **d** mature metaphase II oocyte

2.3.2 Follicle Development

Folliculogenesis within the ovary is a very complex process with a high rate of follicle loss. Follicles periodically leave the resting primordial pool to join the growing pool but, in the absence of follicle-stimulating hormone (FSH), undergo atresia. After puberty, once the hypothalamus–pituitary–ovarian axis has been activated during the follicular phase, elevated FSH levels rescue the growing cohort of follicles. The ovarian paracrine signaling induces the continued growth of follicles from this cohort in a process called *initial recruitment* [23]. The recruited growing follicles, known as primary follicles, will subsequently grow into secondary and antral follicles. Figure 2.4 illustrates the different stages of follicle development. Ultimately, a single follicle will be selected from this cohort to become the dominant follicle. It will release a mature oocyte after exposure to increased levels of LH (luteinizing hormone). Almost 90% of follicles undergo apoptosis or programmed cell death without ever becoming meiotically competent [24].

Critical to this process is the interaction between the somatic cell components and the oocyte itself. Follicle growth from the primordial to the preovulatory stage can be divided into two distinct stages based on responsiveness to the gonadotropins, FSH and LH. The first stage is relatively slow in humans, taking over 120 days [13, 25] and is not directly dependent on gonadotropin levels. Key growth mediators at this early stage may include TGF-β(beta), activin, bone morphogenetic proteins (BMPs), anti-Müllerian hormone (AMH), insulin, estrogen, and androgens. Follicle and oocyte diameters increase, follicles growing in size from 30 to 40 μm in primordial follicles to 100–200 μm in pre-antral follicles (see Fig. 2.4). The single layer of

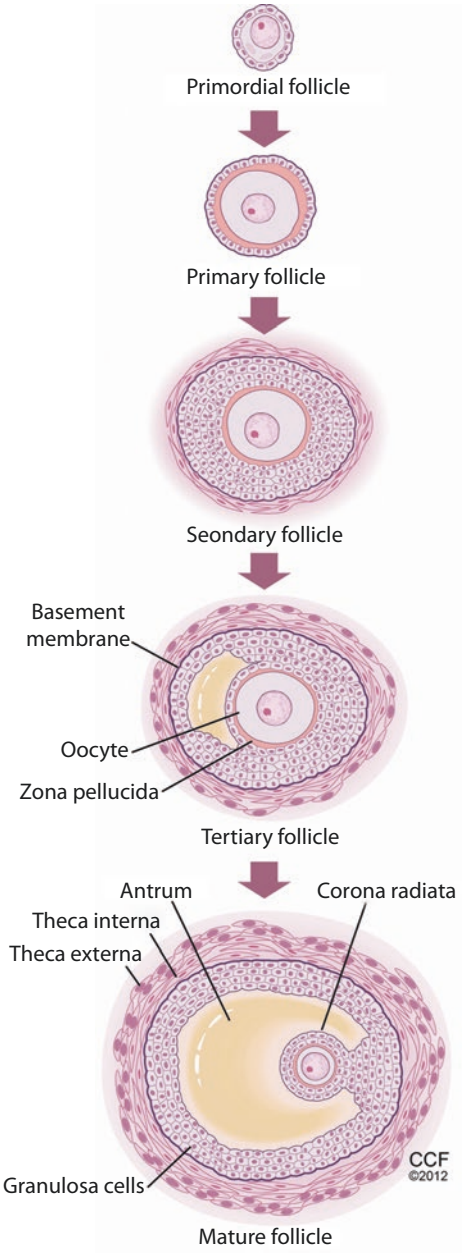


Fig. 2.4 Depicted is a sequential illustration of folliculogenesis within the human ovary. The process begins with the oocyte enclosed within single layer of granulosa cells known as the primordial follicle and ends with a fully developed multilayered follicle with antrum containing a secondary oocyte

squamous granulosa cells present in the primordial follicles starts to proliferate and the oocyte becomes surrounded by several layers of cuboidal granulosa cells. Precursor thecal cells are recruited from surrounding stroma and a basement membrane forms around the follicle.

The second stage of follicular growth is far more rapid. The follicle is now responsive to the gonadotropins, FSH and LH. Granulosa cell secretions result in the formation of a fluid-filled cavity or antrum. During the early antral stage of follicle development, follicle size increases from 200 μm to 2–5 mm. Follicle size increases to 20 mm by the time of oocyte ovulation.

The formation of a fluid-filled antrum and synthesis of steroid hormones mark the transition to the antral stage of follicle development. During this stage and with the influence of FSH, granulosa cells differentiate and become capable of aromatizing androgen, secreted by thecal cells, into estrogen. The local estrogenic environment, combined with high FSH levels, promotes further granulosa cell proliferation and an increase in FSH receptors. This, in turn, makes the follicles even more sensitive to FSH. The negative feedback of rising estrogen levels on the hypothalamus–pituitary axis limits FSH secretion. Thus, only follicles with increased FSH receptors will be able to continue to develop, while other follicles in the cohort will undergo atresia. It is through this mechanism that a single dominant follicle is selected. Continued growth of the selected follicle occurs despite the midcycle fall in FSH concentrations as a result of granulosa cells now acquiring LH receptors [26–28]. While granulosa cells of the early antral follicle are only responsive to FSH, late antral stage follicles become responsive to both FSH and LH and continue to secrete high levels of estradiol [29, 30]. The layers of specialized granulosa cells bordering the oocyte are known as cumulus cells, which are also called *corona radiata*. These cells not only support cytoplasmic maturation, but are pivotal in maintenance of meiotic arrest and induction of ovulation. The preovulatory follicle, also known as a Graafian follicle, measures over 18 mm in size, and oocyte diameter is close to its final size of about 120 μm [31]. The multilayer follicle is enclosed in a basement membrane that separates it from the underlying vascularized thecal cell layer.

2.3.3 Oocyte Growth

Coordinated growth of this diplotene-arrested oocyte and follicle is dependent on bidirectional communication between the oocyte and the surrounding granulosa cells [32, 33].

This communication occurs via gap junctions connecting the granulosa cells and the oocyte [33–39]. These membrane channels enable the sharing of small essential molecules, including inorganic ions, second messengers, small metabolites, and secreted paracrine factors that allow growth of both cell types [39–42].

The oocyte is unable to transport certain amino acids, carry out biosynthesis of cholesterol, or undergo glycolysis without a supply of the necessary factors by the granulosa cells. Similarly, evidence suggests that granulosa cell proliferation as well as select other metabolic processes require oocyte-derived secretions [43–46]. In vitro studies on cultured follicles demonstrate that severing of gap junctions and intercellular communications triggers premature ovulation and eventual degeneration of the released oocyte [47].

Gap junctions are comprised of a variety of connexin proteins [37]. The basic structure of connexins consists of four membrane-spanning domains, two extracellular loops, a cytoplasmic loop, and cytoplasm N-and C-termini. Different connexins contain different properties, providing increased complexity in the regulation of designated molecules. Gene knock-out experiments in the mouse model have identified specific gap junction proteins and their critical role in folliculogenesis. Absence of connexin-37 interferes with antral follicle formation [48, 49], while follicles in mice lacking the gene for connexin-43 arrest in the early pre-antral stage and are unable to produce meiotically competent oocytes [50]. Phosphorylation and several different protein kinases appear also to be associated with the activation and regulation of gap junctions [51, 52].

The oocyte is metabolically active from its early growth stage, synthesizing the maternal RNA pool necessary to support early embryonic events after fertilization. Oocytes synthesize over 400 different proteins. Shortly after follicle activation and entry into the growing pool, the oocyte secretes a thick glycoprotein coat [53, 54]. This matrix coat, known as the zona pellucida, encircles the oocyte and is composed of three zona pellucida proteins, ZP1, ZP2, and ZP3. Thickness of the zona pellucida increases as the oocyte grows, reaching about 15 μm. The zona pellucida plays an important role in protecting the oocyte/embryo as it traverses the reproductive tract. The zona mediates sperm binding, confers species specificity, and serves as a block to polyspermic fertilization. Release of cortical granules by the oocyte cytoplasm at the time of fertilization results in a hardening of the zona and deters additional sperm from penetrating into the oocyte.

2.3.4 Resumption of Meiosis and Ovulation

Meiotic progression of the oocyte is highly dependent on a delicate balance between factors keeping the oocyte in meiotic rest and factors promoting oocyte maturation [55–61]. Cyclic AMP is one of the intracellular signaling molecules that keep the oocyte in meiotic arrest. Cyclic AMP, produced by granulosa cells, is transported via gap junctions to the oocytes. As long as the cAMP threshold is maintained, meiosis is inhibited.

Meiotic competence is also linked to oocyte size, presumably because increasing volume corresponds to increasing cytoplasmic accumulation of synthesized proteins. Oocytes that measure less than 70–80 μm have lower rates of meiotic competence compared to 100 μm fully grown follicles, which are usually able to resume meiosis. Activation of maturation or M-phase promoting factor (MPF) is required to resume meiosis [62, 63]. MPF levels increase with oocyte growth and eventually reach a threshold, at which point the oocyte becomes *meiotically competent*. MPF is composed of a regulatory unit, cyclin B, and its protein kinase CDK1 (also called $p34^{cdc2}$).

The onset of the LH surge triggers a cascade of events culminating in the ovulation of the oocyte from the Graafian follicle and initiation of oocyte maturation. LH induces a shift in steroidogenesis by granulosa cells to progesterone [64]. Cumulus cell expansion just prior to ovulation results in the severing of cumulus: oocyte connections, thus reducing intracellular cAMP levels in the oocyte levels and therein reducing its inhibitory influence on meiosis. Subsequent MPF activation drives the oocyte towards meiosis, starting first with breakdown of the germinal vesicle (GVBD). Neosynthesis of cyclin B may be a rate-limiting step, accounting for the observed lag time between

resumption of meiosis and GVBD, as well as transition from metaphase I to metaphase II.

After the breakdown of the germinal vesicle, bivalents start to become organized and then align at the metaphase plate, forming a meiotic spindle. The oocyte remains arrested at the metaphase II stage until sperm penetration. An intracellular Ca^{2+} signal triggered by either the binding of sperm to oocyte receptors or else the release of a soluble sperm-derived factor during oocyte: sperm fusion initiates the destruction of endogenous cyclin [63]. The oocyte is now able to complete meiosis, with chromosome segregation beginning at the metaphase–anaphase transition. Defective chromosome segregation at this stage can lead to aneuploidy in the resulting egg and embryo.

2.4 Additional Regulatory Mechanisms

Clear maintenance of the primordial follicle pool, follicle recruitment, follicle atresia and selection of a dominant follicle, combined with controlling oocyte maturation, and synchronous growth of the follicular unit, is a complex process. In this section, we discuss a few of the most important ovarian paracrine factors involved in the coordination of events during folliculogenesis (Table 2.1).

wk pc weeks post coitum, *AMH* anti-Müllerian hormone, *BMP* bone morphogenetic protein, *GDF* growth differentiation factor, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *SCF* stem cell factor, *COX-2* cyclooxygenase isoform, *PGE2* prostaglandin E(2)

2.4.1 cAMP

As mentioned earlier, FSH and LH, working in concert, are two of the most important hormones involved in folliculogenesis. An important component of this system is the second messenger cAMP, which amplifies the signal induced by FSH and LH. This amplification allows for a much larger response to the FSH and LH hormones than they are able to create alone [65]. Two important sets of enzymes control intracellular cAMP homeostasis: adenylyl cyclases, which generate cAMP, and the phosphodiesterases (PDEs), which break down cAMP. Binding of FSH and LH to their respective receptors, along with adenylyl cyclase, activates the generation of cAMP from ATP in follicular granulose cells. The cAMP molecules activate a cascade of protein kinases, dissociating their catalytic component, which then works to phosphorylate transcription factors. These factors bind to DNA upstream of genes at positions known as cAMP response elements, regulating various follicular events such as the growth of the dominant follicle [66]. In the oocyte, a high concentration of this second messenger prevents meiosis.

2.4.2 TGF-β Growth Factor Family

Several key regulators involved in folliculogenesis belong to the transforming growth factor-β (TGF-β) family, many of which utilize gap junctions for communications. Important members of this group include AMH, growth differentiation factor-9 (GDF-9), BMPs, activin, and inhibin.

2.4.3 Anti-Müllerian Hormone

AMH is secreted by granulosa cells when a woman is reproductively active. In studies utilizing the mouse model, AMH has been shown to have two important roles during folliculogenesis. First, AMH inhibits the recruitment of additional primordial follicles when there is already a growing cohort of follicles. By this means, AMH prevents women from speeding too quickly through their oocyte reserve. Second, AMH decreases the response of these growing follicles to FSH. Studies in humans have suggested that each follicle has a unique FSH threshold that must be met in order to proceed to the preovulatory stage. AMH may influence how responsive a follicle is to the FSH surge during cyclic recruitment [67].

AMH has also been used as a measure of a woman's ovarian reserve [68]. AMH levels decrease along with the size of a woman's follicle pool. Concomitantly, lower AMH levels fail to adequately inhibit the primordial pool, and as a result there is an increase in the rate of depletion of the follicular reserve [69].

Table 2.1 Human oogenesis and folliculogenesis beginning with the primordial germ cells (key modulators and their points of action are shown)

Developmental event	Key regulators
Primordial germ cells	
3 wk pc	BMP4, BMP8b
Formation	c-KIT, SCF
Migration and proliferation	
Colonization of the forming gonad	
Post-migratory survival	
Oogonia	
Proliferation by mitosis	
Oocyte meiotic prophase 1	
Preleptotene: replication of DNA	
Leptotene: start of homologous pairing	
Zygotene	
Pairing of homologs	
Synapsis	
Pachytene	
Crossing over	
Recombination	
DNA repair	
Diplotene: meiotic arrest	cAMP
Primordial follicle	Activins, AMH, TGF β, BMPs, insulin, estrogen, androgens, c-KIT
16–18 wk pc	
Diplotene-arrested oocyte	
Single flat granulose cell layer	
Primary follicle	Activins, AMH
Cuboidal granulose cell layer	
Pre-antral secondary follicle	Activins, Inhibins, AMH
Late folliculogenesis	
Granulosa proliferation	GDF-9, BMP-15
Theca precursor formation	
Antral/Graafian follicle	Inhibins, AMH, cAMP, FSH/FSH receptor, LH/LH receptor
Formation of antrum	
Formation of preovulatory follicles and corpus luteum	
Cumulus expansion	LH signaling, PGE2
Meiotic resumption	MPF
Ovulation	COX-2, LH
Oocyte maturation and fertilization	Ca^{2+}

2.4.4 Growth Differentiation Factor-9 (GDF-9) and Bone Morphogenetic Proteins

BMPs play a variety of roles in oogenesis and are produced by a variety of cells. While little is known about specific BMP function in the human ovary, results from numerous animal studies have contributed to our understanding of the biological activities of BMPs during follicular development. Evidence from the rat model proposes that BMPs influence the primordial-primary transition as well as the subsequent transition to a secondary follicle. It has also been suggested that a drop in BMP levels may be indicative of dominant follicle selection [70, 71]. In the rat, thecal cells of secondary follicles have expressed BMP-4 and BMP-7, while their respective receptors expressed on granulosa cells [71]. They serve to modulate the actions of FSH on the synthesis of the essential steroids estradiol and progesterone [72].

Oocyte paracrine signaling is responsible for activating pathways involved in regulating cumulus cell differentiation. BMP-15 and GDF-9 are two factors secreted by the oocyte to control its local microenvironment and ultimately oocyte quality [45]. BMP-15 works alongside GDF-9 to activate signaling pathways responsible for the regulation of cumulus cell differentiation and maintenance of their phenotype [45]. The absence of these oocyte-specific factors has been demonstrated to result in sterility [73, 74]. During in vitro culture of ovarian cortical slices, GDF-9 supplementation was observed to increase the number of secondary oocytes present after 7 days of growth and also enhanced follicle density after 14 days of culture. GDF-9 may also serve to prevent atresia [75].

2.4.5 Activin and Inhibin

Activin and inhibin are produced by granulosa cells in the follicle and work in an antagonistic relationship. Activin, produced by many different tissues, including the gonad and anterior pituitary, stimulates the release of FSH by acting as a transcription factor activator of the FSHβ-subunit gene [76]. Meanwhile, inhibin hinders the secretion of FSH from the pituitary, balancing the actions of activin. While it is believed that inhibin plays an important role in the production of steroids and gonadotropins, little else is known about its role in the recruitment and differentiation of thecal cells [77].

2.4.6 c-Kit and Kit Ligand

Another important signaling pathway involved in the regulation of primordial follicles involves the c-Kit receptor and its granulosa cell produced ligand (frequently referred to as KL, stem cell factor or SCF) [78]. Little is known about the specific role of this receptor/ligand combination in the early development of follicles. The presence of the mRNA encoding for c-Kit and KL has been detected in early antral follicles present in human ovarian tissue, oocytes, and granulosa cells [78]. Much of what is known about c-Kit comes from research in the mouse model. Here, c-Kit and KL play a role in PGC survival, activation, migration, proliferation of granulosa cells, recruitment of theca cells, maintenance of meiotic competence, and the development of follicles [79].

2.5 Future Directions and Challenges

Increasing our understanding of the complex regulation of ovarian folliculogenesis and the interactions between the oocyte and granulosa-thecal cell layers has contributed significantly to the advancement of infertility treatment. Medications and ovarian stimulation regimens have been designed which permit the manipulation of a women's menstrual cycle, resulting in the production of multiple mature competent oocytes. This, combined with advances in our ability to treat male factor infertility, has dramatically altered pregnancy outcomes with in vitro fertilization over the last two decades.

One hurdle that has been challenging to overcome has been poor ovarian reserve. The continuous loss and eventual elimination of a woman's follicle pool through accelerated atresia is considered to be the impetus leading to menopause. This is based on the belief set forth over 50 years ago that oogenesis cannot occur in the adult ovary and so, at birth, the female ovary contains a finite oocyte pool [80]. Recently, exciting data have emerged that question this basic established dogma [81, 82]. The intriguing study, first presented in

2004 by Johnson and colleagues, questions the concept of a "non-renewing oocyte pool" [83]. An increasing body of evidence indicates that oogenesis may in fact occur in the adult mammalian ovary [82, 84, 85]. The potential existence of germ cells in the adult ovary and the development of techniques to manipulate such cells may open up new avenues for fertility treatment [86].

Another challenge in reproductive medicine has been fertility preservation for young women diagnosed with cancer. Radiation and chemotherapy during cancer treatment can result in fertility loss through damage to the ovarian follicle reserve. Cryopreservation of ovarian tissue offers hope to patients, but how best to use this tissue to restore fertility is still problematic. Ovarian tissue transplant after the patient is in remission has been met with limited success. Additionally, the possibility of reintroducing the cancer always remains. An alternate solution is the isolation of ovarian follicles from cryopreserved tissue, followed by in vitro maturation of the follicles [87]. The long time interval necessary for human follicle growth from the primordial to the preovulatory stage (~120 days) and the intricate signaling mechanisms necessary for proper follicle growth are quite difficult to mimic in vitro. Maintenance of the spheroid follicle architecture during prolonged growth in conventional 2D culture systems is not possible. Moreover, as the follicle flattens, oocyte:granulosa cell connections are disrupted and critical bi-directional communications between the oocyte and its surrounding somatic cells are lost. Design of 3D follicle culture models has therefore been the focus of much research [87–94].

The challenge of maturing human follicles in vitro and creating competent oocytes may take years to accomplish and will be fueled by our growing understanding of the complex interactions between the oocyte and its supporting granulosa and thecal cell components. The successful culture of human follicles in vitro will ultimately herald a new age in reproductive medicine and the treatment of infertility.

2.6 Male Gametogenesis

Male gametogenesis or spermatogenesis is a temporal event whereby relatively undifferentiated germ cell called spermatogonia slowly evolves into a highly specialized testicular spermatozoa over a span of several weeks. Spermatogenesis takes place in the testis. Spermatozoa are transported to the epididymis where they attain maturity and motility before being released into the seminal ejaculate along with the other accessory gland secretions. In this section we will cover the following topics:

1. Organization of the testis, describe the structure of the testis and function mainly the production of hormone and the spermatozoa and the role of supporting cells, i.e. Leydig cells and the Sertoli cells.
2. Define the terms and explain the process of spermatogenesis, the main steps involved explain the types of spermatogonia, spermatocytogenesis and the processes of mitosis and meiosis, spermiogenesis, nuclear development, release of spermatozoa in the lumen or spermiation. Explain what is the cycle or wave of seminiferous epithelium and efficiency of spermatogenesis.
3. Describe the structure of spermatozoa.
4. Explain the regulation of spermatogenesis, difference between the intrinsic regulation and extrinsic influences on spermatogenesis.
5. Epididymis and its role in storage and maturation of sperm.
6. Process of sperm entry into cervical mucus, physiological process of capacitation and acrosome reaction and subsequent fertilization.
7. The new 2010 World Health Organization Guidelines and concluding remarks

Clinical Case

A 41-year-old male presents with his partner for infertility of 1 year duration. He is referred because his sperm count is low and his FSH is high but his testosterone is normal. He asks if there is anything you can do.

2.7 Organization of the Testis

The testes are ellipsoid in shape, measuring 2.5 × 4 cm in diameter and engulfed by a capsule (tunica albuginea) of strong connective tissue [95]. Along its posterior border, the testis is loosely connected to the epididymis, which gives rise to the vas deferens at its lower pole [96]. The testis has two main functions: it produces hormones, in particular testosterone, and it produces the male gamete—the spermatozoa. The spermatozoa express unique

antigens that are not formed until puberty. The blood–testis barrier develops as these autoantigens develop. The blood–testis barrier makes the testis an immunologically privileged site. The testis is incompletely divided into a series of lobules. Most of the volume of the testis is made up of seminiferous tubules, which are looped or blind-ended and packed in connective tissue within the confines of the fibrous septae (see Fig. 2.5). The fibrous septae divide the parenchyma into about 370 conical lobules consisting of the seminiferous tubules and the intertubular tissue. The seminiferous tubules are separated by groups of Leydig cells, blood vessels, lymphatics, and nerves. The seminiferous tubules are the site of sperm production. The wall of each tubule is made up of myoid cells of limited contractility and also of fibrous tissue. Each seminiferous tubule is about 180 μm in diameter, the height of the germinal epithelium measures 80 μm, and the thickness of the peritubular tissue is about 8 μm. The germinal epithelium consists of cells at different stages of development located within the invaginations of Sertoli cells, namely, spermatogonia, primary and secondary spermatocytes, and spermatids. Both ends of the seminiferous tubules open into the spaces of the rete testis. The fluid secreted by the seminiferous tubules is collected in the rete testis and delivered in the ductal system of the epididymis.

2.7.1 Supporting Cells

The supporting cells of the testes refer to cells that are part of the cellular development that leads to a mature sperm. The two most important cells are the Leydig and Sertoli cells.

2.7.2 Leydig Cells

The Leydig cells are irregularly shaped cells with granular cytoplasm present individually or more often in groups within the connective tissue [97, 98]. Leydig cells are the prime source of the male sex hormone testosterone. The pituitary hormone, luteinizing hormone (LH), acts on Leydig cells to

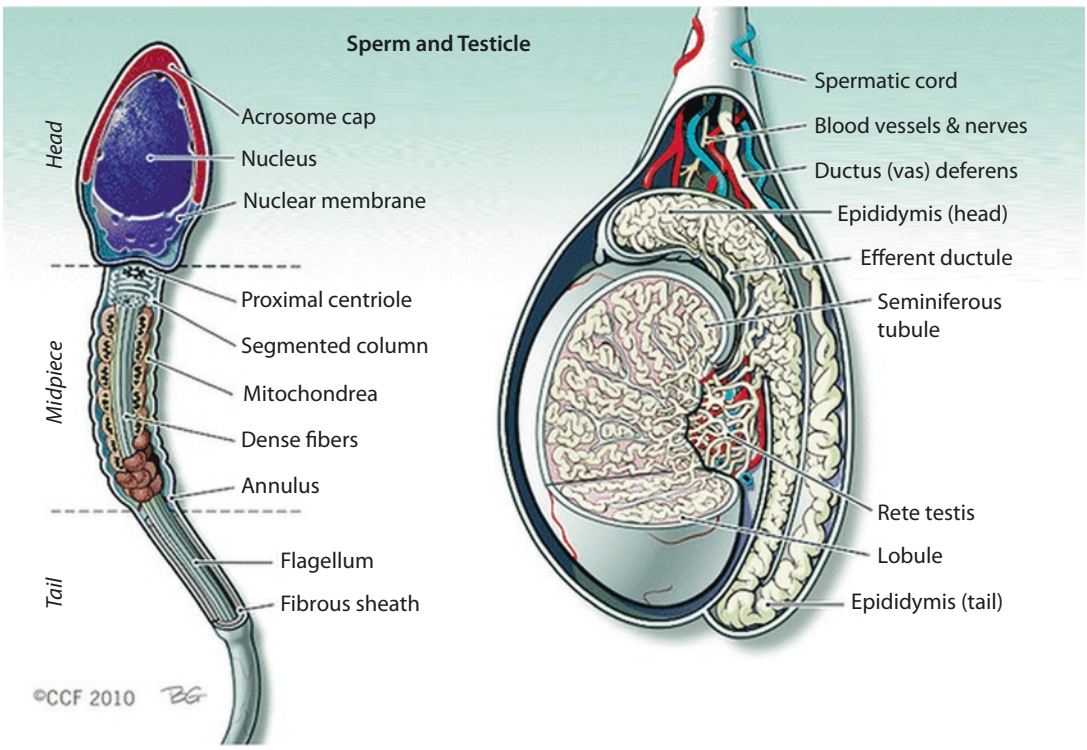


Fig. 2.5 The human testis and the epididymis. The testis shows the tunica vaginalis and tunica albuginea, seminiferous tubule septae, rete testis, and the overlying head, body, and tail of the epididymis. To the *left* is a diagrammatic representation of a fully mature spermatozoon (All Rights Reserved Sperm Chromatin, ed. Zini A and Agarwal A, Biological and Clinical applications in Male Infertility and Assisted Reproduction, Springer Science + Business Media 2011)

stimulate the production of testosterone. This acts as a negative "feedback" on the pituitary to suppress or modulate further LH secretion. Compared with the testosterone levels in the blood, the intratesticular concentration of testosterone is many times higher, especially near the basement membrane of the seminiferous tubule.

2.8 The Sertoli Cell

The seminiferous tubules are lined with highly specialized Sertoli cells that rest on the tubular basement membrane and extend into the lumen with a complex ramification of cytoplasm (see Fig. 2.6). Spermatozoa are produced at puberty,

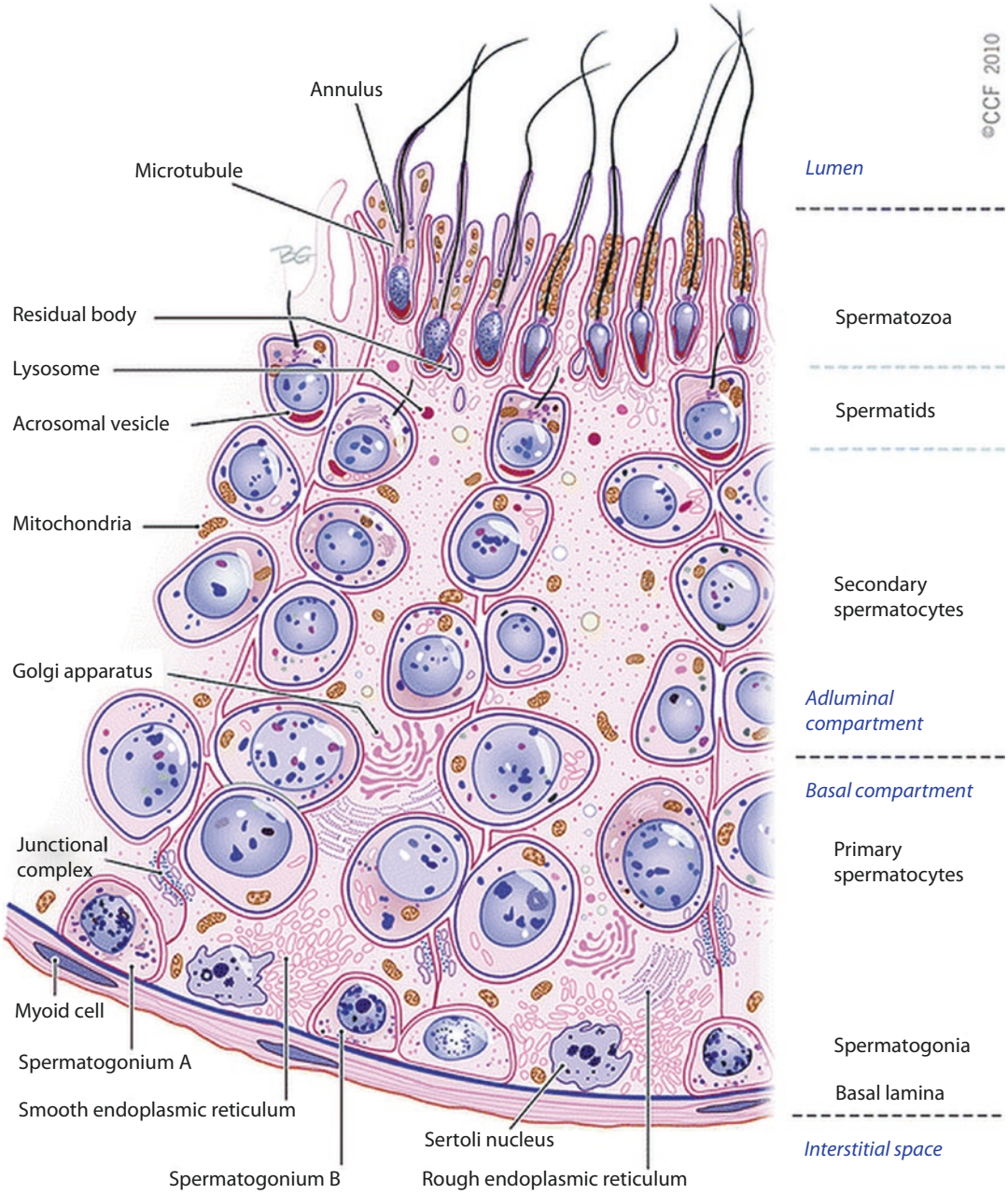


Fig. 2.6 Section of the germinal epithelium in the seminiferous tubule. Sertoli cells divide the germinal epithelium into a basal and adluminal compartment, via the Sertoli cell. Spermatozoa are released into the lumen (All Rights Reserved Sperm Chromatin, ed. Zini A and Agarwal A, Biological and Clinical applications in Male Infertility and Assisted Reproduction, Springer Science + Business Media 2011)

but are not recognized by the immune system that develops during the first year of life. The seminiferous tubule space is divided into basal (basement membrane) and luminal (lumen) compartments by strong intercellular junctional complexes called "tight junctions." These anatomic arrangements, complemented by closely aligned myoid cells that surround the seminiferous tubule, form the basis for the blood–testis barrier. The blood–testis barrier provides a microenvironment for spermatogenesis to occur in an immunologically privileged site. Sertoli cells serve as "nurse" cells for spermatogenesis, nourishing germ cells as they develop. These also participate in germ cell phagocytosis. Multiple sites of communication exist between Sertoli cells and developing germ cells for the maintenance of spermatogenesis within an appropriate hormonal milieu. FSH binds to the high-affinity FSH receptors found on Sertoli cells signaling the secretion of androgen-binding protein. High levels of androgens are also present within the seminiferous tubule.

The two most important hormones secreted by the Sertoli cells are AMH and inhibin. AMH is a critical component of embryonic development and is involved in the regression of the Müllerian ducts. Inhibin is a key macromolecule in pituitary FSH regulation. Some of the functions of the Sertoli cell are (1) maintenance of integrity of seminiferous epithelium, (2) compartmentalization of seminiferous epithelium, (3) secretion of fluid to form tubular lumen to transport sperm within the duct, (4) participation in spermiation, (5) phagocytosis and elimination of cytoplasm, (6) delivery of nutrients to germ cells, (7) steroidogenesis and steroid metabolism, (8) movement of cells within the epithelium, (9) secretion of inhibin and androgen-binding protein, (10) regulation of spermatogenic cycle, and (11) providing a target for hormones LH, FSH, and testosterone receptors present on Sertoli cells.

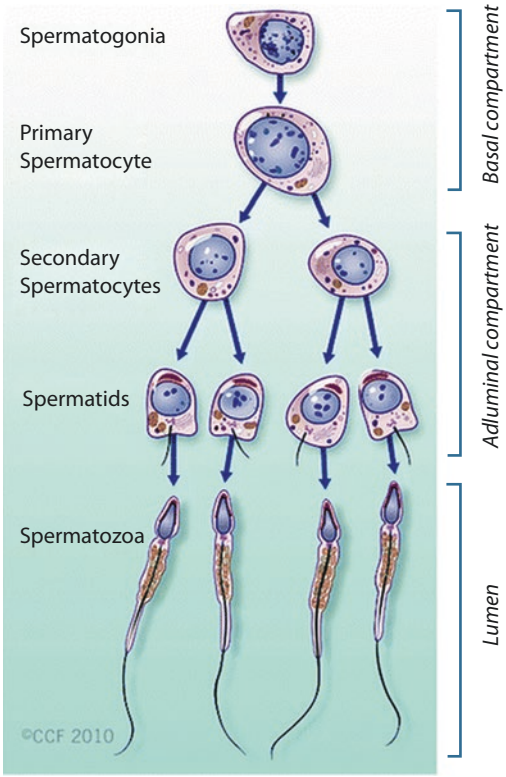
2.9 Spermatogenesis

The process of differentiation of a spermatogonium into a spermatid is known as spermatogenesis. It is a complex, temporal event whereby primitive, totipotent stem cells divide to either renew them or produce daughter cells that become into a specialized testicular spermatozoa over a span of weeks (see Fig. 2.7). Spermatogenesis

Fig. 2.7 A diagrammatic representation of major events in the life of a sperm involving spermatogenesis, spermiogenesis, and spermiation during which the developing germ cells undergo mitotic and meiotic division to reduce the chromosome content (All Rights Reserved Sperm Chromatin, ed. Zini A and Agarwal A, Biological and Clinical applications in Male Infertility and Assisted Reproduction, Springer Science + Business Media 2011)

Major Events in the Life of a Sperm

- Spermatogenesis
- Mitosis
- Meiosis
- Spermiogenesis
  - » Head
  - » Midpiece
  - » Tail
- Capacitation
- Lifespan of a spermatozoa
  - » Puberty through life
  - » 30 x 10^6 per day
  - » 60 to 75 days for sperm production
  - » 10 to 14 days transport (epididymis)
  - » 20 to 100 million per milliliter of ejaculate



involves both mitotic and meiotic proliferation as well as extensive cell remodeling. Spermatogenesis can be divided into three major phases: (1) proliferation and differentiation of spermatogonia, (2) meiosis, and (3) spermiogenesis, a complex metamorphosis that transforms round spermatids arising from the final division of meiosis into a complex structure called the *spermatozoon*. In humans, the process of spermatogenesis starts at puberty and continues throughout the entire life span of the individual. It takes place in the lumen of seminiferous tubules. In fact, 90% of the testis volume is determined by the seminiferous tubules and their constituent germ cells at various stages of development. Once the gonocytes have differentiated into fetal spermatogonia, an active process of mitotic replication is initiated very early in the embryonic development. This appears to be under FSH control and develops the baseline number of precursor cells of the testicle.

Within the seminiferous tubule, germ cells are arranged in a highly ordered sequence from the basement membrane to the lumen. Spermatogonia lie directly on the basement membrane, followed by primary spermatocytes, secondary spermatocytes, and spermatids as they progress toward the tubule lumen. The tight junction barrier supports spermatogonia and early spermatocytes within the basal compartment and all subsequent germ cells within the luminal compartment.

Spermatogenesis can also be disturbed by a number of external factors, including nutrition, therapeutic drugs, increased scrotal temperature, and X-radiation.

2.9.1 Types of Spermatogonia

Spermatogonia represent a population of cells that divide by mitosis, providing a renewing stem cell population as well as spermatogonia that are committed to enter the meiotic process. Germ cells are staged by their morphologic appearance; there are dark type A (A_{dark}), pale type A (A_{pale}), and type B spermatogonia; primary spermatocytes (preleptotene, leptotene, zygotene, and pachytene); secondary spermatocytes; and spermatids (Sa, Sb, Sc, Sd_1, and Sd_2). Other proliferative spermatogonia include A_{paired} (A_{pr}), resulting from dividing $A_{isolated}$ (A_{is}), and subsequently dividing to form $A_{aligned}$ (A_{al}). Differentiated spermatogonia include type A1, A2, A3, A4, intermediate, and type B, each a result of the cellular division of the previous type. In humans, four spermatogonial cell types have been identified; these are A_{long}, A_{dark}, A_{pale}, and B [99, 100]. In the rat, type $A_{isolated}$ (A_{is}) is believed to be the stem cell [101]; however, it is not clear which human type A spermatogonia is the stem cell. Some investigators have proposed that the type A_{dark} spermatogonia represent the reserve or nonproliferative spermatogonial population that gives rise to A_{pale} [100]. Type B spermatogonia possess considerably more chromatin within the inner nuclear envelope than intermediate or type A spermatogonia (see Fig. 2.8). Type B spermatogonia represent the cells that differentiate and enter into the process of meiosis, where they are called primary spermatocytes [100]. They are the differential precursors to preleptotene spermatocytes. This last mitotic division helps maintain a pool of stem cells so that the process can continue indefinitely.

Spermatogonia do not separate completely after meiosis but remain joined by intercellular bridges, which persist throughout all stages of spermatogenesis and are thought to facilitate biochemical interactions, allowing synchrony of germ cell maturation [102].

2.9.2 Spermatocytogenesis

The purpose of spermatogenesis is to produce genetic material necessary for the replication of the species through mitosis and meiosis. Spermatocytogenesis takes place in the basal compartment. Primary spermatocytes enter the first meiotic division to form secondary spermatocytes. The prophase of the first meiotic division is very long; thus, the primary spermatocyte has the longest life span. Secondary spermatocytes undergo the second meiotic division to produce spermatids. Secondary spermatocytes are short-lived (1.1–1.7 days).

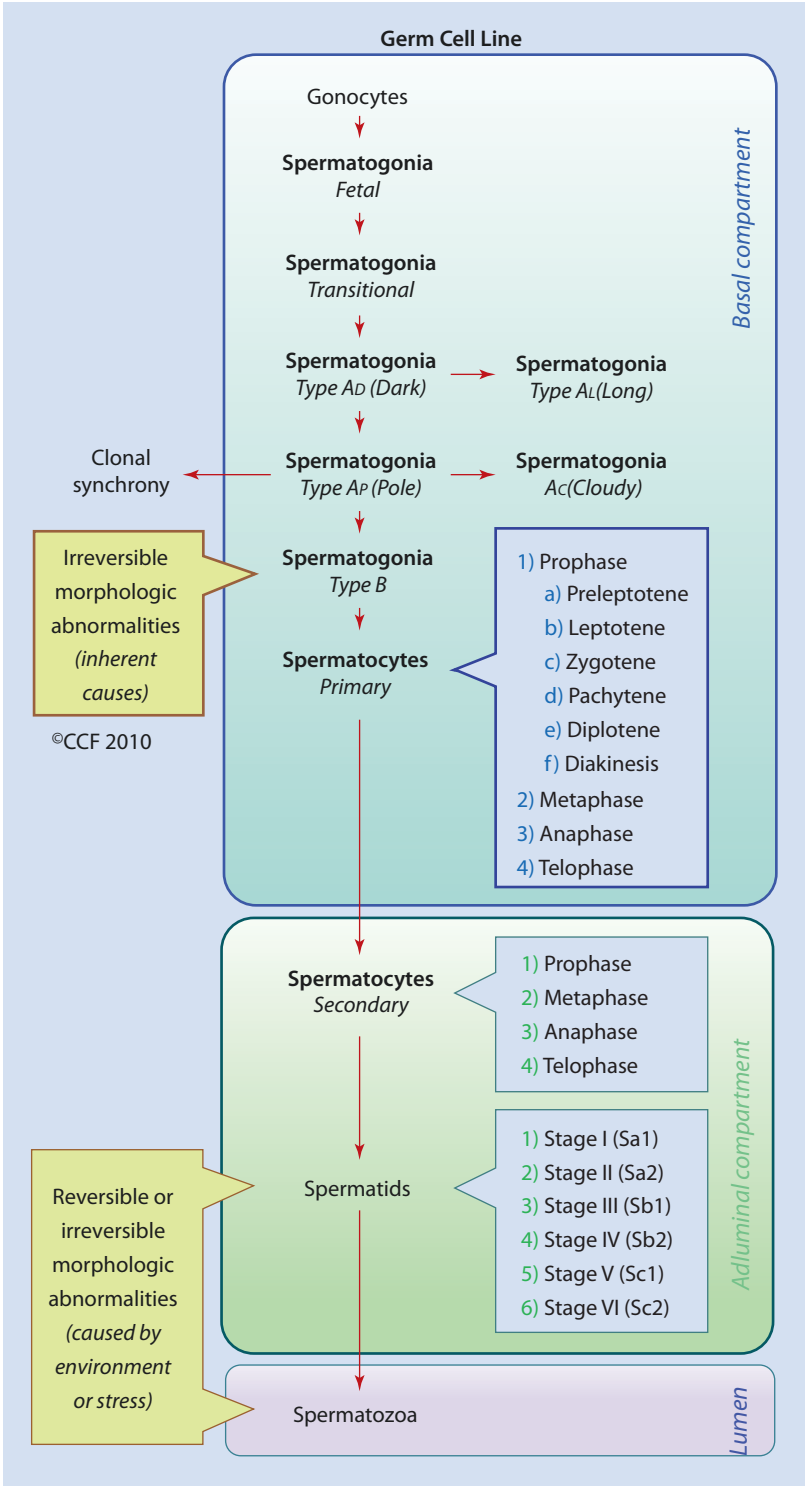


Fig. 2.8 Schematic representation of the development of a diploid undifferentiated germ cell into a fully functional haploid spermatozoon along the basal to the adluminal compartment and final release into the lumen. Different steps in the development of primary, secondary, and spermatid stages are also shown and the irreversible and reversible morphological abnormalities that may occur during various stages of spermatogenesis (All Rights Reserved Sperm Chromatin, ed. Zini A and Agarwal A, Biological and Clinical applications in Male Infertility and Assisted Reproduction, Springer Science + Business Media 2011)

2.9.3 Mitosis

Mitosis involves proliferation and maintenance of spermatogonia. It is a precise, well-orchestrated sequence of events involving duplication of the genetic material (chromosomes), breakdown of the nuclear envelope, and equal division of the chromosomes and cytoplasm into two daughter cells [103]. DNA is also spatially organized into loop domains on which specific regulatory proteins interact during cellular replication [103–105]. The mitotic phase involves spermatogonia (types A and B) and primary spermatocytes (spermatocytes I). Developing germ cells interconnected by intracellular bridges produce the primary spermatocyte through a series of mitotic divisions. Once the baseline number of spermatogonia is established after puberty, the mitotic component will proceed in order to continue to provide precursor cells and to start the process of differentiation and maturation.

2.9.4 Meiosis

Meiosis is a complex process with specific regulatory mechanisms of its own [106]. The process commences when type B spermatogonia lose their contact with the basement membrane to form preleptotene primary spermatocytes. Thus, each primary spermatocyte can theoretically yield four spermatids, although fewer actually result, because some germ cells are lost due to the complexity of meiosis. The primary spermatocytes are the largest germ cells of the germinal epithelium. Meiosis is characterized by prophase, metaphase, anaphase, and telophase. In this, two successive cell divisions yield four haploid spermatids from one diploid primary spermatocyte. As a consequence, the daughter cells contain only half of the chromosome content of the parent cell. After the first meiotic division (reduction division), each daughter cell contains one partner of the homologous chromosome pair, and they are called *secondary spermatocytes*. These cells rapidly enter the second meiotic division (equational division), in which the chromatids then separate at the centromere to yield haploid early round spermatids. Meiosis assures genetic diversity and involves primary and secondary spermatocytes, which give rise to spermatids.

The meiotic phase involves primary spermatocytes until spermatids are formed; during this process, chromosome pairing, crossover, and genetic exchange are accomplished until a new genome is determined. In turn, a post-meiotic phase involving spermatids all the way up to spermatozoa develops, ending in the formation of the specialized cell.

2.9.5 Spermiogenesis

Spermiogenesis is a process during which the morphologic changes occur during the differentiation of the spermatid into the spermatozoon. It begins once the process of meiosis is completed. Six different stages have been described in the process of spermatid maturation in humans, as $S_{a\text{-}1}$ and $S_{a\text{-}2}$, $S_{b\text{-}1}$ and $S_{b\text{-}2}$, and $S_{c\text{-}1}$ and $S_{c\text{-}2}$(see Fig. 2.9). Each of these stages can be identified by the morphologic characteristics. During the $S_{a\text{-}1}$ stage, both the Golgi complex and mitochondria are well developed and differentiated, the acrosomal vesicle appears, the chromatid body develops in one pole of the cell opposite from the acrosomal vesicle, and the proximal centriole and the axial filament appear. During $S_{b\text{-}1}$ and $S_{b\text{-}2}$ stages, acrosome formation is completed, the intermediate piece is formed, and the tail develops. This process is completed during the S_c stages.

2.9.6 Nuclear Development

During spermatogenesis, a number of changes occur to the nucleus and its contents. The nucleus of the spermatozoa elongates and flattens from step 1 to step 8 of spermiogenesis [107], giving the head its characteristic oval shape. The necessity of this compaction has been debated, but it is widely believed to facilitate the penetration of the oocyte and to help optimize the swimming capability of the spermatozoa [108].

The compaction of the nucleus includes modification of the chromatin material. During the last post-meiotic phase of spermiogenesis, the histone molecules, around which the DNA is organized, are converted into transitional proteins, which are then converted to protamines [109]. Protamines contain large amounts of cysteine, which aid in the formation of protamine disulfide bonds as the

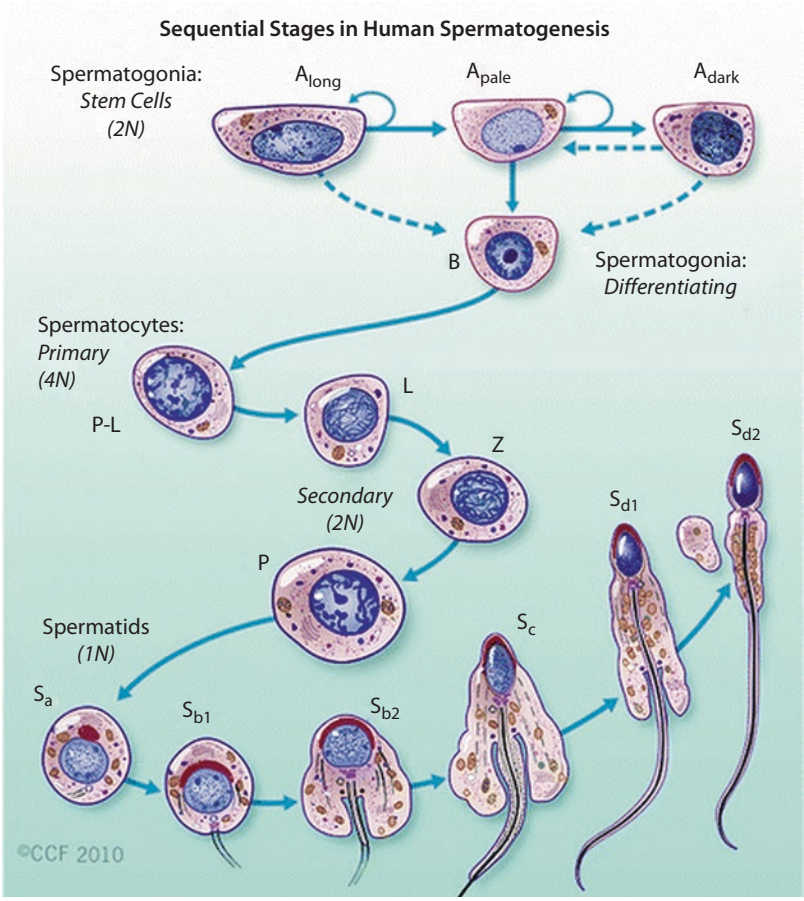


Fig. 2.9 Differentiation of a human diploid germ cell into a fully functional spermatozoon (All Rights Reserved Sperm Chromatin, ed. Zini A and Agarwal A, Biological and Clinical applications in Male Infertility and Assisted Reproduction, Springer Science + Business Media 2011)

sperm matures in the epididymis [110–112]. Within 2–4 h of fertilization, the protamines in the chromatin of the spermatozoa are replaced by histones from the oocyte.

2.9.7 Spermiation

The process whereby a mature spermatid frees itself from the Sertoli cell and enters the lumen of the tubule as a spermatozoon is known as *spermiation*. Spermiation involves the active participation of the Sertoli cell. This may also involve actual cell movement as the spermatids advance towards the lumen of the seminiferous tubules [112]. The mature spermatids close their intracellular bridges, disconnect their contact to the germinal epithelium, and become free cells called *spermatozoa*. Portions of the cytoplasm of the Sertoli cell known as *cytoplasmic droplet* may remain as part of the spermatozoon during the process of spermiation. This is a morphological feature present on the immature sperm in semen [113].

2.9.8 The Cycle or Wave of Seminiferous Epithelium

A cycle of spermatogenesis involves the division of primitive spermatogonial stem cells into subsequent germ cell types through the process of meiosis. Type A spermatogonial divisions occur at a shorter time interval than the entire process of spermatogenesis. Therefore, at any given time, several cycles of spermatogenesis coexist within the germinal epithelium. In humans, spermatocyte maturation takes 25.3 days, spermiogenesis 21.6 days, and the total estimated time for spermatogenesis is 74 days. Spermatogenesis is not random throughout the seminiferous epithelium. Germ cells are localized in spatial units referred to as *stages* and represent consistent associations of

germ cell steps [114, 115]. In rodent spermatogenesis, one stage can be found in a cross-section of seminiferous tubule.

Each stage is recognized by development of the acrosome, meiotic divisions and shape of the nucleus, and release of the sperm into lumen of the seminiferous tubule. The cycle of spermatogenesis can be identified for each species, but the duration of the cycle varies for each species [100]. The stages of spermatogenesis are sequentially arranged along the length of the tubule. This arrangement of the stages of spermatogenesis is such that it results in a "wave of spermatogenesis" along the tubule. This wave is in space but the cycle is in time [115]. Along the length of the seminiferous tubule there are only certain cross-sections where spermatozoa are released. In the rat, all stages are involved in spermatogenesis, but spermatozoa are released only in stage VIII.

The stages of spermatogenesis are organized spatially as well as in time [115]. Thus, a position in the tubule that is occupied by cells comprising stage I will become stage II, followed by stage III, until the cycle repeats. In humans, the duration of the cycle is 16 days, and the progression from spermatogonia to spermatozoa takes 70 days, or four and a half cycles of the seminiferous cycle. During spermatogenesis, cytoplasmic bridges link cohorts of germ cells that are at the same point in development, and these cells pass through the process together. Groups of such cells at different stages can be observed histologically on cross-section, and many germ cell cohorts are seen only in association with certain other germ cells. This has led to the description of six stages of the seminiferous tubule epithelium in men. To add another level of complexity, the steps of the spermatogenic cycle within the space of seminiferous tubules demonstrate a specific spatial organization, termed *spermatogenic waves*. In humans, this wave appears to describe a spiral cellular arrangement as one progresses down the tubule. This spatial arrangement probably exists to ensure that sperm production is a continuous rather than a pulsatile process.

2.9.9 Efficiency of Spermatogenesis

Spermatogenic efficiency varies between different species but appears to be relatively constant in man. The time for the differentiation of a spermatogonium into a mature spermatid is estimated to be 70 ± 4 days [116]. In comparison to animals, the spermatogenetic efficiency in man is poor. The daily rate of spermatozoa production is calculated at 3–4 million per gram of testicular tissue [117]. A higher number of spermatozoa should be expected in the ejaculate than the 20 million/mL described by the World Health Organization manual in 1999 [118] and the 15 million/mL in 2010 [119]. A majority of the cells developed (>75%) are lost as a result of apoptosis or degeneration; of the remaining, more than half are abnormal. Therefore, only about 12% of the spermatogenetic potential is available for reproduction [120]. An age-related reduction in daily sperm production in men, which is associated with a loss of Sertoli cells, is also seen.

2.10 Structure of the Spermatozoa

Spermatozoa are highly specialized and condensed cells that neither grow nor divide. A spermatozoon consists of the head, which contains the paternal material (DNA) and the acrosome, the neck, and the tail, which provides motility. The spermatozoon is endowed with a large nucleus but lacks the large cytoplasm characteristic of most body cells. Men are unique in the morphologic heterogeneity of the ejaculate.

2.10.1 Head

The head of the spermatozoa is ovular, measuring about 4.0–5.5 μm in length and 2.5–3.5 μm in width. The normal length-to-width ratio is about 1.50–1.70 [118]. Under bright field illumination, the most commonly observed aberrations include head shape/size defects, including large, small, tapering, piriform, amorphous, vacuolated (>20% of the head surface occupied by unstained vacuolar areas), and double heads, or any combination [118].

The head also contains the acrosome, which is a cap-like structure represented by the Golgi complex and covers about two thirds of the anterior head area [118]. The apical thickening seen in many other species is missing; however, the acrosome shows a uniform thickness/thinning toward the equatorial segment and covers about 40–70% of the sperm head. During fertilization of the egg, the fusion of the outer acrosomal membrane with

the plasma membrane at multiple sites releases the acrosomal enzymes at the time of the acrosome reaction. The anterior half of the head is devoid of the plasma and outer acrosomal membrane and is covered only by the inner acrosomal membrane. The posterior region of the sperm head is covered by a single membrane called the *postnuclear cap*. The overlap of the acrosome and the postnuclear cap results in an equatorial segment which does not participate in the acrosome reaction.

2.10.2 Neck

This forms a junction between head and tail. It is fragile and the presence of decapitated spermatozoa is a common abnormality.

2.10.3 Tail

The sperm tail is 40–50 μm long and arises at the spermatid stage. The tail contains the motility apparatus of the spermatozoa and propels by waves generated in the neck region and pass along the tail like a whiplash. It is formed during spermiogenesis due to the differentiation of the centriole into three parts, which can be clearly observed under scanning electron microscopy: the midpiece, the main or principal piece, and the endpiece. The mitochondria are organized helically around the midpiece. The mitochondrial sheath of the midpiece is relatively short but slightly longer than the combined length of the head and neck. An axial core comprising of two central fibrils is surrounded by a concentric ring of nine double fibrils, which continue to the end of the tail. The additional outer ring comprises nine coarse fibrils. The principal piece, the longest part of the tail, provides most of the propellant machinery. The coarse nine fibrils of the outer ring diminish in thickness and finally disappear, leaving only the inner fibrils in the axial core for most of the length of the principal piece. The fibrils of the principal piece are surrounded by a fibrous tail sheath, which consist of branching and anastomosing semicircular strands or ribs held together by their attachment to two bands that run lengthwise along opposite sides of the tail. The tail terminates in the endpiece with a length of 4–10 μm and a diameter of less than 1 μm. The small diameter is due to the absence of the outer fibrous sheath and a distal fading of the microtubules. Common tail abnormalities include tail absence, bent tails, distended or irregular/bent midpiece, abnormally thin midpiece (no mitochondrial sheath), and short, multiple, hairpin, and broken tails, tails of irregular width, coiling tails with terminal droplets, or any of these combinations [118].

2.11 Regulation of Spermatogenesis

The spermatogenic process is maintained by different intrinsic and extrinsic influences.

2.11.1 Intrinsic Regulation

Leydig cells secrete hormone (testosterone), neurotransmitters (neuroendocrine substances), and growth factors to neighboring Leydig cells, blood vessels, lamina propria of the seminiferous tubules, and Sertoli cells [97, 120, 121]. They help maintain the nutrition of the Sertoli cells and the cells of the peritubular tissue and influence the contractility of myofibroblasts, thereby regulating the peristaltic movements of seminiferous tubules and transportation of the spermatozoa. Leydig cells also help in the regulation of blood flow in the intertubular microvasculature [95]. In addition, different growth factors are delivered from Sertoli cells and various germ cells participating in a complicated regulation of cell functions and developmental processes of germ cells. Altogether these factors represent an independent intratesticular regulation of spermatogenesis.

2.11.2 Extrinsic Influences

The local regulation of spermatogenesis is controlled by the hypothalamus and hypophysis. Pulsatile secretion of gonadotropin-releasing hormone of the hypothalamus initiates the release of LH from the hypophysis; in response, the Leydig cells produce testosterone. Testosterone not only influences spermatogenesis, but is also distributed throughout the body. It thus provides feedback to the hypophysis that regulates the secretory activity of Leydig cells. Stimulation of Sertoli cells by FSH is necessary for maturation of the germ cells.

Complete qualitative spermatogenesis requires both FSH and LH. The interaction between the endocrine and paracrine mechanisms determines the functions within the testis [122]. Inhibin secreted by Sertoli cells functions in the feedback mechanism directed to the hypophysis. Thus, both growth and differentiation of testicular germ cells involves a series of complex interactions both between somatic and germinal elements [123, 124].

2.12 The Epididymis

The epididymis lies along the dorsolateral border of each testis. It is made up of the efferent ductules, which emanate from the rete testis, and the epididymal ducts. The epididymis opens into the vas deferens, which then passes through the inguinal canal into the peritoneal cavity and opens into the urethra adjacent to the prostate. It is divided into three functionally distinct regions: head, body, and tail or caput epididymis, corpus epididymis, and cauda epididymis. Their functions can be described simplistically as increasing the concentration, maturation, and storage of the spermatozoa.

Much of the testicular fluid that transports spermatozoa from the seminiferous tubules is resorbed in the caput, increasing the concentration of the spermatozoa by 10–100-fold. The epididymal epithelium secretes the epididymal plasma in which the spermatozoa are suspended. As the newly developed spermatozoa pass through these regions of the epididymis, many changes occur, including alterations in net surface charge, membrane protein composition, immunoreactivity, phospholipid and fatty acid content, and adenylate cyclase activity. Many of these changes are thought to improve the structural integrity of the sperm membrane and also increase the fertilization ability of the spermatozoa. The capacities for protein secretion and storage within the epididymis are known to be extremely sensitive to temperature and reproductive hormone levels, including estrogens.

As many as half of the spermatozoa released from the testis die and disintegrate within the epididymis and are resorbed by the epididymal epithelium. The remaining mature spermatozoa are stored in the cauda epididymis, which contain about 70% of all spermatozoa present in the male tract. The capacity for sperm storage decreases distally; spermatozoa in the vas deferens may only be motile for a few days. In humans, it is not a perfect storage organ, and the spermatozoa do not remain in a viable state indefinitely. After prolonged sexual activity, caudal spermatozoa first lose their fertilizing ability, followed by their motility and then their vitality; they finally disintegrate. Unless these older, senescent spermatozoa are eliminated from the male tract at regular intervals, their relative contribution to the next ejaculate(s) increases, thus reducing semen quality, even though such ejaculates do have a high sperm concentration. The transit time of sperm through the fine tubules of the epididymis is thought to be 10–15 days in humans.

As spermatozoa traverse the epididymis, they are exposed to a continuously changing milieu of the luminal fluid derived from the rete testis and modified by the secretory and absorptive activity of the epididymal epithelium. In nonhuman mammals, there is compelling evidence that the epididymal epithelium does provide essential factors for sperm maturation [125, 126]. In humans, most of the information is obtained from treatment of pathologic cases rather than from normal fertile men. Both epididymal maturation and capacitation are necessary before fertilization. The epididymis is limited to a storage role because spermatozoa that have never passed through the epididymis and that have been obtained from the efferent ductules in men with congenital absence of vas deferens can fertilize the human oocyte in vitro and result in pregnancy with live birth (as well as with intracytoplasmic sperm injection with sperm obtained after testicular biopsy).

2.13 Sperm Entry into Cervical Mucus

At the moment of ejaculation, spermatozoa from the cauda epididymis are mixed with secretions of the various accessory glands in a specific sequence and deposited around the external cervical os and in the posterior fornix of the vagina. Spermatozoa in the first fraction of the ejaculate have significantly better motility and survival than the later fractions. The majority of the spermatozoa penetrate cervical mucus within 15–20 min of ejaculation [127]. The ability to migrate across the semen–mucus interphase is highly dependent on the specific movement pattern of the spermatozoa [128]. At the time of sperm penetration into the

cervical mucus, further selection of the spermatozoa occurs based on the differential motility of the normal vs. abnormal spermatozoa. This is further modified once the "vanguard" spermatozoon is within the cervical mucus [129]. The receptivity of the cervical mucus to the penetration by the spermatozoa is cyclic, increasing over a period of about 4 days before ovulation and decreasing rapidly after ovulation. Maximum receptivity is seen the day before and on the day of the LH peak [130]. Spermatozoa enter the uterine cavity from the internal cervical os by virtue of their own motility. From here the spermatozoa traverse to the site of fertilization in the ampulla of the fallopian tube or the oviduct.

2.14 Capacitation and Acrosome Reaction

Animal studies in rats and rabbits indicate that spermatozoa that are stored in the female tract are unable to penetrate the ova. They have to spend time in the female tract before they acquire this ability. Capacitation is a series of cellular or physiological changes that spermatozoa must undergo in order to fertilize [131]. It represents a change in the molecular organization of the intact sperm plasma membrane that is characterized by the ability to undergo the acrosome reaction, to bind to the zona pellucida, and to acquire hypermotility.

Capacitation involves the removal of seminal plasma factors that coat the surface of the sperm; modification of the surface charge; modification of the sperm membrane and of the sterols, lipids, and glycoproteins and the outer acrosomal membrane lying immediately under it. It also involves an increase in intracellular-free calcium [132]. Changes in sperm metabolism, increase in 3′,5′-cyclic monophosphate, and activation of acrosomal enzymes are believed to be components of capacitation. Sperm capacitation may be initiated in vivo during migration through cervical mucus [133]. Capacitation may be an evolutionary consequence of the development of a storage system for inactive sperm in the caudal epididymis.

Capacitation can also be achieved by culture medium supplemented with appropriate substrates for energy and in the presence of protein or biological fluid such as serum or follicular fluid. Usually it takes about 2 h for sperm to undergo capacitation in vitro. Further modifications occur when capacitated sperm reach the vicinity of the oocyte.

The acrosome reaction confers the ability to penetrate the zona pellucida and also confers the fusogenic state in the plasmalemma overlying the nonreactive equatorial segment, which is needed for interaction with the oolemma. There are distinct fusion points between the outer acrosomal membrane and the plasma membrane. The fusion begins posteriorly around the anterior border of the equatorial segment, which is always excluded from the reaction. The changes, termed *acrosome reaction*, prepare the sperm to fuse with the egg membrane. The removal of cholesterol from the surface membrane prepares the sperm membrane for the acrosome reaction [134]. In addition, D-mannose-binding lectins are also involved in the binding of human sperm to the zona pellucida [135].

Thus, these series of changes are necessary to transform the stem cells into fully mature, functional spermatozoa equipped to fertilize the egg.

2.15 Concluding Remarks

Spermatogenesis involves a complex series of events that produces the fully functional spermatozoa capable of fertilization. However many events whether pre-testicular, testicular or post-testicular can significantly influence both the quality and the number of spermatozoa that are released in the ejaculate. According to the WHO 2010 guidelines, the reference values have significantly changed especially for sperm concentration, motility and normal sperm morphology and there is an ongoing debate on the declining sperm counts and their clinical implication in the management of male infertility.

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Normal Puberty and Pubertal Disorders

Meir Jonathon Solnik and Joseph S. Sanfilippo

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3.1 Introduction

Sexual development involves a complex series of events that, if orchestrated in an appropriate sequence, results in the normal transition from childhood to young adulthood. Although from an evolutionary perspective, the ultimate goal is propagation of a species, this pivotal time in the life of an adolescent often represents one of physical and emotional challenges, potentially heightened even when subtle variances to societal norms occur.

Written for an overview of the subject, this segment will detail how pubertal development has been categorized and the neuro-physiological changes that ensue. The focus will then shift towards processes that result in precocious (central and GnRH-independent causes) and delayed development, highlighting more common etiologies and disease states. How and when to evaluate abnormal presentations of development and how to ideally treat them is discussed. This period of change allows for a pivotal entry point towards establishing longitudinal care with the clinician. However, a thorough understanding of the appropriate timing of events and an awareness of the stressors that frequently complement these changes is then essential for a wide range of specialists who can bring a unique perspective when caring for such young patients when normal puberty drifts awry.

Clinical Case

A 17-year-old girl consults you because she has not had her period yet. Her mother accompanies her. She had normal breast development at 11 years of age. Her mother reports that her growth charts including her growth spurt were normal. She is presently 5′2′.

3.2 The First Visit

The first gynecologic encounter represents a critical event and possible first pseudo-adult exposure to health care, which can have either a positive or negative influence on her own care needs. A thorough examination should begin with a detailed intake, including age and order of onset of symptoms, progression of secondary sexual characteristics, and assessment of linear growth for at least the prior 6 months.

Psychosocial history should be obtained focusing on relationships with peers, authority figures, parents, teachers, coaches, as well as siblings. A nutrition history focused on fad diets, fast food, athleticism, and overt eating disorders should be assessed. Significant weight changes may also help direct the clinician towards a more appropriate list of differentials.

Vaginal bleeding, for example, although not the typical initial presenting symptom of precocity, may signal poor hygiene, neglect, or abuse and should be investigated promptly. Height and weight should be charted on a linear growth curve and followed over the course of years to watch for trends and rate of change. Predicted final height has traditionally been based on the methodology described by Bayley and Pinneau [1]. Target height (cm) considers genetic potential and is calculated from the averages of the height of the child's parents: male—[father's height + mother's height + 13]/2 and female: [father's height − 13 + mother's height]/2.

The most important and least invasive portion of the physical examination is a visual exam of her axilla, breast, and external genitalia. Only if there is suspicion of a pelvic mass or significant pathology should a pelvic or recto-abdominal exam be performed. Cervical cytology is no longer indicated in this group of girls, nor is testing for sexually transmitted infection, unless there is suspicion for abuse. A vaginal smear in an estrogenized system will reveal increased numbers of superficial squamous cells. An estrogen-secreting tumor may be suspected when greater than 40% of the cells are superficial and when rapid increase in height is noted.

Physical findings suggestive of central precocious puberty (CPP) include Tanner stage II breast development with darkening of the areola, labial fullness with a dullness of the vaginal mucosa, and leukorrhea. Coarse pubic hair, acne, oily skin, clitoromegaly, and deepening of the voice are signs of androgen production, which may occur in the setting of heterosexual development and should likewise be investigated. Tall stature and adult-type body odor are other indications for the evaluation of precocious puberty. A complete neurological exam, psychological evaluation, and skin assessment should be performed initially and with subsequent visits as well. Simple findings such as elevated blood pressure, suggestive of non-classic congenital adrenal hyperplasia (CAH), or skin changes consistent with café-au-lait spots are most helpful and easy to notice.

3.3 Normal Puberty

The activation of the hypothalamic–pituitary–gonadal (HPG) axis represents the commencement of reproductive life in the adolescent female, originally described by Ernest Knobil in 1980 at the University of Pittsburgh [2]. In the higher cortical centers, from the arcuate nucleus of the hypothalamus, gonadotropin-releasing hormone (GnRH) is synthesized and released [3]. Through its effect on the anterior pituitary, GnRH regulates the synthesis, storage, and release of the pituitary gonadotropins, follicle-stimulating hormone (FSH), and luteinizing hormone (LH). These hormone levels approach that of an adult in the fetal circulation by mid-gestation. However, with increasing maternal steroid hormone production towards term, gonadotropin levels decline. Shortly after delivery, as the maternal source of estrogen is withdrawn, gonadotropin levels are noted to increase as a result of the release from the negative-feedback circuit [4].

This sequence of events demonstrates the functional capability of the HPO axis early in development and results in follicular growth in the prepubertal ovary and an increase in circulating estradiol. This effective and exquisitely sensitive negative-feedback system, often referred to as the gonadostat, develops rapidly. In the years preceding puberty, gonadotropin levels remain low in response to suppression by low levels of circulating estrogen (10 pg/mL).

It is thought that the two primary inhibitory influences on the pulsatile release of GnRH and the down-regulation of the HPO axis during childhood are the (1) intrinsic central nervous system (CNS) inhibition via γ(gamma)-aminobutyric acid (GABA) and the (2) negative-feedback system driven by ovarian steroid hormones [5, 6]. With continued maturation of the CNS after birth, a more profound internal inhibitory effect can be noted in reference to GnRH-secreting neurons. In premature infants with less developed neuronal pathways, pituitary gonadotropins are higher than in term counterparts, presumably due to a weaker inhibitory influence [7]. The presence of a nonsteroidal regulator of these pathways is further substantiated by the ability of patients with gonadal agenesis to secrete moderate levels of gonadotropins in response to GnRH [8].

The normal age range of puberty is 7–13 years for white girls and 6–13 for Black girls [9]. Mean age at menarche is 12.9 (+/−1.2) years in white girls and 12.1 (+/−1.2) years in Black girls [8]. On average thelarche occurs 1.2 years before pubarche. Menarche usually correlates with pubarche stage 4 and generally is 2–2.5 years after thelarche [10].

3.4 Onset of Puberty

Pulsatile secretion of GnRH from the arcuate nucleus of the hypothalamus leads to gonadarche, documented by profound increases in sex steroid hormone production [3]. Early pubertal changes are temporally associated with an increase in GnRH pulse frequency, primarily during the sleep cycle [11]. As menarche approaches, GnRH pulses increase in amplitude and can be detected throughout the day, similar to that of an adult [12, 13].

Both genetic and environmental effects may play a role with the initiation of pubertal development. It has been suggested that appropriate weight gain and percent body fat are required for these events to occur [14]. This concept is substantiated by data from adolescent females who suffer from chronic illness, malnutrition, or have low body mass indices due to vigorous exercise. These young girls frequently experience delays in sexual maturation and may present with primary amenorrhea, resulting from hypothalamic hypogonadism. Accordingly, normal menstrual cycles resume with reversal of their nutritional status [15]. Investigators who followed healthy females throughout puberty found that body composition did not change prior to, but rather along with, the increase in GnRH secretion [16].

Plasma concentrations of leptin, an adipocyte-derived hormone, correlate well with body composition and have been shown to rise throughout puberty in female patients [17]. Specific leptin deficiencies have been shown to prevent sexual maturation, which can then be triggered by restoring

normal levels [18]. Nevertheless, the role of leptin in pubertal development has not been clearly elucidated. The concept of intrauterine growth restriction, imprinting, and subsequent developmental disorders follows a common thread, since early exposure to a spendthrift, "low-calorie" environment may have a contrary effect in childhood, as suggested by the Barker hypothesis, resulting in early onset menarche and adrenarche [19–21].

Another molecule that may play a role in the reversal of the HPO downregulation is neuropeptide Y (NPY). Circulating levels are regulated by steroid hormones as well as nutritional status, with a net influence in gonadotropin synthesis through an alteration in GnRH pulsatility and pituitary response to GnRH [22]. Increased levels of NPY have been documented in females with eating disorders such as anorexia nervosa and bulimia [23], representing another possible correlation with percent body fat and reproductive potential.

Kisspeptin is a strong stimulator of the HPO axis, acting through GnRH neuronal activity, and may be a key player in early pubertal development [24]. Although the exact mechanisms on the gonadotropic axis are not well defined, receptor mutations have been identified in women with precocious puberty, and when administered to women with hypothalamic amenorrhea, kisspeptin agonists have successfully stimulated gonadotropin secretion.

Insulin-like growth factor I plays a role and appears to be under the control of Gonadotropin Releasing Hormone (GnRH), furthermore this appears to be tied into the Growth hormone Axis [25, 26]. Low levels of estrogen appear to stimulate bone growth in part through the Growth hormone-Insulin like Growth Factor I axis [27]. Please see Fig. 3.1 outlining the neuroendocrine basis for pubertal development [28].

3.5 Characteristics of Sexual Development

The predictable and ordered series of events, which have historically been referred to as the standard for sexual development and somatic growth, were initially described by Tanner and Marshall more than 30 years ago (Sexual Maturity Rating (SMR) Scale) [29] (Fig. 3.2). Although the activation of the HPO axis results in normal onset of sexual development, alternate sources of steroid hormone production may signal abnormally early development in adolescent girls. Such agents include organic pesticides, soy-based products, and shampoos containing placental extract. Investigators have suggested several possible pathways by which these agents influence development, including direct activation of the HPO axis and steroid hormone-like activity [30–32]. These publications raise the notion of endocrine-disrupting chemicals, and although there is little doubt that persistent exposure may adversely affect developmental pathways and promote disease progression, the association with pubertal development remains tentative and weakly causative from an epidemiological perspective.

3.6 Thelarche

The first sign of development in the majority of white females is breast budding. According to Tanner and Marshall, this initial event occurs between 8 and 13 years of age in most females, with a mean of 10.6 years. The transition period from stage II to stage V breast development may last 4.2 years [29].

3.7 Adrenarche

Pubic hair growth typically occurs after thelarche, but may occur concomitantly, with the activation of the hypothalamic–pituitary axis. Although adrenarche may also present before breast development in a normally maturing female, adult hair distribution should not be detected at this early stage, as it may represent an excess of androgen production. Accordingly, breast maturation should not be so advanced in the absence of pubic hair development, a potential sign of androgen insensitivity syndrome. Adrenarche typically occurs between the ages of

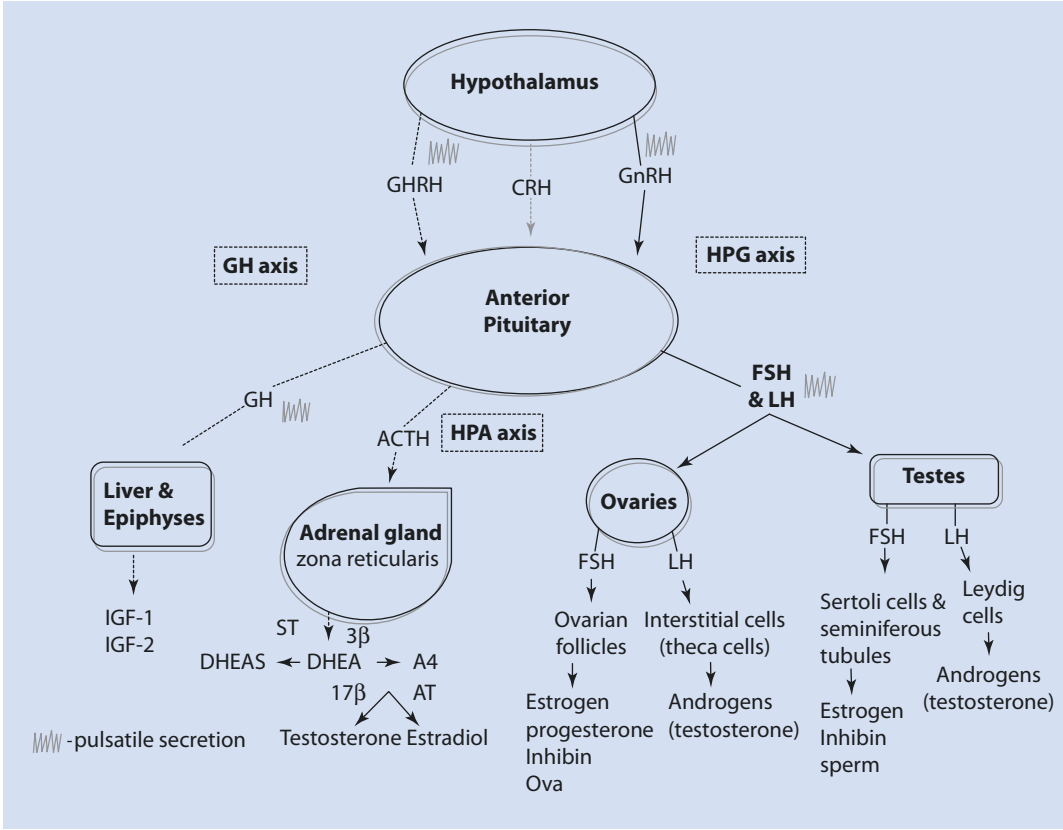

Fig. 3.1 Simplified diagram of the hypothalamic–pituitary–gonadal (HPG) axes, hypothalamic–pituitary–adrenal (HPA) axes, and growth hormone (GH) axes. The hypothalamus releases gonadotropin-releasing hormone (GnRH), corticotropin-releasing hormone (CRH), and growth hormone-releasing hormone (GHRH), which stimulate the anterior pituitary gland to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH), adrenocorticotropic hormone (ACTH), and growth hormone (GH), respectively. GnRH, LH, GHRH, and GH are released in a pulsatile fashion that varies with pubertal stage. In the HPG axis, FSH stimulates the ovarian follicles to produce estrogen (from androgenic precursors produced from theca cells), inhibin, progesterone, and ova. Estrogen provides both a positive and negative feedback on GnRH. In females, a critical amount of estrogen is needed to produce a positive feedback to stimulate the LH surge that leads to ovulation. In males, FSH stimulates Sertoli cells and seminiferous tubules to produce estrogen, inhibin, and sperm. LH stimulates theca cells in females and Leydig cells in males to produce androgens. On the HPA axis, ACTH stimulates the zona reticularis of the adrenal gland to secrete dehydroandrosterone (DHEA). DHEA is then converted to dehydroandrosterone sulfate (DHEAS) via sulfotransferase (ST), and to androstenedione (A4) via 3b-hydroxysteroid dehydrogenase (3b). A4 is then converted to testosterone via 17b-hydroxysteroid dehydrogenase (17b) and estradiol via aromatase (AT). In the GH axis, GH stimulates the liver and epiphyses of bone to produce insulin-like growth factor 1 (IGF-1) and insulin-like growth factor 2 (IGF-2)

11 and 12, with adult hair distribution by age 14. Androgen levels change without a corresponding change in ACTH and cortisol secretion throughout life. So the means by which adrenal androgens are produced is not as clearly delineated and appears to occur independent of the hypothalamic–pituitary axis.

3.8 Growth Spurt

The growth spurt (peak growth velocity), during which adolescents achieve approximately 20% of their adult final height, occurs with the onset of puberty [29]. Peak growth velocity (2–3 cm/year) precedes menarche and typically occurs earlier in

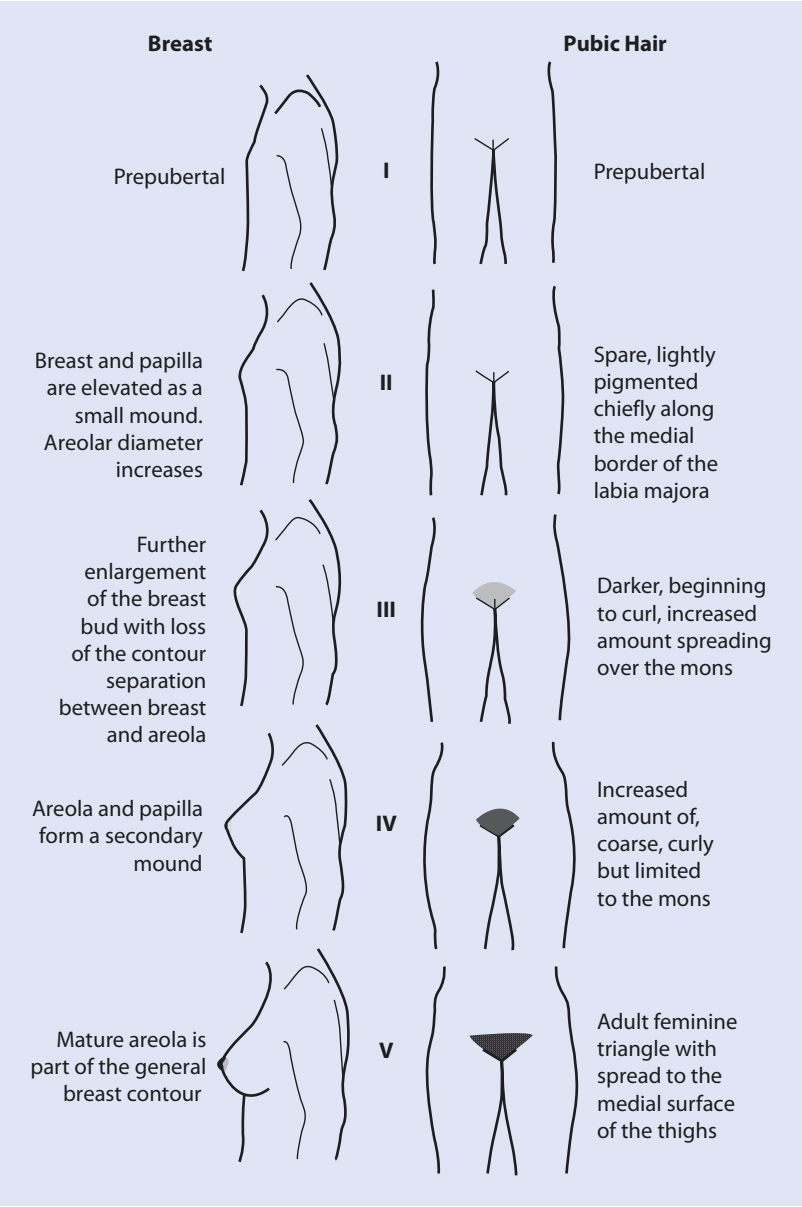

Fig. 3.2 Timing of events of puberty. 1969 data from a study of British schoolchildren. 1997 data from a study of American schoolchildren. Reproduced with permission from Solnik JM, Sanfilippo JS. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007; adapted from [22]

girls than in boys. Rapid growth of the extremities occurs first, followed by a gradual lengthening within the vertebral column. The timing of the growth spurt varies according to ethnicity.

3.9 Menarche

According to Tanner, girls in the United Kingdom in 1969 had their first menses at the average age of 13.5 years, with a range of 9–16 years [29]. The mean age of menarche for a Caucasian adolescent in the USA is approximately 12.7 years. At the time of menarche, most have achieved Tanner stage IV breast development, and the interval from initial breast development to menarche on average is 2.3 years [29].

There seems to have been a decline in the average age of menarche in the first half of the twentieth century, in part due to the improvement in general health and nutrition [33]. Nonetheless, few reports have documented any

further changes since the mid-twentieth century.

There is good evidence that African-American girls have an earlier onset of puberty compared to Caucasian girls [34, 35]. This was well demonstrated by the Pediatric Research in Office Settings (PROS) study published by Herman-Giddens in 1999 [34]. This multicenter, cross-sectional study evaluated over 17,000 female patients between the ages of 3 and 12 years of age [34]. On average, African-American females show early signs of puberty up to 1.5 years earlier than their Caucasian counterparts. By 7 years of age, 27.2% of African-American girls and 6.7% of Caucasian girls showed breast or pubic hair development. Menarche was achieved almost a year earlier. The mean age for onset of breast development was 8.87 in African-American girls and 9.96 years in Caucasian girls. At each consecutive stage of development, African-Americans were more advanced per year than Caucasians. Girls of other ethnic backgrounds may also have a characteristic difference in onset of pubertal maturation. However, only Caucasian and African-American girls were included in this study.

PROS was the first large publication to address current and demographically relevant standards for assessing normal and abnormal onset of puberty. Updated guidelines have since been proposed and recommend a formal evaluation for precocious puberty be initiated in African-American girls who present before the age of 6 and Caucasians who present before the age of 7. Although this provocative investigation has drawn much criticism, it does invite us to reconsider the current standards (Fig. 3.2).

3.10 Precocious Puberty

One challenge each clinician faces is when to initiate the assessment of a child suspected of having precocious puberty. Historical accounts from the nineteenth century report a relative later age of onset of menstruation (16–17 years), presumably due to malnutrition. The definition of precocious puberty since remained stable, such that any female who presented prior to 8 years of age was observed, if not evaluated, for progression of sexual characteristics [29]. As referred to earlier, the traditional definition was challenged by Herman-Giddens, who strongly suggested that normal pubertal development may begin as early as 6 years of age [34]. Causes of precocious puberty are listed in Table 3.1.

Table 3.1 Causes of precocious puberty

Central precocious puberty (GnRH dependent)
1. Idiopathic
2. Central nervous system tumors
(a) Craniopharyngioma
(b) Trauma
(c) Infection
(d) Primary hypothyroidism
3. Syndromes associated with elevated gonadotropins
(a) Silver's syndrome (dwarf-like characteristics)
Peripheral precocious puberty (GnRH independent)
1. Exogenous steroid hormone exposure (estrogens)
2. Ovarian tumors
(a) Granulosa cell
(b) Functional cyst
3. Adrenal tumors
4. McCune-Albright syndrome
Heterosexual precocious puberty
1. Exogenous steroid hormone exposure (androgens)
2. Adrenal and ovarian androgen-producing tumors

3.10.1 Effects of Precocious Puberty on Adult Height

Low levels of estrogens have been shown to promote bone growth, as is manifest by rapid growth velocity during the growth spurt. Conversely, high levels promote closure of the epiphyseal plates. Girls who present early in the course of precocious puberty are generally taller than their age-respective cohorts due to increased levels of steroids and the actions of IGF-I. This growth is premature and limited, so that the final height in untreated patients will likely be less than 155 cm [1]. As a result, by the time most adolescents achieve menarche, they have likely reached their final height. Notwithstanding

the apparent risk for short stature, a significant number of untreated patients with idiopathic disease will likely attain relatively normal adult height, greater than the third percentile [1]. Some specialists in the field believe that the diagnosis of precocious puberty cannot be assigned unless symptoms are also associated with an accelerated growth spurt.

3.10.2 Central Precocious Puberty

CPP is more frequently noted among girls, with an incidence of 1:5000–1:10,000 [36]. It results from the premature activation of the hypothalamic GnRH neurons. Approximately 70–95% of such cases are idiopathic in nature; however, other potential etiologies must first be considered, since the level of urgency and need for management of individual causes will vary [37, 38]. For a full list of etiologies, see Table 3.1.

3.10.3 Laboratory Findings

Baseline gonadotropin levels in the pubertal range with a predominant LH response are suggestive of CPP. Random daytime levels may be of less use in early central pubertal development because the initial increase in pulsatility occurs at night. To help distinguish CPP from GnRH-independent forms of precocious puberty, a GnRH stimulation test should be performed. To accomplish this, 100 μg of GnRH (gonadorelin acetate) is administered intravenously, and gonadotropin levels are drawn at baseline and at 20, 40, and 60 min. One of the earliest signs of physiologic puberty is the nocturnal, pulsatile secretion of GnRH with a subsequent increase in serum LH. There is a corresponding rise in LH for each pulse of GnRH secreted. These same events occur with early onset, and an LH:FSH ratio >1 would be expected. Serum estradiol levels would be detected in the pubertal range as well. In order to maintain the diagnosis of CPP, androgen (DHEA, DHEA-S, testosterone) and 17-hydroxyprogesterone (17-OHP) levels should be drawn.

3.10.4 Imaging Studies

Imaging studies play a key role in the evaluation of children with precocious puberty, because a rapid increase in growth and bone age are typically seen in children with rapidly increasing levels of sex steroid hormones. Linear growth and skeletal maturation are often a more accurate assessment of pubertal development than progression of secondary sexual characteristics.

Bone age is typically evaluated by radiographic plain films of the left hand and wrist. This is a simple and noninvasive test that is well tolerated by most children. Bone age advance over chronological age is diagnostic for precocious puberty, and a disparity of greater than 2 years is more suspicious for a progressive disorder [39]. Given the higher prevalence of CNS abnormalities, especially in girls who present with particularly early onset or who have a known history of childhood seizures, neuroimaging is always indicated to rule out space-occupying lesions, malignant neoplasms, and other CNS anomalies, even in the absence of neurological complaints.

Pelvic ultrasound, however, is typically one of the easiest and most useful studies since it provides a good picture of ovarian function (developing follicles capable of producing estradiol, increased cortical volume suggestive of excess androgen production) or neoplastic processes. Ultrasound may also demonstrate subsequent steroid hormone influence on other reproductive organs. A diagnostic approach to precocious puberty is given in Fig. 3.3

3.10.5 Treatment

The ultimate therapeutic goal with Central Precocious Puberty is to suppress the HPO axis and return the hormonal environment to that of the prepubertal state (serum estradiol <10 pg/mL). Most important is the normalization of linear growth velocity and bone maturation. The outcome for patients with CPP can vary significantly, which further limits our ability to predict who will benefit most from therapy.

3.10.6 Hypothalamic Suppression

Initial attempts to achieve such a degree of hypothalamic suppression included the use of progestins; however, these were unsuccessful at limiting progressive changes and their use has since been abandoned [40]. The most commonly used GnRH analogs to treat CPP in the USA are leuprolide,

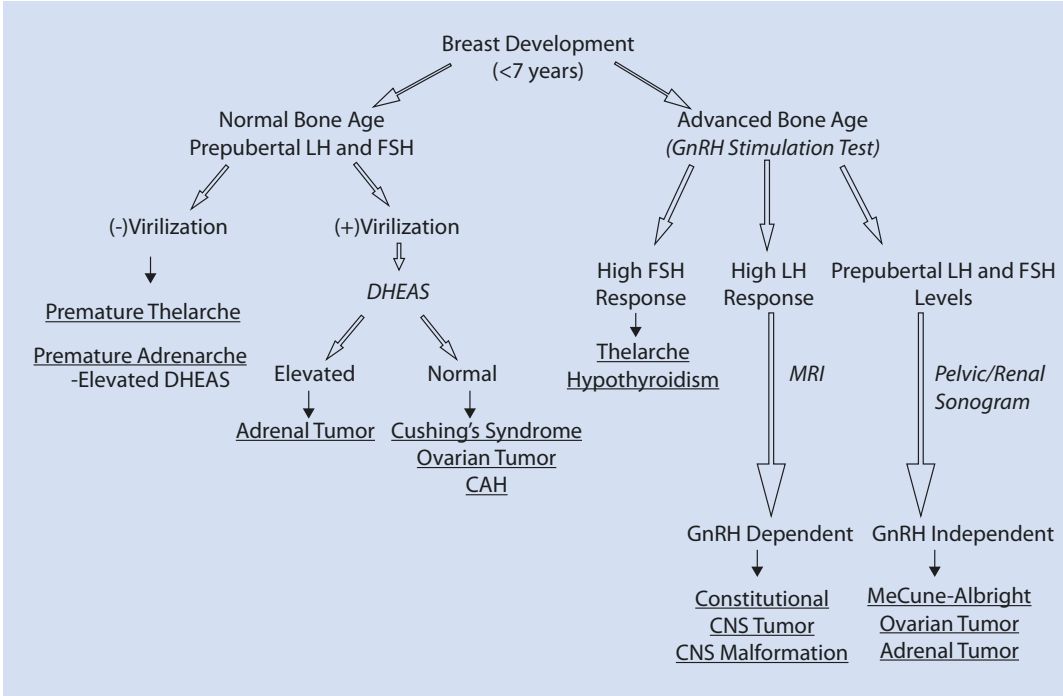

Fig. 3.3 Evaluation of central, peripheral, and incomplete precocious puberty

nafarelin, and histrelin. Children with precocious puberty generally require higher doses to achieve suppression, which can be monitored with serum estradiol levels and GnRH stimulation tests. In order to improve compliance, subcutaneous formulations can be used. Early treatment protocols using long-acting GnRH agonists reported significant regression of secondary sexual characteristics and overall improvement in final height compared to nonrandomized controls [41]. A few randomized series have, however, been published that addressed the effect of GnRH agonists on final height in girls who presented with early or slowly progressive puberty [42, 43]. They confirmed results from previous observational and non-randomized reports that documented very little effect of hypothalamic suppression on improving final height in patients presenting at a later age. Children presenting with either "early puberty" or advanced "slowly progressive puberty" were likely to achieve reasonable adult height without hypothalamic suppression.

One theory that may help explain impaired growth during GnRH analog therapy in this group is early growth plate senescence related to estrogen exposure prior to onset of treatment [44]. So it may be this rate-limiting step, patients presenting beyond the window of opportunity, that limits final height.

Significant consideration should be given to promptly initiating therapy in girls presenting early with advanced bone age, as they will likely benefit most from GnRH agonist therapy [45–48]. Adan et al. suggested the following as risk factors for decreased stature and appropriate indications for therapy, especially at an earlier age of onset: (1) predicted adult height below 155 cm (may include those with a predicted height over 155 cm if the LH/FSH ratio is consistent with CPP) and (2) bone age advance over chronological age greater than 2 years [48]. Hormonal monitoring of therapy can be performed with the GnRH stimulation test at 3, 6, and 12 months after initiation, with annual follow-up thereafter.

Although the optimal time of discontinuing therapy remains unclear, many recommend that suppression stop at a bone age of 12–12.5 years. Other elements to consider include the total duration of therapy and growth velocity over the months preceding. Routine evaluation of secondary sexual characteristics, weight, and sonographic measurements of pelvic structures should be performed on an ongoing basis as well. Bone mineral density may be affected by prolonged use

of GnRH agonists, so attention to bone health should not be omitted.

3.10.7 Recombinant Growth Hormone

Some children with precocious puberty will have early closure of their epiphyseal plates despite the use of GnRH analogs. As a result, these girls will grow up to be short adults without further intervention. The use of growth hormone as an adjunct to GnRH agonists in girls with precocious puberty has been evaluated by several observational and randomized series and has been found to improve final height prognosis [49]. Although the use of growth hormone in certain patients is frequently prescribed among pediatric endocrinologists, the studies evaluating efficacy are troubled by obstacles quite similar to those seen with the analysis of GnRH agonists on final height. It is important to be aware that growth hormone has not yet been approved by the U.S. Food and Drug Administration for treatment of girls with short stature as a result of precocious puberty

3.10.8 GnRH-Independent Precocious Puberty

When precocious puberty occurs independent of pituitary gonadotropins, the source of estrogen production must be established. One common source is surreptitious ingestion of exogenous hormones, such as those found in oral contraceptive pills or anabolic steroids. Other less common sources include primary hypothyroidism. However, the most common origin of GnRH-independent estrogen production is frequently the ovary itself.

3.10.9 Autonomous Ovarian Estrogen Production

Ovarian tumors are uncommon but important childhood neoplasms that present with precocious puberty in approximately 10% of cases [50]. Granulosa cell tumors are the most common estrogen-producing neoplasms detected. However, other tumors, such as thecal cell tumors, gonadoblastomas, teratomas, cystadenomas, and ovarian cancers, may be responsible. Intra-abdominal masses are often palpable, but imaging with sonography or magnetic resonance imaging may help characterize the tumor, and surgical exploration is generally warranted.

Laboratory criteria that help distinguish these processes from a central source include low baseline gonadotropin levels and a prepubertal response to the GnRH stimulation test. Similar to CPP, estradiol levels will be high and bone age advanced (see Fig. 3.2). Treatment is based on surgical extirpation of the source, which results in regression of pubertal changes.

3.10.10 McCune-Albright Syndrome

McCune-Albright syndrome, also known as polyostotic fibrous dysplasia, is a genetic disease affecting the bones and pigmentation of the skin. The hallmark of McCune-Albright syndrome in girls is precocious puberty, and this condition accounts for approximately 5% of all girls with precocious puberty. These patients have estrogen-producing ovarian follicular cysts that develop independent of gonadal hormone stimulation, a condition termed autonomous follicle development.

Children with this rare disorder also have fibrous dysplasia in their bones, which leads to fractures, deformities, and X-ray abnormalities. Facial bone deformities may result in appropriate concerns for cosmesis. In addition, these children have cafe-au-lait spots, which are light tan birthmarks. McCune-Albright syndrome is often associated with several other endocrinopathies, including hyperthyroidism, acromegaly, pituitary adenomas, and adrenal hyperplasia [51].

3.10.11 Treatment

In contrast to girls with CPP, girls with McCune-Albright syndrome exhibit a lack of GnRH pulsatility, gonadotropin levels are low, and estradiol is produced from autonomous follicle development. Treatment protocols for McCune-Albright syndrome are aimed at inhibiting peripheral estradiol production with aromatase inhibitors or blocking the effects at the receptor level with selective estrogen receptor modulators (SERMs).

Aromatase inhibitors offer several theoretical benefits for the treatment of McCune-Albright syndrome. Unfortunately, results from studies evaluating testolactone have been inconclusive [52–54].

It has been suggested that continuous estrogen exposure from a peripheral source may secondarily induce the HPO axis, such that a central component may occur simultaneously [55]. These findings help to explain the lack of therapeutic benefit of aromatase inhibitors in certain patients with McCune-Albright syndrome. Evaluation and management of these complicated patients should then be based on algorithms used for CPP.

The SERM, tamoxifen, was studied in a prospective, multicenter trial, for the 12-month treatment of 25 girls with McCune-Albright syndrome. This treatment decreased the incidence of vaginal bleeding and also decreased bone velocity and bone maturation [56]. Other causes of precocious puberty can be found in Table 3.1.

3.10.12 Premature Thelarche

Early breast development in the absence of other signs of sexual maturity is typically a benign, self-limited event. Initial laboratory evaluation will reveal prepubertal gonadotropin levels and normal bone age. GnRH stimulation will result in a predominant FSH response. Continued observation is nonetheless mandatory, and breast development, which may be unilateral or bilateral, will likely regress, but may persist until normal onset of puberty.

3.10.13 Premature Adrenarche

Adult pubic hair growth before the age of 6 may result from an abnormal adrenal secretory response, which promotes androgen production (17-hydroxypregnenolone, DHEA, and DHEA-S). Like premature thelarche, the diagnosis can only be made after longitudinal evaluation, in the absence of other signs of sexual development. Although mild increases in bone age may occur, no treatment is necessary, as these children will likely achieve normal adult height [57].

A fasting 17-OHP level is generally sufficient to rule out nonclassic CAH unless there is significant bone age advance. Only patients with conclusively high levels of 17-OHP warrant treatment. There is evidence to suggest that girls with premature adrenarche may be at risk for developing polycystic ovarian syndrome (PCOS).

3.11 Delayed Puberty and Primary Amenorrhea

Delayed puberty in girls is defined as lack of thelarche by 13 years of age or when more than 4 years pass between thelarche (SMR Stage Tanner 2) and menarche. Primary amenorrhea is diagnosed when girls who have developed secondary sexual characteristics do not reach menarche by 16 years of age. Constitutional delay is the most common etiology accounting for 53% of all cases [58]. Please see Fig. 3.4 outlining a flow chart for evaluating girls with delayed puberty [59].

Most girls with delayed puberty have normal ovaries (eugonadal) and their sexual development is constitutionally delayed. Hypogonadism, which can occur, can be the result of ovarian failure, termed hypergonadotropic hypogonadism, or because normal ovaries are not stimulated to secrete hormones, referred to as hypogonadotropic hypogonadism.

3.11.1 Hypergonadotropic Hypogonadism

Hypergonadotropic hypogonadism is the single most common etiology of pubertal delay. The essential condition required to make this diagnosis is elevated gonadotropins, both FSH and LH. Recent advances have enhanced our understanding of neuroendocrine mechanisms affecting puberty, genetics, environmental roles have contributed to this understanding. These have led to novel therapies [60]. Details of such are beyond the scope of this chapter.

Turner syndrome is the most commonly (1:2000 live born females) diagnosed condition within this subset. Karyotype may reveal 45,X or a mosaic, which may occur in up to 40–50% of patients with gonadal dysgenesis. DNA analysis is crucial, because the presence of the Y chromosome places patients at risk for gonadal neoplasias such as gonadoblastoma and dysgerminoma. Mixed gonadal dysgenesis, 45,X/46,XY (the most common karyotype), is also representative of the abnormal sex chromosome group.

Several forms of primary and secondary ovarian failure with normal sex chromosomes also exist. Pure gonadal dysgenesis, Swyer's syndrome, typically presents with delayed puberty. Chemotherapy and/or radiation therapy may

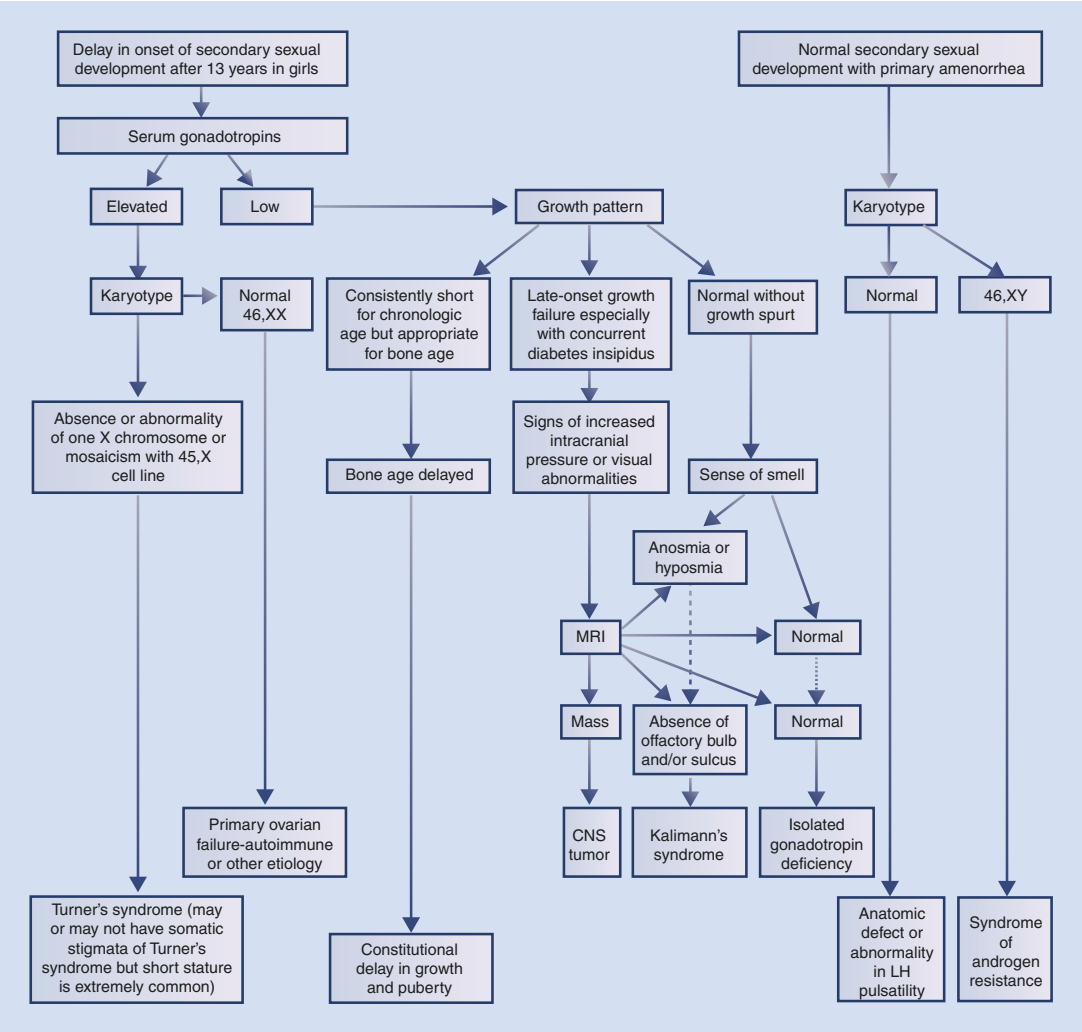

Fig. 3.4 Flowchart for the evaluation of delayed puberty in girls. (From Melmed S, Polonsky K, Larsen PR, et al. Williams textbook of endocrinology. 12th edition. Philadelphia: Saunders; 2011. p. 1137)

result in gonadal dysfunction and delayed development in an otherwise genetically and phenotypically normal female.

Other causes of ovarian failure include autoimmune oophoritis, galactosemia, gonadotropin-resistant ovary syndrome, steroidogenesis enzyme deficiency, infection, or gonadotropin receptor gene mutation. Autoimmune disorders associated with hypergonadotropic hypogonadism include Hashimoto's thyroiditis and Addison's disease. Patients with 17α(alpha)-hydroxylase deficiency present with adrenal insufficiency, hypertension, and lack of sex steroids, including androgens.

3.11.2 Hypogonadotropic Hypogonadism

This condition results from a failure of the hypothalamic–pituitary–ovarian (HPO) axis and deficiency of GnRH. The most common cause of this is constitutional delay, a diagnosis of exclusion. Bone age and height age are delayed, unlike that seen in hypothyroidism where bone age is delayed more than height age. Unfortunately, the only way to discern constitutional delay from idiopathic hypogonadotropic hypogonadism is by longitudinal observation.

Central etiologies of hypogonadotropic hypogonadism include brain tumors, Kallmann syndrome, hypothyroidism, and chronic disease states (Crohn's disease, Cushing's disease, anorexia nervosa, or malnutrition).

3.11.3 Primary Amenorrhea with Otherwise Normal Sexual Development

Genetically normal patients with a normally functioning HPO axis who present with primary amenorrhea typically have anomalies of the genital outflow tract, such as imperforate hymen or vaginal septum (Table 3.2). One of the most common causes of primary amenorrhea in these patients is Mayer–Rokitansky–Kuster–Hauser (MRKH) syndrome. This condition is characterized by a blind vaginal pouch in an otherwise normally sexually developed adolescent and results from the failure of development of the Müllerian (paramesonephric) duct system in genotypic females.

Androgen insensitivity syndrome is another common cause of primary amenorrhea. Androgen insensitivity syndrome, previously termed testicular feminization, is the result of an abnormal androgen receptor. This maternal X-linked recessive disease occurs in individuals with XY genotypes and normal but partially or completely undescended testicles that produce testosterone. Defects in androgen action, manifesting as complete androgen insensitivity syndrome appear to be associated with mutations of the Androgen Receptor (AR) gene [61]. Because of the abnormal androgen receptors, high levels of circulating testosterone result in appropriately timed puberty in females who appear phenotypically normal. A harbinger is sparse or absent pubic hair.

Primary amenorrhea or delayed menarche is frequently associated with hyperandrogenia, secondary to either PCOS or adult-onset CAH. These patients have otherwise normal puberty, but will also present with signs of androgen excess ranging from hirsutism and acne to virilization.

Syndromic Hypergonadotropic Hypogonadism is an autosomal recessive ovarian dysgenesis and has been termed "Perrault syndrome" when associated with deafness [62].

Congenital galactosemia has been associated with primary ovarian insufficiency. This appears to reflect a metabolic toxicity state affecting ovarian function [63]. Inactivation of FSH and LH receptors also can result in primary amenorrhea and premature ovarian failure resulting in delayed puberty [64].

Table 3.2 Causes of primary amenorrhea

CAUV (congenital absence of uterus and vagina)
Hypergonadotropic hypogonadism
1. Abnormal sex chromosomes
(a) Turner syndrome
2. Normal sex chromosomes
(a) 46,XX gonadal dysgenesis
(b) 46,XY gonadal dysgenesis
(c) Pseudo-ovarian failure
Hypogonadism
1. Hypergonadotropic hypogonadism
(1) Abnormal sex chromosomes
(a) Turner syndrome
(2) Normal sex chromosomes
(a) 46,XX gonadal dysgenesis
(b) 46,XY gonadal dysgenesis
(c) Pseudo-ovarian failure
2. Hypogonadotropic hypogonadism
(1) Congenital abnormalities
(a) GnRH deficiencies
(b) Gene mutations
(2) Constitutional delay
(3) Acquired abnormalities
(a) Endocrine disorders
(b) Pituitary tumors
(4) Systemic disorders
3. Eugonadism
(1) Anatomic abnormalities
(a) CAUV
(b) Imperforate hymen
(c) Transverse vaginal septum
4. Intersex disorders
(1) Androgen insensitivity
Polycystic ovarian syndrome

3.11.4 Evaluation

A complete evaluation should be undertaken for any adolescent who meets the standard criteria for delayed puberty or primary amenorrhea:

- Lack of any pubertal development by 13 years of age
- When more than 4 years have passed between thelarche and menarche
- No menses by 16 years of age with secondary sex characteristic

Extensive neonatal and family history, including ages of pubertal development and attainment of final height extending to members beyond the nuclear family, is helpful. History pertinent to information of prior exposure to exogenous steroid hormones or chemotherapy must also be elicited. Review of systems to evaluate for chronic illnesses and pattern of exercise and diet may uncover the diagnosis.

Physical examination and height and weight plotted on a growth chart should be completed as well as blood pressure, thyroid exam, Tanner staging, and abdominal exam. A complete neurological evaluation, including assessing the ability to smell, should also be performed.

Hypergonadotropic hypogonadism patients often present with short stature. Patients with Turner syndrome (45,XO), the most common presentation of hypergonadotropic hypogonadism, may present during infancy with lymphedema or during childhood with typical features such as short stature, webbed neck, and shortened fourth metacarpals. Cardiovascular anomalies and renal abnormalities such as aortic coarctation, bicuspid aortic valves, and horseshoe kidney can be determined with imaging studies.

Patients with mixed gonadal dysgenesis (mosaic XY/XO) may present phenotypically similar to those with Turner's syndrome; however, virilization or ambiguous genitalia may also be evident in the presence of the Y chromosome. Patients with 46,XX complete gonadal dysgenesis are normal to tall in stature and are most commonly phenotypically female. Sexual infantilism with lack of Müllerian structures and 46,XY karyotype is consistent with Swyer's syndrome.

Hypogonadotropic hypogonadism can be seen in adolescents who are extremely athletic or malnourished. Minimal body fat from either cause is associated with reversible hypothalamic dysfunction. Patients with CNS tumors may present with persistent headaches or visual field defects. Marked centripetal obesity and moon facies are typical of Cushing's syndrome. Anosmia along with hypothalamic hypogonadism is consistent with the diagnosis of Kallmann syndrome (isolated GnRH deficiency). A prolactinoma may present with hyperprolactinemia and galactorrhea.

Adolescents with primary amenorrhea who have normal development of other secondary sexual characteristics are usually of normal stature. Pelvic or recto-abdominal examination is performed in these patients to exclude anatomic abnormalities of the reproductive tract. Examples include vaginal septum and the blind vaginal pouch associated with MRKH and androgen insensitivity syndrome.

3.11.5 Imaging

Bone age can be assessed similarly to adolescents presenting with precocious puberty. Stature, consequently, will be decreased in patients with hypergonadotropic hypogonadism, with the exception of pure gonadal dysgenesis (46,XX).

If gonadotropin levels are low with delayed puberty, then a central cause must be determined. Likewise, an elevated prolactin suggests a pituitary or hypothalamic problem. In these cases, MRI scan of the brain and pituitary gland is indicated to exclude abnormality of the hypothalamic–pituitary axis such as pituitary or hypothalamic tumors.

Adolescents with primary amenorrhea but otherwise normal sexual development require pelvic ultrasound to evaluate the internal reproductive organs and detect the presence of a fluid collection consistent with hematocolpos related to a vaginal septum. Abdominal and pelvic MRI is helpful in evaluating renal or skeletal anomalies in patients with vaginal agenesis.

3.11.6 Treatment of Delayed Puberty

Specific Therapy

Therapy for hypogonadotropic disorders is focused on treating the primary etiology whenever possible. If an intracranial lesion compresses the pituitary stalk, then surgical therapy is indicated. If prolactinoma is the cause, then bromocriptine

becomes the first line of therapy. Medical therapy generally restores menses and fertility, and although surgical treatment may portend good results initially, there is a high incidence of recurring hyperprolactinemia. Surgery may therefore be postponed unless the condition is refractory to medical management.

Treatment for competitive athletes and patients with anorexia nervosa becomes somewhat more challenging. Since body weight with total body fat of at least 12–14% has been associated with return of menses, many clinicians feel that patients should be encouraged to change their lifestyle and improve their diet before introducing pharmaceutical management. Whereas patients with idiopathic or irreversible gonadal failure who have not demonstrated sexual development begin therapy at 14–15 year of age, therapy may be initiated later for these patients.

Estrogen Therapy

If the cause of delayed puberty is determined to be irreversible or idiopathic (i.e., constitutional delayed), sex steroid hormone replacement is indicated. The goal of therapy involves induction of breast development, bone growth, and menses. Hormone replacement for patients with ovarian failure is important not only to induce pubertal development, but also to decrease the risk of subsequent osteoporosis and cardiovascular disease due to prolonged hypoestrogenic state.

Timing is quite important when starting hormone replacement. In most instances, therapy is initiated when patients present with delayed puberty during their early teenage years. However, some Turner's syndrome patients will be referred for evaluation during childhood. If these patients begin estrogen therapy too early, their potential growth may be limited by epiphyseal closure.

Hormone replacement for treatment of delayed puberty is begun with low-dose estrogens, typically 0.3 mg conjugated estrogens for 6–12 months. The main goal is to induce normal breast development, as too high of an estrogen dose can result in the development of tuberous breasts [65]. Subsequent goals include regulation of menses and maintenance of bone mass. This can be achieved by increasing the dosage of estrogen slowly after the first year until menstruation occurs. Progestin therapy (e.g., medroxyprogesterone acetate [5–10 mg] at end of estrogen cycle) is initiated approximately 3 months after the increase in dosage of estrogen, typically when breakthrough bleeding occurs. The most common formulation includes continuous estrogen therapy with sequential progestins given orally in the latter part of the cycle to create regular menses. Alternative forms include transdermal estrogen replacement and micronized progestins that have less negative impact on lipid profiles. Gonadotropins have also been used to induce ovulation, but are costly and more difficult to administer, especially in the adolescent patient population.

Novel Therapies

Kisspeptin-10 in men has been described. This may have application in the future for females; it appears to stimulate the hypothalamic pituitary ovarian axis and has been advocated as one selective option for treatment of idiopathic hypothalamic hypogonadism [66].

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Fertilization and Implantation

Levent Mutlu and Hugh S. Taylor

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4.1 Introduction

Infertility is a common problem with significant psychological, economic, and medical implications. It is estimated to affect one in eight women [1]. There are approximately 70 million infertile couples worldwide, most of whom reside in developing countries [2]. In the USA, the CDC estimated that of married women ages 15–44, 1.5 million (6%) are infertile, and of all women ages 15–44 years old, 7.4 million have used infertility services [3]. Two of the most common mechanisms of infertility are failure of fertilization and failure of implantation. Fertilization is the union of a sperm and an oocyte, while implantation is the attachment of a developed blastocyst to the uterine endometrium. Although the majority of our knowledge on this subject relies on other mammalian fertilization models, with advancing technology and a deeper understanding of gamete biology, transport, and implantation, our knowledge and available treatment options and medical care for infertile couples have significantly improved.

In this chapter, we will summarize the current evidence on molecular and cellular interactions that are essential for successful fertilization and implantation and their relevance to current clinical reproductive medicine.

Clinical Case

A 27–year-old woman is undergoing in vitro fertilization (IVF) for idiopathic infertility. Her investigation revealed no abnormality. Her partner has normal sperm parameters. She undergoes in vitro fertilization (IVF). Eleven oocytes are obtained with fertilization resulting in 9 embryos. Seven embryos develop to the blastocyst stage. One is transferred but no pregnancy occurred. She has 2 subsequent frozen thawed cycles with no pregnancy. She has 2 remaining embryos. She consults you for an explanation and plan.

4.2 Sperm Transport in the Male Reproductive Tract

A mature and healthy spermatozoon is essential for reproductive success. The male gonads, commonly referred to as testes, have two important functions for reproduction. The first is to produce a constant supply of spermatogenic stem cells and mature them through meiosis into spermatozoa, a process called spermatogenesis. The second is to produce sex hormones, which have diverse metabolic and reproductive functions essential for spermatogenesis and male endocrinology. Even though these functions are accomplished in two distinct anatomical compartments of the testes, the interstitial compartment and seminiferous tubules, respectively, they are very reliant on each other.

Seminiferous tubules are the most abundant anatomical component of the testicle. Each testicle contains approximately 360 m of seminiferous tubules. This convoluted tubular structure is lined by Sertoli cells, which act to nourish and aid the process of spermatogenesis. In between the Sertoli cells, germline stem cells undergo differentiation into spermatogonia and ultimately mature into spermatozoa. In order to protect spermatogenesis, Sertoli cells form tight junctions between each other called the blood-testis barrier. This barrier helps regulate the entry of hormones and nutrients, and most importantly, prevents an immune response to the developing spermatozoon.

The interstitial compartment of the testes contains Leydig cells, blood vessels, myofibroblastic cells, and nerves, which all contribute to spermatogenesis via hormone production and control of the local environment. Leydig cells produce the majority of the androgens needed for spermatogenesis and male reproductive function.

It is well known that ultimate control of spermatogenesis and production of gonad-derived steroid hormones comes from the anterior pituitary gonadotropins LH and FSH. Luteinizing hormone (LH), secreted by the anterior pituitary gland, stimulates Leydig cells to produce androgens. Intra-testicular testosterone exerts its effect through binding to testosterone receptors found in Sertoli, Leydig, and peritubular cells.

The other gonadotropin, follicle-stimulating hormone (FSH), stimulates Sertoli cells to produce androgen-binding protein, which binds androgen and optimizes local androgen levels that are essential for spermatogenesis. Other secretory functions of the Sertoli cell include, but are not limited to, production of aromatase, which converts androgens to estrogens, and release of inhibin/activin, which regulate the release of FSH.

Spermatogenesis takes approximately 72 days. After production in the seminiferous tubules, spermatozoa are transported to the rete testis, efferent ducts, caput epididymis, corpus epididymis, and finally to the cauda epididymis, where they are stored until ejaculation [4]. The epididymis is not merely a storage site for spermatozoa; in fact, it is the site where spermatozoa undergo physiological modifications that result in the acquisition of progressive motility and the ability to undergo capacitation [5]. Spermatozoa in the epididymis contain free sulfhydryl groups rather than disulfide bonds, and the oxidation of those free groups helps stabilize the sperm structures [6]. Spermatozoa are able to become motile in the epididymis, but their motility is suppressed by acidification of the epididymal lumen [7]. During the transport in epididymis the sperm interacts with the seminal fluid and enriched with cholesterol, glycophospholipids. This process decreases the sperm membranes fluidity to prevent premature acrosomal reaction [8]. Activation of cannabinoid receptors on the sperm surface also helps to keep spermatozoa in an immotile state [9]. Additionally, various secretory proteins in the epididymal lumen may contribute to sperm maturation [10]. Extracellular vesicular proteins containing MIF and aldose reductase can be transported from the apical surface of the epididymal cells to spermatozoa thereby allowing spermatozoa to acquire new surface proteins that are controlled by androgen involved in further maturation of the spermatozoa [11].

Human spermatozoa seem to rely on glycolysis for ATP production and the activity of glycolytic enzymes is modified during epididymal maturation [6, 12].

With ejaculation, the spermatozoa are rapidly transported from the epididymis through the ductus deferens where they are mixed with seminal vesicle and prostatic secretions. In fact, only 5% of the ejaculate volume is composed of spermatozoa. The bulk of the ejaculate is composed of secretions from the seminal vesicle (70%) and prostatic secretions (25%). Less than 1% of the ejaculate volume comes from the bulbourethral glands.

Seminal vesicle secretions have an alkaline nature and contain rich nutritional substances, which serve as an initial energy source for spermatozoa, and proteins responsible for the "coagulum" formation, which is important to stabilize the deposited sperm in the female reproductive tract. An important component of prostatic secretions is a prostate-derived serine protease, prostate-specific antigen (PSA), responsible for liquefaction of the coagulum so that the sperm can swim freely once in the vaginal vault.

Components of ejaculate differ among individuals, as well as within a single individual. Initially, prostatic, non-coagulable secretions are followed by the sperm-rich non-coagulable component. Subsequently, seminal vesicle secretions predominate, which results in coagulation of the ejaculate. The initial non-coagulable spermatozoa have the advantage of entering the female reproductive tract earlier than the spermatozoa that are trapped in the coagulum. An average ejaculate contains 200–500 million spermatozoa, most of which are mature and motile [13].

In addition to spermatogenesis, penile anatomy is also important for reproductive success. Various anatomical abnormalities like penile curvature and uncorrected urethral openings can cause male factor infertility.

4.3 Sperm Transport in the Female Reproductive Tract

After ejaculation, mature spermatozoa are deposited near the external cervical os, or in the anterior vaginal fornix. The ejaculate coagulates within a minute and forms a loose gel in humans rather than a compact gel that is observed in rodents. The principal proteins involved in coagulation are semenogelin I and semenogelin II, which are secreted from seminal vesicles [14]. The gel formation minimizes the back-flow of deposited sperm into the vagina and protects spermatozoa against the harsh vaginal environment, yet a median of 35% of spermatozoa are still lost through retrograde flow down and out of the vagina [15]. The coagulate is then enzymatically digested in about 30–60 min. PSA is the main enzyme that is involved in this digestion. Although the alkaline nature of the seminal plasma protects the spermatozoa from the acidic vaginal environment, this protection is only transient and sperm is protected for 2 h. Alkaline environment of the ejaculate increases the pH of the sperm cytoplasm and sperm become mobile [16]. Spermatozoa have to leave the coagulant quickly to escape from inactivation or immune attack; they are only able

to remain motile in the vagina for a few hours. Within a few minutes of vaginal deposition, human sperm begins to leave the seminal pool and enter the cervical canal [17]. Cervix and the uterotubal junction are two mechanical barriers that spermatozoa need to breach. The amount of sperm that transverse the cervix depends on multiple factors, including sperm concentration, morphology, motility, molecular characteristics of the sperm surface, as well as genetic factors of the spermatozoa. Only the highest quality spermatozoa can breach these barriers, which is an evolutionary protective mechanism against polyspermy [18]. Majority of the remaining spermatozoa that do not enter the female reproductive tract are either inactivated by the acidic environment or phagocytized.

As the spermatozoa enter the cervical canal, they encounter the cervical mucus. At the time of ovulation, under the influence of estrogen, the cervical mucus is highly hydrated and the cervical pH becomes alkaline, which are optimal for spermatozoa transport and activation [19]. Failure of these physiological changes are of clinical importance since hyperandrogenic women have acidic cervical pH likely contributing to infertility [20]. Additionally, coitus on the day of maximal mucus hydration is more closely related with pregnancy success than using other indicators such as basal body temperature [21]. If the conception does not occur during this period, then, under the effect of progesterone, the cervical mucus gets thicker and creates an unfavorable environment for passage of spermatozoa.

Cervical mucus acts as a selective gate for sperm transport. Cervical mucus also acts as a barrier to abnormal sperm transport, selecting for the more vigorous and motile sperm [19, 22]. Additionally, due to flow of uterine secretions, cervical mucus is aligned to form a microarchitecture in cervical mucosal grooves. The microarchitecture is thought to guide spermatozoa to the uterus [23]. Spermatozoa carrying fragmented DNA are filtered in the cervical mucus, likely as a result of their inadequate membrane surface characteristics and motility. This selection helps to prevent sperm with poor DNA quality to reach to oocyte that would otherwise result in poor quality embryos [24].

Like the vagina, the cervix also contains immunologic barriers such as immunoglobulins, the complement system, and neutrophils, that together act to combat entry of microorganisms. However, with the aid of the seminal plasma proteins which coat spermatozoa against immune attack, highly motile spermatozoa can escape this barrier without difficulty.

As sperm enter the uterus, they are able to quickly transverse the cavity. Uterine smooth muscle contractions, which are directed caudally, increase in intensity during the late follicular phase [25]. The smooth muscle contractions appear to be limited to the layer of myometrium directly beneath the endometrial layer [26]. Thus, it appears that both active flagellar beating and uterine contractions aid in transportation through the uterine cavity. It was also suggested that these contractions could draw watery cervical mucus into the uterus. As the uterine lumen is small in volume and cervical mucus is plentiful during the peri-ovulatory phase, this would easily drag the spermatozoa through the uterine lumen [27].

The final part of the sperm transport is the passage through the uterotubal junction. In most mammals, it is narrow and may be filled with mucus. Although mucus has been shown in the uterotubal junction in humans, this does not appear to be a rate-limiting factor for sperm transport [17]. Only a few spermatozoa traverse the oviduct at any given time and move towards the ampulla, the most common site of fertilization. Movement is facilitated by oviduct contractions and fluid flow.

4.4 Sperm Capacitation

Although mature, spermatozoa are not able to fertilize an oocyte immediately after spermatogenesis. A series of molecular and physiological events that begin in the cervix and occur in the female reproductive tract give the spermatozoa the ability to fertilize the oocyte; this process is known as capacitation [28]. Capacitation encompasses plasma membrane reorganization, ion permeability regulation, membrane hyperpolarization, cholesterol loss, and changes in the phosphorylation state of many proteins [29]. Capacitation normally takes place within the female reproductive tract; however, it can be mimicked in the laboratory by incubation of sperm in a defined medium containing bicarbonate, a cholesterol acceptor like albumin, calcium, and an energy source such as pyruvate, glucose, or lactate [24].

Spermatozoa have two principal structural compartments, the head and the tail, which behave relatively independent from each other during capacitation. However, some studies suggested that they are functionally related, and as capacitation starts in the tail (hyperactivation), it subsequently triggers capacitation in the other compartment, the sperm head (acrosomal reaction) [24].

Hyperactivation, which occurs in the tail (or flagellum), increases the speed, velocity, and rate of flagellum beating when compared to spermatozoa prior to capacitation. The spermatozoa exhibit asymmetrical flagellar beats with increased amplitude of principal flagellar bend and a typical high velocity figure-eight pattern of movement. This pattern generates enough propulsive power to allow spermatozoa to navigate through the viscous oviduct fluid and penetrate the outer layers of the oocyte, namely, the cumulus oophorus and corona radiata [30]. Hyperactivation is triggered by an alkaline environment and subsequently increased intracytoplasmic calcium levels. Calcium enters into the spermatozoa from both the external milieu by sperm ion channels and release from intracellular stores [16, 31]. In addition to calcium alterations, changes in membrane permeability to potassium, sodium, protons, bicarbonate, and chloride contribute to sperm capacitation [6]. Mutations of these ion channels were found to be associated with male subfertility [32].

The spermatozoon head, where the "acrosomal reaction" occurs, is further divided into two parts: the acrosomal region and nucleus. The acrosomal region contains various enzymes that play a critical role in penetrating the zona pellucida and fusion with the oocyte, whereas the nucleus carries the paternal genetic code. The acrosomal reaction is the last step of capacitation and occurs when the spermatozoon approaches the oocyte, normally in the ampulla of the oviduct. It is described as acrosomal exocytosis and is a prerequisite for fertilization because it allows the sperm to penetrate the oocyte zona pellucida. The acrosome contains various hydrolytic enzymes like proteases, arylsulfatases, phosphatases, phospholipases, hyaluronidase, and acrosin. Calcium is obligatory for acrosomal exocytosis. There are various theories on the source of calcium increase needed for acrosomal exocytosis. One theory is that the depletion of calcium from the acrosome activates Ca^{++} channels, which allows the entry of Ca^{++} from the surrounding medium [33]. Other theories suggest that the exposure of the sperm head to the zona pellucida proteins or progesterone releases calcium stores, which are found in the redundant nuclear envelope at the posterior end of the sperm head. The increase in calcium starts from the head-tail junction and the calcium wave propagates towards the head [31]. An increase in calcium levels leads to a concomitant increase in cAMP levels, with the release of the vesicular fusion proteins; the acrosome completely discharges its enzymatic contents to penetrate the zona pellucida and fuses with the oocyte plasma membrane. The fertilization process will be discussed further, after a discussion of the oocyte.

An understanding of the capacitation process has useful clinical applications in some couples with infertility. Since the fertilizable life of a sperm is decreased once it has been capacitated, with evaluation of acrosomal status and in vitro capacitation, the timing of conception can be precisely controlled and aid in the treatment infertility.

4.5 Oocyte Development

4.5.1 Early Follicular Development

During early embryonic development, at the seventh week of gestation, gonadal stem cells derived from the yolk sac endoderm migrate to the gonadal ridges. After this migration, primordial germ cells undergo mitosis and substantially increase in number becoming "oogonium." During embryonic development, oogonium form nests and are not initially surrounded with somatic cells. These oogonial cells are then individually surrounded with flat pre-granulosa cells, forming primordial follicles. The process of forming oogonium from primordial germ cells continues until around the third trimester. Concomitantly, around the 11th week of gestation, oogonia begin to enter their first meiotic division to become primary oocytes, but this division gets arrested at the dictyotene stage. Primary oocytes will stay arrested at the dictyate stage of prophase I until postnatal life when the female enters menarche. With each menses/ovulation, only a few primary oocytes will continue to develop while the others remain arrested. The number of primary oocytes is highest in the 20th gestational week [34], estimated to be around 7 million, and steadily

decreases thereafter. It was previously believed that no oocytes were produced in reproductive-age women; however, recent data suggest the possibility of oogonial stem cells, which can give rise to new oocyte-like structures in the adult [35–38]. Further studies are needed to understand their biology and contribution to the "oocyte pool." This pool of available oocytes is the ultimate determinant of menopausal age under physiologic conditions. Depletion of the oocyte reserve starts in utero and continues thereafter [39, 40]. At the time of puberty, there are on average 200,000 primary oocytes remaining in the ovary [41].

Follicles provide support for the oocyte, and folliculogenesis occurs concomitantly with oocyte development. Initially, primordial follicles (immature oocytes surrounded by flat granulosa cells) develop and reach their maximal numbers around the same time as the peak in primary oocytes, around 20 weeks gestational age. These follicles will either continue to develop or spontaneously regress or undergo apoptosis, with only a fraction remaining by puberty [39]. Primordial follicles, also known as pre-antral follicles, are not responsive to gonadotropins and therefore rely on other factors for their development. The factors that initiate follicular development prior to attainment of gonadotropin sensitivity have not been fully determined; however, kit ligand, LIF, EGF, KGF, BMP-4, AMH, and bFGF have been shown to contribute to this process [42–47]. AMH is expressed in granulosa cells of small growing follicles and inhibits transition of primordial follicles to primary follicles. It also reduces follicle sensitivity to FSH, thereby inhibiting FSH-induced pre-antral follicle growth. In animal models, it also inhibits kit ligand and bFGF, which are known stimulatory factors for primordial follicle recruitment [48, 49]. Therefore, AMH seems to play a pivotal role in preventing follicle exhaustion and recruitment at a younger age by suppressing primordial follicles [49, 50].

As early follicular development is independent of gonadotropin stimulation, this stage of follicular development can occur before puberty, as well as during reproductive ages. However, they spontaneously regress or undergo apoptosis [51]. Only after the development of antrum, the follicle becomes responsive to the gonadotropins [44]. With puberty, maturation of the hypothalamic pituitary axis and pulsatile release of FSH and LH, antral follicles continue their development until either ovulation or atresia [52, 53]. With puberty, maturation of the hypothalamic pituitary axis and pulsatile release of FSH and LH allow antral follicle development to progress until either ovulation or atresia [53].

4.6 Cumulus Cells and Oocyte Interactions During Ovulation

Follicles contain both an oocyte and a number of cells surrounding it, including an inner layer of cumulus and outer layer of granulosa cells. The oocyte actively regulates adjacent cumulus cell (CC)/granulosa cell (GC) metabolism and creates the optimal environment for its own development. The oocyte–CC interaction is achieved by direct contact via gap junctions and by a paracrine effect of oocyte-secreted factors (OSF). Because cumulus cells lie closer to the oocyte than granulosa cells, the oocyte regulates the adjacent CC cells more than the distant granulosa cells. Two distinct factors that have been determined as OSF are growth differentiation factor 9 (GDF9) and BMP-15 [54].

The effect of OSF on granulosa and cumulus cells can be summarized as follows:

1. OSF increases DNA synthesis in both CC and GC and increases cell proliferation
2. Inhibition of CC luteinization
3. Inhibition of CC apoptosis
4. Regulation of CC metabolism
5. Promotion of CC mucification and expansion

In conclusion, the oocyte tightly controls the adjacent microenvironment for its optimal development. Under the effect of OSF, CC/GC are transformed into supportive cells for oocyte development. CC/GC have different physiological properties than mural granulosa cells, which are not affected by the OSF. Mural granulosa cells express FSH receptors and later in follicular development they are involved in steroid hormone secretion, follicular expansion, and finally, ovulation.

4.7 Late Follicular Development and Oocyte Pickup

In the follicular phase, under the influence of hypothalamic GnRH pulse frequency, anterior pituitary gonadotrophs release FSH. FSH binds to its receptors on the primary follicular granulosa cells and induces proliferation. Under continuous FSH stimulus,

pre-antral follicles escape from follicular atresia and continue to develop. A dominant follicle is then selected and continues to grow and develop under continued FSH and eventually LH stimulus, while other follicles begin to undergo atresia. After the LH surge in midcycle, the oocyte of the dominant follicle completes its first meiotic division (it had been arrested in meiosis prophase I since initial development in gestation) and shortly thereafter is expelled from the ovary. At this stage, the oocyte is surrounded by a thick glycoprotein layer, the zone pellucida, and overlying granulosa cells, which altogether form the cumulus oophorus complex. The oocyte and granulosa cells are functionally connected through gap junctions, which are thought to play an important role in local regulation of the oocyte. Shortly after ovulation, the cumulus oophorus complex is taken up by the infundibular part of the fallopian tubes. The infundibula contain fimbriae that are finger-like projections that constantly sweep the ovarian surface. The fimbriae guide the ovulated COC into the fallopian tube. Myometrial contractions together with tubal epithelial ciliary beatings are thought to contribute to this process. Within minutes, the cumulus oophorus oocyte complex can be found in the ampullary region of the tube.

During oocyte transport, the spermatozoa are moving up the fallopian tubes to meet the cumulus oophorus oocyte complex. Unlike spermatozoa which maintain fertilizing capability for days, the oocyte loses its capability to become fertilized after 12 hours in the female reproductive tract. The differential timing of oocyte and sperm viability demonstrates the clinical importance of proper timing intercourse to assure sperm availability at ovulation.

4.8 Fertilization

4.8.1 Sperm Penetration through the Cumulus Oophorus

To fertilize the oocyte, the capacitated sperm has to pass through the cumulus oophorus, a specialized layer of cuboidal granulosa cells that surround the oocyte (Fig. 4.1). These cells are

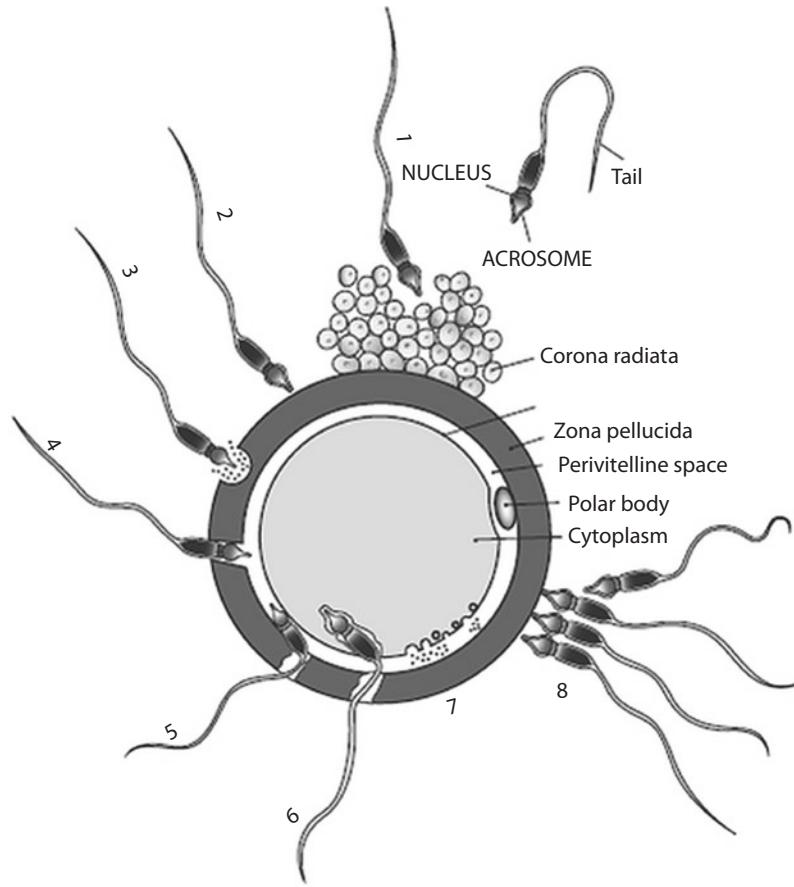

Fig. 4.1 Fertilization process. *1* Sperm penetration of cumulus cells, *2* attachment to zona, *3* exocytosis of acrosomal contents, *4* penetration to the zona pellucida, *5* entry into perivitelline space, *6* binding and fusion with the egg plasma membrane, *7* cortical reaction, and *8* block to polyspermy. Reproduced with permission from Esfandiari N. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

formed by follicular cells, which are adherent to the oocyte prior to ovulation and originate from the squamous granulosa cells present at the primordial stage of follicular development. These cumulus cells are attached to each other with an extracellular matrix that is mainly composed of hyaluronic acid, heparin sulfate, and chondroitin sulfate [55]. Although cumulus-free oocytes surrounded only by a zona pellucida are able to induce an acrosomal reaction, cumulus cells seem to foster the reaction before the sperm reach the zona pellucida [56]. Sperm hyperactivated motility also helps penetration through this initial barrier.

4.9 Structure of Zona Pellucida and Sperm Penetration

Once spermatozoa pass through the cumulus oophorus, they bind zona pellucida, which is the thick extracellular coat of the egg. (Fig. 4.2) [57]. The sperm to zona pellucida binding is a species-specific process. This concept is the basis of the "hamster zona binding test." Human sperm cannot bind to hamster eggs with an intact zona pellucida, which led to the thought that the zona pellucida contains species-specific receptors. Human sperm can only bind to hamster eggs after this glycoprotein layer is removed. Although there are some species–species exceptions, the zona pellucida is an important barrier between interspecies fertilization. In the clinical setting, the sperm penetration assay, the sperm–zona pellucida binding, the acrosome reaction, and the hyaluronan binding can be utilized in workup of subfertile men [58].

With the aid of electron microscopy and advanced molecular techniques, our understanding of the zonal structure has increased. There are three major glycoproteins that compose the zona pellucida and have distinct roles in this structure: ZP1, ZP2, and ZP3 [59]. The ZP2 and ZP3 proteins form a filamentous structure that is then cross-linked with ZP1 proteins [60, 61].

In a classical model, ZP3 binds sperm and initiates the acrosomal reaction (see Fig. 4.1). Mutagenesis of O-glycosylation sites of ZP3 has been shown to decrease sperm receptor activity, suggesting that ZP3 serves as the sperm receptor in zona pellucida [62]. As sperm binds to ZP3, the outer acrosomal membrane fuses with the sperm plasma membrane that subsequently causes membrane blebs and results in the releasing of acrosomal enzymes that lyse the zona pellucida. This reaction exposes the inner acrosomal membrane that can bind to ZP2 (Fig. 4.3) [63]. Eventually, sperm penetrate the zona pellucida and enter into the perivitelline space. Various other models have shown that this acrosomal reaction could occur when sperm encounter cumulus cells [64]. However, as mentioned above, some oocytes do not have cumulus cells and can still be fertilized.

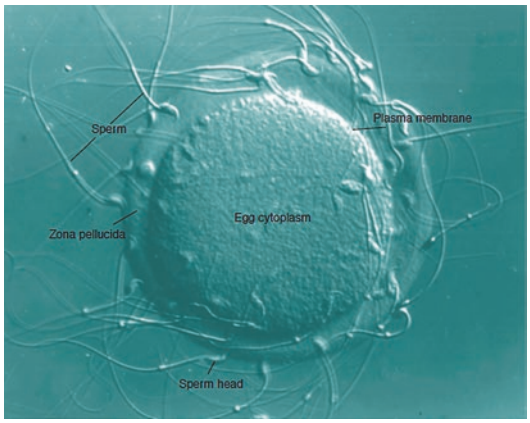

Fig. 4.2 Light microscopy of mouse sperm binding the zona pellucida of an unfertilized egg. (Source: Wassarman PM, Jovine L, Litscher ES. A profile of fertilization in mammals. Nat Cell Biol. 2001 Feb;3(2):E59–64. Used with permission from Nature Publishing Group)

4.10 Cortical Reaction to Block Polyspermy

As the spermatozoa enter the perivitelline space, they initiate the cortical reaction (see Fig. 4.1). Release of proteolytic enzymes from egg cortical granules causes cleavage of ZP2, with subsequent dissociation of ZP2 from ZP3 [65]. Thus, after the cortical reaction, sperm can no longer bind to ZP3 (Fig. 4.3) [63]. Additionally, cleaved ZP2 cannot bind a spermatozoon that had previously undergone an acrosomal reaction. In conclusion, neither a sperm with an intact acrosome nor a sperm that has undergone an acrosomal reaction would be able to bind to the zona pellucida after the cortical reaction [63]. This is the principal mechanism preventing polyspermy.

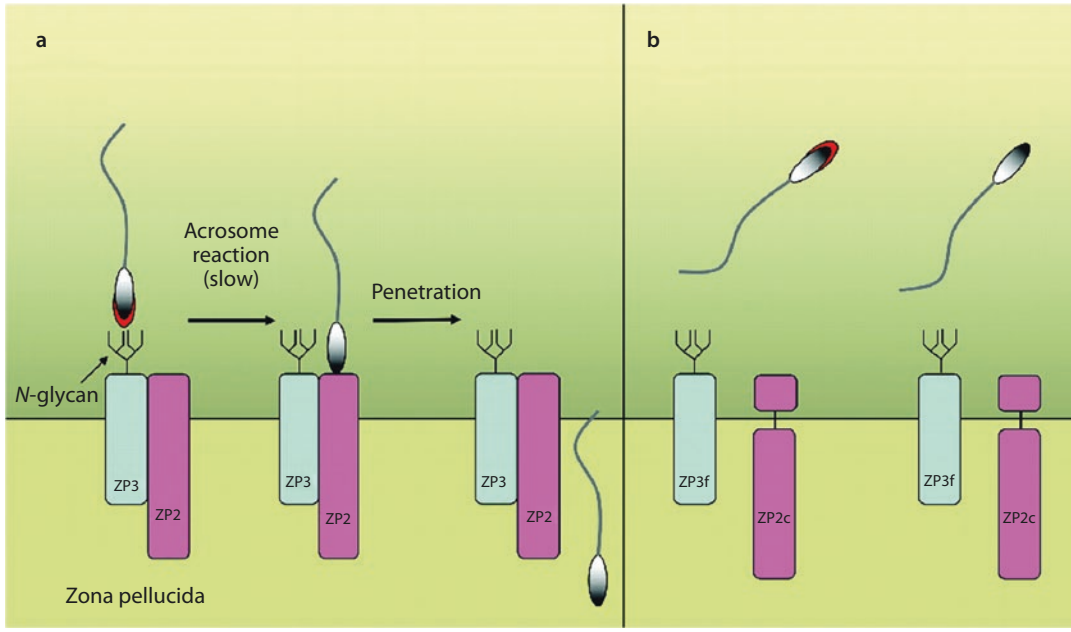

Fig. 4.3 Sperm and ZP3 binding. Acrosome intact spermatozoon shown with red crescent on its head, whereas acrosome reacted spermatozoon does not. A- ZP3 binds spermatozoon and induces acrosome reaction, thereby releasing of acrosomal enzymes that lyse the zona pellucida. Acrosome-reacted spermatozoon binds ZP2 via their exposed inner acrosomal membrane and penetrate the zona pellucida, ultimately fusing with the oocyte. B- Immediately after fertilization cortical granules release proteases to the perivitelline space that clip ZP2 and converts it to cleaved ZP2 (ZP2c) that can no longer bind acrosome-reacted sperm. Cleaved ZP2 dissociates from ZP3, resulting in subtle modification of ZP3 to convert it ZP3f that lacks sperm receptor and acrosome inducing capability. (Source: Clark GF, Reproduction. 2011 Sep.; 142(3):377–81. Used with permission from Bioscientifica Ltd.)

Although through murine models we have learned an impressive amount about the process of fertilization, there are still many questions that need to be answered and further research on the exact mechanism of sperm binding is needed.

4.11 Sperm-Oocyte Membrane Fusion

After a spermatozoon penetrates the zona pellucida and enters the perivitelline space, the oocyte membrane and the spermatozoon membrane unite (see Fig. 4.1). At this stage, the spermatozoon has already undergone an acrosomal reaction, which exposed the inner acrosomal membrane and modified the membrane composition of both equatorial and post-acrosomal regions of the spermatozoon. The fertilizing spermatozoon binds to the microvillar region of the oocyte membrane with its equatorial segment [66]. Sperm tail movement decreases or stops within a few seconds of sperm-oocyte fusion [67]. Subsequently, the posterior region of the sperm head and the tail are incorporated into the egg. Unfortunately, the details of molecular interactions in sperm-egg fusion are not fully known. Initially, ADAM family members that are found on the sperm membrane, specifically fertilin and cyritestin, gained much attention. However, gene knockout studies questioned their fundamental roles in sperm-egg fusion. Currently, cyritestin, fertilin α(alpha), fertilin β(beta), CRISP1, izumo proteins, α(alpha)6β(beta)1 integrin, GPI-anchored proteins, CD151, CD9, and CD81 on the plasma membrane are thought to be involved in sperm-oocyte membrane fusion and are the subjects of ongoing research [68, 69] (Fig. 4.4).

4.12 Oocyte Activation

Mammalian oocytes become arrested at the metaphase of the second meiotic division. After sperm-oocyte fusion, the oocyte continues

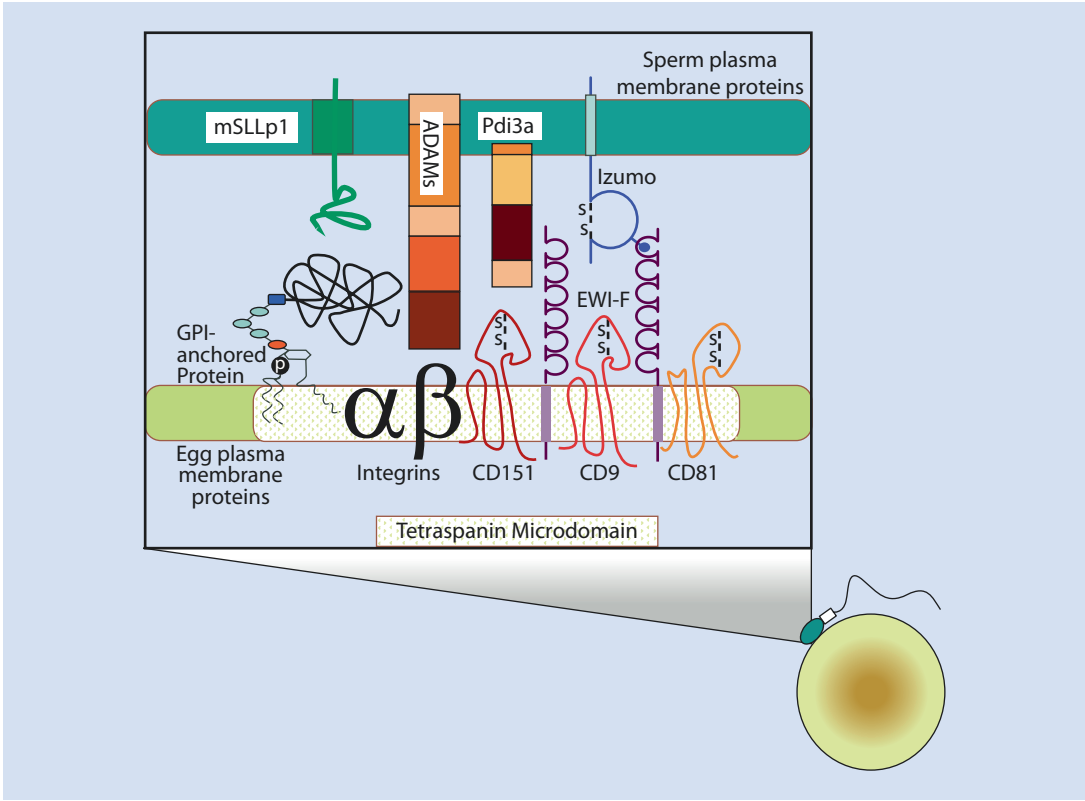

Fig. 4.4 Model for molecular interactions during sperm-egg binding. GPI-anchored proteins, integrins, CD151, CD9, CD81 on the oocyte membrane and ADAM proteins, Pdi3a chaperone refolding Izumo on the sperm membrane are involved in sperm-oocyte membrane fusion. (Source: Nixon B, Aitken RJ, McLauglin EA. Cell Mol Life Sci. 2007 Jul;64(14):1805–23. Used with permission from Springer)

meiotic division, releases cortical granules, progresses cell cycle, forms its pronucleus, and recruits maternal mRNA that are all essential for gamete formation [70]. These morphologic and biochemical changes that occur in the oocyte are collectively called "oocyte activation." Another important hallmark of oocyte activation is calcium oscillations. It has been shown that injecting calcium into mice oocytes is enough to trigger embryo development up to the blastocyst stage [71]. In a mammalian oocyte, the calcium oscillations are a direct result of inositol triphosphate-mediated calcium release. Sperm-derived phospholipase-zeta (PLC-ζ(zeta)) is also responsible for oocyte activation [72]. Another protein that has been shown to activate oocytes is post-acrosomal sheath WW domain-binding protein. Its exact signaling mechanism is not clearly known, but it presumably acts through calcium signaling [73]. Regardless of the signaling pathway, oocyte activation is essential for pronucleus formation and subsequent embryo formation.

Oocyte activation clearly has clinical importance. A deficiency in oocyte activation was regarded as the principal cause of fertilization failure or low fertilization rate after ICSI. Recently it has been suggested that PLC-ζ(zeta) could be used as an alternative oocyte-activating agent, including male factor infertility, similar to other artificial oocyte activators [74, 75].

4.13 Male Pronucleus Formation and Genomic Union

The final step of fertilization is the union of sperm and egg pronuclei, producing a diploid cell, the zygote. Dynactin, nucleoporins, vimentin, dynein, and microtubules are involved in

bringing the two pronuclei together. It was proposed that a nuclear pore complex is inserted into the nuclear envelopes of the newly forming pronuclei. Dynactin and vimentin filaments are then incorporated into this nuclear pore complex. Formation of the complex probably starts after egg activation. The sperm aster then extends the microtubule "plus ends" away from the male pronucleus, some of which reach the female pronuclear envelope. With the aid of the dynactin-dynein motor complex, the two pronuclei are apposed [76]. Subsequently, the two nuclear envelopes disappear and the DNA undergoes replication. Homologous chromosomes are paired and aligned on the newly formed mitotic spindle. Eventually, the zygote is ready to undergo its first mitotic division.

4.14 Early Embryonic Development

In mammals, the zygote undergoes mitotic division (known as cleavage) as it travels through the fallopian tube, and eventually develops into a blastocyst once in the uterus (Fig. 4.5). The symmetrical cell divisions and cleavage create a ball of totipotent cells (blastomeres) that are still enclosed in the zone pellucida. When the zygote is approximately 16 cells, blastomeres form a closely packed group of cells with a smooth outer surface. This early developmental event is called compaction. The smooth surface is created by the formation of adherens and tight junctions between the blastomeres. At this time, two types of polarity originate in the zygote. The first type of polarity is cellular polarity. Cellular polarity occurs as the formation of microvilli on the external surface of the outer blastomeres separate from the basolateral surface [77].The second type of polarity is developmental polarity. Developmental polarity is represented by the ability of the blastomeres in the internal compartment, the inner cell mass, to remain pluripotent, whereas the outer blastomeres begin to form trophoblast cells as they continue to divide [78]. This begins formation of the blastocyst and typically occurs around day 5 of fertilization. As cleavage continues, outer blastomeres express tight junction proteins, including ZO-1 and uvomorulin, gap junction proteins such as Connexin-43, and differentially position Na-K ATPase pumps selectively along

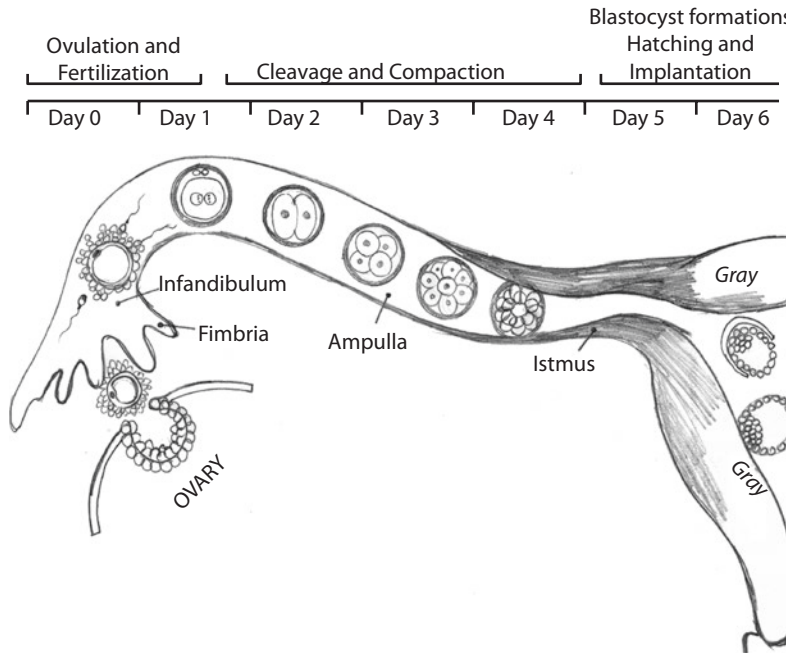

Fig. 4.5 Schematic drawing showing the major events from ovulation to the implantation of blastocyst during the first week of human life. Reproduced with permission from Esfandiari N. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

the apical-basolateral axis. The outer blastomeres have a highly restricted developmental fate, eventually becoming the cells of trophoectoderm. The polarized expression of Na-K-ATPase in trophoectoderm creates a trans-trophoectoderm sodium gradient, which drives the osmotic accumulation of water into the nascent blastocoelic cavity. Growth factors like TGF-α(alpha) and EGF increase expression of Na-K-ATPase, which subsequently stimulate further expansion of the blastocoelic cavity (blastocoel). Meanwhile, the inner blastomeres continue to divide, and with the expansion of the blastocoel, they create a cluster of cells that impend into blastocoel. This totipotent cell cluster is commonly called the inner cell mass. The inner cell mass will eventually give rise to the embryo and extraembryonic tissues . The outer layer of blastomeres, which have developed into trophoblasts/trophoectoderm, eventually give rise to the placenta. It is at this point that the developing embryo is called a "blastocyst."

Although difficult to completely exclude, this initial exponential division and formation of the blastocyst seems to be relatively independent of maternal contribution.

Around day 6 after ovulation, the embryo/blastocyst reaches the uterine cavity and is initially still covered with zona pellucida. For proper implantation, it must shed the zona pellucida. Trophoblast-derived trypsine-like enzymes, strypsin and plasmin, are thought to lyse the zona pellucida, allowing the embryo to hatch from the zona pellucida and begin to attach to the uterine endometrium [79, 80].

4.15 Trophoblastic Development and Invasion

The blastocyst is lined with a layer of trophoectoderm, which, as stated above, will give rise to the placenta. Although the inner cell mass is destined to produce embryonic and extraembryonic tissues, it stimulates trophoectodermal growth. In vitro, the removal of the ICM causes maturation of the trophoblastic cells, inducing them to turn into trophoblastic giant cells that are unable to invade the endometrium. For proper endometrial attachment, the blastocyst should remain attached to the trophoectoderm cells that are adjacent to the inner cell mass. Attachment of trophoblastic cells remote from the ICM has been associated with abnormal placental shape and eccentric insertion of the umbilical cord [81].

Prior to blastocyst attachment, for a successful pregnancy in the window of implantation, the uterine epithelium has to retract its cilia and express pinopodes. If all necessary molecular events occur, the blastocyst is firmly attached to the uterine epithelium around 6–7 days postconception.

The trophoblastic cells in contact with the inner cell mass start to proliferate and invade the uterine epithelium. As they invade, they fuse with each other and form multinucleated giant trophoblastic cells, known as syncytiotrophoblasts. An inner layer of mononucleated trophoblastic cells also develops called cytotrophoblasts. With fusion, the multinucleated syncytiotrophoblastic cells cannot proliferate, so the cytotrophoblastic cells function as a reservoir. Throughout the pregnancy, cytotrophoblastic cells divide and replenish the mature syncytiotrophoblastic cells. In addition to replenishing syncytiotrophoblasts, cytotrophoblasts give rise to various other cell types of the placenta, which are discussed below [82]

The fusion kinetics of cytotrophoblasts changes during pregnancy. In early pregnancy, two mononuclear cytotrophoblasts fuse to become a syncytiotrophoblast. However, later in the pregnancy, cytotrophoblasts fuse with already formed syncytiotrophoblasts [82]. Here we will discuss the process of early embryo development; the process of implantation will be discussed in more detail below.

Around 14 days postconception, cytotrophoblastic cells invade beyond the syncytiotrophoblastic cell layer and come into contact with maternal decidual cells. They form a column of cells with a proliferating core, and as the cells proliferate, more mature cells are passively pushed towards the maternal decidua [83]. More immature cells have α(alpha)6β(beta)4 integrin on their surface, which help bind basal membrane components like collagen IV and laminin.

However, as they move further in the column and become closer to maternal decidual cells, they change their expression of surface integrins (integrin α1/β1, α5/β1, or α-v/β3/5), which helps them attach to the maternal extracellular matrix [82, 84].

In addition to adhesion molecules, trophoblasts secrete variety of enzymes that regulate invasion. MMP-2 and MMP-9 degrade collagen IV, which is the main collagen component of the basement membrane, and are therefore regarded as key enzymes in the implantation process, enabling the invasion of the trophoblast cells through the decidua and into the maternal vasculature [85, 86]. Tissue inhibitor of matrix metalloproteinases (TIMP), particularly TIMP-1, TIMP-2, and TIMP-3, were also detected in the trophoblastic cells and decidual tissues. TIMPs are normally inhibitory metalloproteinases and their regulation through trophoblastic and decidual cytokines control MMP activity [87–89]. Other lytic enzymes involved in extracellular matrix degradation are urokinase and tissue-type plasminogen activator (uPA and tPA, respectively). Both uPA and tPA are produced by trophoblasts, and their activity is controlled by plasminogen activator inhibitors (PAI) [88]. Another trophoblast protein, adrenomedullin, decreases PAI levels and subsequently increases plasminogen activators. Additionally, adrenomedullin increases trophoblastic proliferation [90].

Trophoblasts also secrete proangiogenic factors, which stimulate new vessel formation during invasion. Neovascularization is essential for the growth and maintenance of the developing embryo. VEGF, PDGF, and PAF are the main angiogenic factors that have been shown to be secreted by trophoblasts. TGF-β(beta) and TNF-α(alpha), which are present in decidua, further stimulate trophoblastic secretion of these angiogenic factors [91].

Complex molecular interactions take place between the decidua and trophoblasts to regulate trophoblastic invasion. In addition to those factors mentioned above, cytokines like EGF, HB-EGF, IGFBP-1, LIF, IL-1 and hormones like hCG and progesterone have also been shown to regulate trophoblast invasion [88].

A number of other important types of trophoblast cells are involved in implantation—namely, extravillous, endovascular, and endoglandular. Small extravillous trophoblasts invade maternal decidua up to the inner one-third of uterine myometrium and reach the maternal spiral arteries. EVTs replace the spiral arteries' tunica media, which contains mainly the smooth muscle, and transform the spiral arteries into low resistance vessels that are no longer reactive to maternal vasomotor substances. This transformation aims to allow adequate maternal exchange with the developing fetus, particularly in the second trimester when maternal blood flow increases to the uterus to support the developing fetus. Apart from replacing the smooth muscle, endovascular trophoblasts, a subset of EVTs, replace the intimal layer of the spiral arteries [92]. Disturbances in this remodeling can result in IUGR and preeclampsia. Lastly, endoglandular trophoblasts invade the uterine glands, orient them towards the intervillous space, and replace the uterine epithelial cells (Fig. 4.6) [81].

Despite all these early trophoblastic changes, free transfer of maternal blood is only established towards the end of the first trimester. The large number of endovascular trophoblasts plugs the distal segments of the spiral arteries during initial invasion. Rather than maternal blood, the intervillous space ultimately contains glandular secretion products and maternal plasma filtrate, which are responsible for intrauterine nutrition, up until approximately 10 weeks gestation [81]. The reasoning behind the initial spiral artery plugging is believed that it helps keep a low oxygen environment and thus decrease free-radical formation during early embryogenesis.

After 10 weeks, the trophoblastic plugs dissolve and maternal blood contributes to intervillous fluid, which provides the appropriate amount of nutrients and oxygen for the developing fetus. These carefully regulated interactions between the invading trophoblasts and the maternal decidua eventually create a functional placenta, which is the main organ of nutrition, respiration, metabolite excretion, and hormone production in the developing fetus.

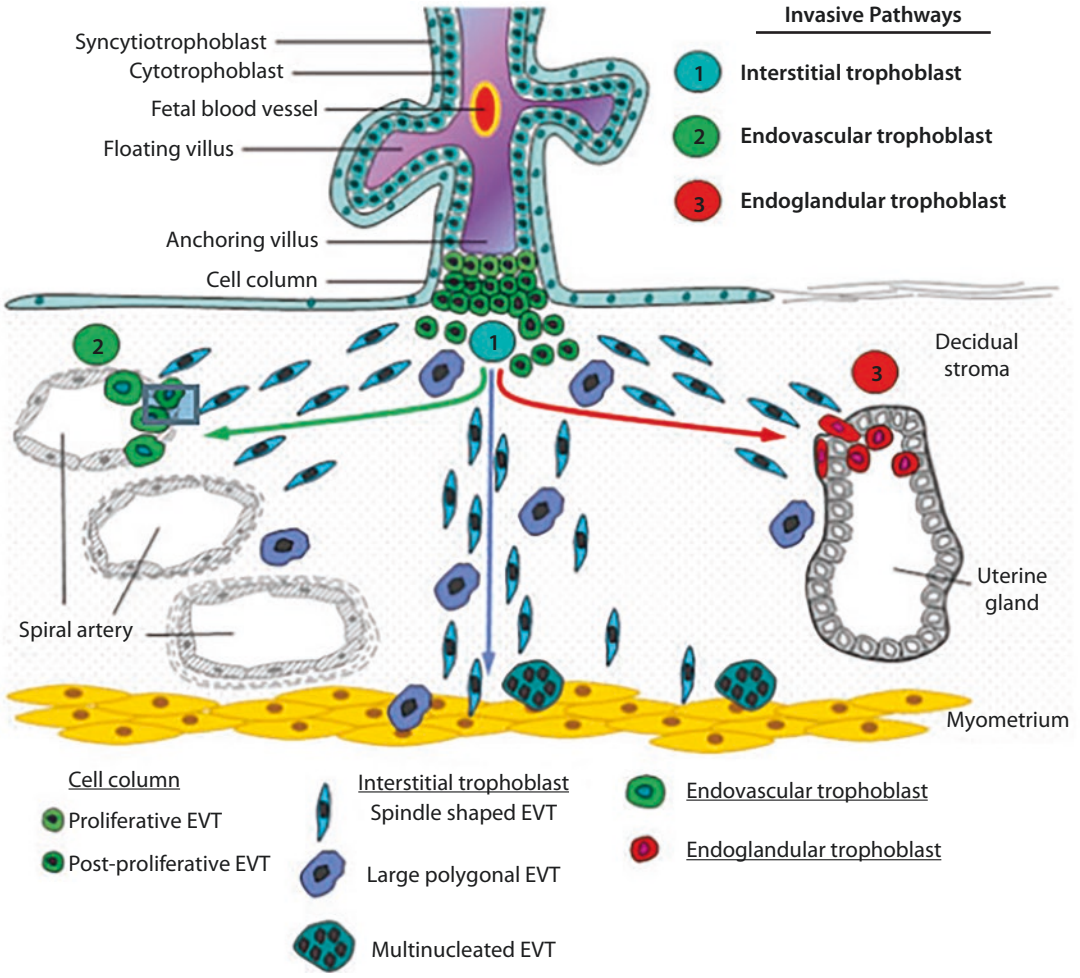

Fig. 4.6 Trophoblastic invasion of maternal decidua. Small interstitial extravillous trophoblasts invade maternal decidua up to the inner one-third of uterine myometrium and replace the tunica media maternal spiral arteries to create low resistance blood flow. Endovascular trophoblasts replace the intimal layer of the blood vessels while endoglandular trophoblasts invade the uterine glands. (Source: Huppertz B, Berghold VM, Kawaguchi R, Gauster M. Am J Reprod Immunol. 2012 May;67(5):349–57. Used with permission from John Wiley and Sons)

4.16 Implantation

Implantation can be divided into three stages: apposition, adhesion, and invasion. Apposition is the initial adhesion of the blastocyst to the endometrial surface. Apposition is unstable and with uterine flushing the blastocyst can be detached from the endometrial surface. Apposition is followed by the adhesion stage, when a stronger connection is established between the embryo and endometrium. Finally, in the invasion stage, trophoblastic cells invade the endometrium.

4.17 Endometrial Receptivity

Successful implantation requires a properly developed blastocyst, a receptive endometrium, and series of molecular interactions. In humans, 75% of the failed pregnancies are considered to be secondary to implantation failure, therefore it is essential to understand the basic molecular interactions involved in the process [93]. Under the influence of estradiol, the endometrium proliferates and reaches a critical thickness to support implantation. After ovulation, in response to

progesterone, the endometrium differentiates and becomes receptive to the newly hatched blastocyst.

Implantation occurs around 6 days after ovulation, ranging between 6 and 12 days [94]. The ideal time for implantation is thought to be around day 7 to day 9 after the LH surge and is called the "window of implantation." This period is characterized by structural and secretory changes in endometrial cells, providing the most favorable conditions for successful blastocyst implantation. The endometrium increases in thickness, becomes more vascularized, and glands become tortuous and increase their secretions rich in cholesterol, fat-soluble vitamins, lipid, and protein. These secretions will serve as an energy source for the embryo, which has no connection to uterine vessels at this point in development. In addition, there is a decrease in uterine fluid content to allow greater contact between the embryo and endometrium. These drastic changes in the uterine environment are mostly the result of progesterone stimulating the differentiation of endometrial cells into decidual cells. Decidual cells contain more intracellular lipids and glycogen deposits than endometrial cells, which cause them to get a polygonal shape as opposed to more rounded endometrial cells.

4.18 Pinopodes

One characteristic feature of receptive endometrium is the presence of pinopodes on the apical surface of endometrial cells. Pinopodes are bleb-like protrusions into the uterine lumen and are found in large numbers between days 19 and 21 in an idealized 28-day menstrual cycle. Although they are expressed throughout the mid- and late secretory phase, they show different morphological features. This suggests that their morphology, rather than their presence, is important for successful implantation [95]. Blastocyst attachment has been shown to occur preferentially on the top of pinopodes, which suggests that receptors necessary for attachment are located on the pinopode surface [96].

Pinopode development has been associated with progesterone [95], HOXA-10, LIF [97], and aVβ3 integrin [98]. HOXA-10, a homeobox gene, is necessary for blastocyst implantation, endometrial stromal cell proliferation, and epithelial cell morphogenesis [95]. Blocking HOXA-10 expression greatly decreases the number of pinopodes.

4.19 Selectins

Selectins are glycoproteins that have a glycosylated extracellular domain, single spanning transmembrane domain, and a cytoplasmic tail. There are three distinct selectins: P selectin, L selectin, and E selectin. Selectins are commonly known for their role in initial leukocyte attachment and subsequent rolling on the endothelial surface. In addition to leukocytes, however, selectins are thought to be responsible for the initial blastocyst-endometrium attachment.

Strong L selectin expression has been shown on the blastocyst surface, whereas on the maternal site, its ligands, namely MECA-79 and HECA-452, are up-regulated during the window of implantation [99]. Although L selectin is found on both luminal and glandular epithelium, expression of L selectin is higher on the luminal epithelium [100]. Initial trophoblast attachment to endometrium is thought to occur with trophoblastic L selectin and endometrial oligosaccharide interactions [101].

4.20 Integrins

Integrins are transmembrane glycoproteins composed of noncovalently linked α(alpha)- and β(beta)-subunits. Each subunit has an extracellular, intracellular, and transmembrane domain. The intracellular domains are linked to the cytoplasmic cytoskeleton and intracellular signaling pathways [96]. They are paired to compose integrin heterodimers; 24 functionally distinct integrins have been identified [102]. Among various other functions, they are mainly involved in cell-to-cell and cell-to-extracellular matrix interactions. Among the many different types of integrins that are expressed constitutively in the endometrium, α1β1, α4β1, αvβ3 are co-expressed between days 20 and 24 in the menstrual cycle. β(beta)3 integrin deserves special attention among other subunits, because its expression starts at cycle day 19 and increases thereafter [103]. Moreover, it is mainly expressed on the endometrial luminal surface, which suggests that αvβ3 integrin, and its

endometrial ligand osteopontin, might serve as a receptor for embryonic attachment [103, 104]. Various studies showed that the αvβ3 integrin is regulated in both a hormonal and paracrine manner. For example, estrogens down-regulate integrin expression, but increasing levels of progesterone in the luteal phase counteract the estrogen effect. Rather than a direct effect, progesterone increases epidermal growth factor and heparin binding growth factor in the uterine stroma, which results in increased αvβ3 levels [103]. The embryo is also actively involved in the β3 subunit regulation, probably with the embryonic IL-1 system [96].

Additionally, HOXA-10 increases the expression of the β3 subunit in endometrial cells [105]. This subunit is the rate-limiting step in αvβ3 integrin production. Considering the important role of αvβ3 integrin in the implantation process, it's not surprising that it is used as a clinical marker of endometrial receptivity [96, 106].

4.21 Mucins

Mucins are heavily glycosylated proteins. Carbohydrates constitute 50–90% of their molecular weight. To date, 18 mammalian mucin genes have been identified [107]. Mainly mucin-1 (MUC1) and to lesser extent mucin-6 (MUC6) are expressed in the human endometrium. They are found on the luminal surface of the epithelial cells in the reproductive tract. Their proposed physiological role in the reproductive tract is to trap bacteria and viruses and expel them. They are resistant to digestive enzymes. Their extracellular portion can be cleaved, and those cleaved molecules can join via sulfide bonds to create a mucin gel. Altogether, mucins produce a formidable barrier in microbial defense. Estrogens increase mucin production. Progesterone has no independent effect on mucin production; yet by counteracting the effects of estrogens, the net effect of progesterone is to decrease mucin levels. Cytokines, particularly TNF-α, have also been shown to be involved in mucin regulation.

Although they provide an important barrier in microbial defense, mucins also constitute a barrier against blastocyst implantation. Mucins extend their projections well beyond endometrial surface receptors, thereby hindering blastocyst access to them [108]. At the site of implantation, mucins' extracellular domain needs to be cleaved. The sheddase family of enzymes, particularly TACE/ADAM17 and MT1-MMP, has been suggested to play a role in this cleavage process [109]. The blastocyst, through the action of secreted cytokines, up-regulates sheddases that cleave mucins in endometrium [108].

Interestingly, during the implantation period, mucin production is increased [110]. It seems to be a paradoxical phenomenon; however, two possible explanations have been suggested. First, after sexual intercourse, the ejaculate may introduce microbial pathogens into the endometrium and the increased mucin levels may act as an additional protective barrier . Second, as the blastocyst is actively involved in sheddase induction, it has to be competent to do so. Mucins may be a protective mechanism against the attachment of unhealthy embryos that would otherwise have resulted in pregnancy failure [110]. Consistent with this view, women with recurrent pregnancy failure have decreased mucin levels compared to a fertile control group [111].

To summarize, mucins prevent embryo attachment and need to be cleaved at the site of embryonic attachment. This process involves a series of interactions that requires a healthy embryo as well as a functional endometrium.

4.22 Cytokines

Cytokines are soluble proteins that have a variety of functions in inflammation, the menstrual cycle, ovulation, and implantation. A disturbance in the normal expression or action of several cytokines results in implantation failure and abnormal placental development in humans. Of known importance are members of the gp130 family, such as LIF, IL-1, IL-11, and IL-15 system [112].

4.23 Leukemia Inhibitory Factor

A member of the gp130 cytokines, LIF acts through its surface receptor complex, LIF receptor (LIFR), and the gp130 receptor chain. Binding of LIF to LIFR results in heterodimerization with gp130 and subsequent activation of downstream signaling pathways that include the JAK/STAT, MAP kinase, and PI3 kinase pathways [96]. Other

members of the gp130 cytokine family, including oncostatin M, ciliary neurotrophic factor, cardiotrophin-1, IL-6, and IL-11, can also bind to the LIFR [112].

LIF was the first cytokine shown to be critical for implantation in mice [113]. Wild-type mice embryos failed to implant in the endometrium of homozygous LIF mutant female mice, and the implantation failure was reversed after LIF supplementation .

LIF mRNA is expressed between menstrual cycle days 18 and 28 in fertile women , and it is expressed by both glandular and luminal epithelium [114]. Among many LIF regulators, progesterone is probably responsible for endometrial LIF induction. When treated with a selective progesterone receptor modulator, mifepristone, decreased levels of LIF are observed in endometrium [115]. In addition to progesterone, IL-1α, TNF, PDGF, TGF-β1, and HB-EGF stimulate LIF expression in cultured endometrial stromal cells. The embryo secretes hCG, IGF-1, and IGF-2 that also increases LIF levels [116].

LIF protein expression is maximal in uterine flushings in the midlate secretory phase of the menstrual cycle at the time of expected implantation. Considering the ease of performing uterine flushings, LIF has been suggested as a marker of uterine receptivity [117, 118]. In women with recurrent implantation failure, LIF levels are lower than in controls, emphasizing the importance of LIF in successful implantation [118, 119]. rhLIF has also been suggested to improve endometrial receptivity in recurrent implantation failure patients; however, the efficacy has not been demonstrated in clinical trials [120].

4.24 Interleukins

IL-1 is one of the key regulatory mediators of the inflammatory response. IL-1α, IL-1β, and the IL-1 receptor antagonist are members of the IL-1 cytokine family. Stromal cells, glandular cells, and macrophages are the reservoir of IL-1 in the endometrium. In vitro, treating endometrial cells with IL-1 increases integrin β3 expression in the epithelial cells [121]. IL-1α knockout mice are, however, fertile, suggesting redundancy in the effects these interleukins have in implantation. IL-1 receptor antagonist expression is decreased during the implantation window. It is possible that down-regulation of IL-1 antagonist works synergistically with IL-1 to affect implantation [122]. Exogenous IL-1 receptor antagonist treatment during implantation can block blastocyst implantation [121]. Overall, the IL-1 system is clearly involved in implantation; however, its exact role in implantation remains unclear.

IL-6 is involved in many immune interactions, and it has been also suggested to play a role in implantation. Endometrial IL-6 mRNA expression increases during the mid- to late secretory phase and decreases in the late secretory phase. Strong immunoreactivity has been observed in uterine glandular and luminal epithelium during the window of implantation [123]. While controversial, IL-6-deficient mice appear to have reduced fertility and decreased implantation rate [124]. The IL-6 receptor is found on the surface of the blastocyst, and IL-6 is probably involved in paracrine/autocrine interactions in the window of implantation. Decreased levels of mid-secretory IL-6 mRNA are found in patients with recurrent spontaneous abortions, also supporting this hypothesis [125].

Another cytokine that has gained attention is IL-11. IL-11 has anti-inflammatory activities, and it is expressed in endometrial glandular and luminal epithelium. Estrogen, progesterone, and local factors increase IL-11 levels. IL-11 advances progesterone-induced decidualization of human endometrial stromal cells. IL-11 and its receptor IL-11R were immunolocalized to decidualized stromal cells in the mid-late secretory phase epithelium. They were also shown on the trophoblastic cells, suggesting a role in normal placentation [112]. Additionally, inadequate IL-11 signaling was found to result in dysregulation in trophoblastic invasion [126].

4.25 Prostaglandins

Prostaglandins (PGs) are lipid mediators of inflammation, and they have a variety of functions in inflammation, menstrual cycle regulation, ovulation, embryo attachment, trophoblastic invasion, and labor. Prostaglandins, leukotrienes, and thromboxanes are members of the eicosanoid family. They are produced from membrane lipids by phospholipase A2 (PLA2) and cyclooxygenase (COX) enzymes. To date, three isoforms of COX enzymes have been discovered:

COX-1, COX-2, and COX-3. COX-1 is constitutive and expressed under normal physiological functions, whereas COX-2 is involved mainly in inflammatory responses. COX-3 is expressed in the human brain and is responsible for fever and response to pain.

Murine studies have shown the importance of prostaglandins (PGs) in implantation. Lack of either PLA2 or COX2 in mice leads to defective PG synthesis; PLA2 knockout mice show pregnancy failure [127]. COX expression is maximal in the menstrual and proliferative phases . Among many regulators, IL-1 deserves further consideration. IL-1 increases COX enzymes and PG production, resulting in increased endometrial integrin levels that are essential for blastocyst implantation [96].

Although prostaglandins' role in the menstrual cycle and pathophysiology in endometriosis is well known, their role in human blastocyst attachment and subsequent invasion needs to be explored further.

4.26 HOX Genes

Homeobox (HOX) genes are highly conserved genes that are involved in embryonic development as well as endometrial growth, differentiation, and receptivity [128]. Both estrogen and progesterone increase HOXA10 and HOXA11 expression. Additionally, HOXA10 and HOXA11 expression reach the highest levels during the window of implantation [129]. Wild-type mice embryos cannot implant to the uteri of HOXA10 or HOXA11 knockout mice. These findings suggest that HOXA10 and HOXA11 play an essential role in endometrial receptivity [130]. In parallel with these findings, pinopodes, β3 integrin, and insulin-like growth factor binding protein were shown to be regulated by HOX genes [131]. As discussed above, these genes are among the few proven to be essential for endometrial receptivity. In humans, there are no documented HOXA10 or HOXA11 mutations. However, in various gynecologic disorders such as endometriosis, PCOS, hydrosalpinx, and uterine fibroids, endometrial HOXA10 and HOXA11 mRNA levels are reduced [132–134]. These findings demonstrate that HOX genes contribute to the defective endometrial receptivity that is observed in those disorders.

4.27 Immune Response to Trophoblast Invasion: Trophoblast–Leukocyte Interactions

As mentioned above, to achieve a successful pregnancy, the blastocyst must be able to attach to the endometrial decidua without complication. The blastocyst has to invade the endometrium and maternal blood vessels in order to ensure adequate blood supply for nutrients and gas exchange. However, because a blastocyst receives half of its genome from the father and the other half from the mother, it is treated as a semiallogenic by the maternal immune system. Therefore, alterations in the reactivity of the maternal immune system must occur at the maternal–fetal interface.

As the blastocyst attaches to the uterine epithelium, the trophoectoderm differentiates into two layers, as previously mentioned, an outer syncytiotrophoblast and an inner cytotrophoblast layer. Two weeks after implantation, the cytotrophoblast layer protrudes through the syncytiotrophoblasts and forms cytotrophoblastic buds. The buds then differentiate into both villious trophoblasts and extravillous trophoblasts. Villous trophoblasts cover the chorionic villi which form the main interface for gas and nutrient exchange between the fetus and mother, and as discussed above, extravillous trophoblasts invade and remodel the spiral arteries.

Maternal and fetal cells are in direct contact during this invasion process, and the immune response deserves a detailed explanation. Maternal leukocytes reside in the uterine endometrium, and it has been estimated that approximately 40% of the decidua is leukocytes. Fortunately, trophoblasts have a distinct MHC expression profile. They do not express the most common HLA antigens such as HLA-A and HLA-B, and even with potent stimulators like IFN-α, they do not express MHC class II antigens recognized by certain leukocytes. The dominant MHC types expressed on trophoblasts are HLA-C, HLA-G, and HLA-E.

Villous syncytiotrophoblasts line blood-filled lacunae and are in direct contact with maternal blood. They do not express MHC-I antigens and are therefore protected from T-cell-mediated responses. Interstitial trophoblasts invade the decidua and express HLA-C, HLA-G, and HLA-E. Endovascular trophoblasts that line the maternal spiral arteries express HLA-C, HLA-G, and

HLA-E. The expression of HLA-G and HLA-E on this cell population confers protection from maternal immune rejection.

Leukocytes are normally found in endometrium and actually 40% of the decidua consists of leukocytes. In endometrial infection, there are a multiple types of leukocytes that are found in the endometrial lining, and they all act through different mechanisms. B-cells respond to antigenic stimulation and produce antibody secreting plasma cells.

Additionally, macrophages can be found and represent approximately 20% of the endometrial leukocytes; they can recognize and respond to HLA-G antigens [135].

T cells represent 10% of the leukocytes found in the endometrium. They require an MHC-II antigen presentation for immune response and, as trophoblasts do not express MHC-II antigens, they cannot directly stimulate T-cell responses. This is how villous syncytiotrophoblasts, which line the maternal blood-filled lacunae, are protected from the maternal T cells. However, maternal endometrial dendritic cells and macrophages can process paternally derived antigens by migrating to lymph nodes where they can initiate an immune response.

Interestingly, antibodies against paternal HLA antigens can be found during pregnancy; however, they are likely formed during birth due to fetal cells crossing the placenta. Fortunately, these antibodies are mainly against HLA-A and HLA-B. As these HLA types are not expressed by the trophoblasts, the presence of these antibodies is not correlated with pregnancy success [92].

Natural killer T (NKT) cells are a subset of T cells that have an immunomodulatory role in infection through cytokine production. Invading trophoblasts are protected from blood NK cells through multiple different mechanisms. Villous syncytiotrophoblasts are likely protected by the absence of NK-activating ligands on the syncytiotrophoblastic surface. Similarly, endovascular and interstitial trophoblasts, which line maternal spiral arteries and decidua, respectively, express HLA-C, HLA-G, and HLA-E. The expression of HLA-G and HLA-E on these cell populations confers protection from blood NK cells.

Uterine NK cells (uNK) are among the most studied endometrial leukocyte type and are known to be involved in endometrial renewal, differentiation, and breakdown in menstrual cycle. Although their exact role in implantation is unknown, their dysregulation has been shown to be associated with recurrent pregnancy loss, preeclampsia, and implantation failure. uNK cells are $CD56^{bright}CD16^{dim}$ and functionally distinct compared to their circulating counterparts. They are spatially and temporally correlated with the implantation site of the embryo and modulate the cytokine, chemokine microenvironment, thereby contributing to physiological changes within the uterine stroma during pregnancy [136]. Their origin is still unknown, yet they are thought to arise from in utero proliferation and differentiation of CD34+ stem cells. They are found in deeper layers of decidua and are not shed during menstruation. Another alternative to their origin is recruitment from CD56+ cells in the blood into the endometrium. Regardless of their origin, their quantity correlates with maternal progesterone levels. Additionally, they are found to accumulate in large numbers at the site of implantation. Their close proximity to trophoblasts suggests that they may be involved in regulating trophoblastic invasion [92]. In addition, lower uNK counts in the endometrium have been correlated with decreased IVF-ET success [137]. In summary, although dysregulation of uNK cells and their cytokine production profile was shown to be related with recurrent pregnancy loss, preeclampsia, and implantation failure, their exact role in implantation is not known [138].

4.28 Clinical Relevance

Infertility is classically defined as the failure of a couple to conceive after 12 months of frequent intercourse in women under 35 years, and after 6 months in women over the age of 35 [139]. Infertility can be due to male factors, female factors, or both. The availability of ICSI/IVF has resulted in pregnancy rates in couples with male factor infertility that are comparable to those without male factor infertility [140].

Key steps for successful fertilization are the oocyte quality and appropriate oocyte maturation. As our understanding of oocyte biology has increased, we are able to mimic endogenous oocyte developmental steps in vitro. One such success in reproductive medicine is improving in vitro maturation (IVM), in which immature oocytes are collected and then maturated for

in vitro fertilization. This technique provides an invaluable opportunity for many infertile patients. Additionally, IVM and IVF provide an opportunity for fertility preservation, including use in patients undergoing gonadotoxic chemotherapy for various cancers [141]. As will be discussed further in the book, morphokinetics of the developing embryo have been used to select viable embryos that are more likely to implant the endometrium and result in successful pregnancy [142].

Defective endometrial receptivity is a significant cause of ART failure [143]. Therefore, it is essential to correctly assess the endometrial receptivity state for successful implantation. Among many others, pinopodes and αvβ3 integrin have been suggested as candidate biomarkers that reflect the window of implantation [144]. Endometrial scratching and G-CSF have been used to improve endometrial receptivity and implantation, however strong evidence favoring these methods is lacking [145–147]. Endometrial receptivity arrays based on the transcriptomic data have been developed to predict the window implantation in efforts to increase implantation success [148, 149]. However, there is currently insufficient evidence from adequately powered prospective clinical trials to validate these markers. Patients with endometriosis, fibroids, PCOS, and hydrosalpinx frequently present with infertility due at least in part to defective endometrial receptivity. Recognizing and treating the underlying etiology can at least partly improve endometrial receptivity in these patients.

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Reproductive Imaging

Miriam S. Krause and Steven T. Nakajima

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5.1 Introduction

Several different techniques are available to image the female reproductive tract: **pelvic sonography**, **saline infusion sonography (SIS)**, **hysterosalpingo-contrast sonography (HyCoSy)**, a **hysterosalpingogram (HSG)**, or a **magnetic resonance imaging (MRI)** procedure. The optimal **imaging** method should be diagnostically accurate, cost-effective, minimally invasive, and reliable. Depending on the disease process or anatomic variant to be depicted, one imaging modality may be better suited than another, or more than one diagnostic test may be needed. Each procedure has advantages and disadvantages and can be the superior modality in certain specific clinical settings; Table 5.1 summarizes these findings. Table 5.2 contains a decision tree to choose the best imaging modality. Laparoscopy and hysteroscopy are additional means to directly visualize the pelvic structures, but are more invasive with surgical risk factors. An important advantage of direct visualization is the ability to perform therapeutic interventions.

5

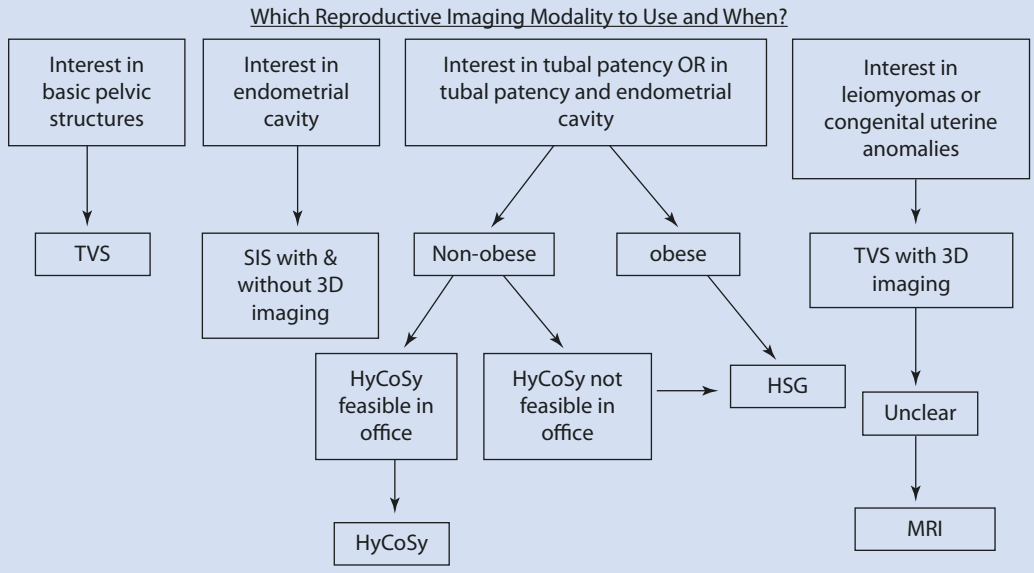
Clinical Case

A 34-year-old woman presents with a history of regular heavy menstrual bleeding. She is a gravida 0. She was tried on progestin medical therapy with no improvement. Pelvic ultrasound was normal. Endometrial biopsy showed hormonal effect with no other abnormality. A saline infusion sonography is ordered.

Table 5.1 Advantages and disadvantages of different imaging modalities

Imaging modality	Advantages	Disadvantages
TVS	Easily available	Limited information on tubal or endometrial pathology
	Relatively inexpensive	Can be limited in obese individuals
SIS	Assessment of endometrial pathology	Limited information on tubal pathology
HyCoSy	Visualization of ovaries, tubes, and endometrial lining	Patient discomfort
		Limited in obese individuals
		May require additional supply items
HSG	Better visualization of the entire fallopian tube	More invasive procedure
	Possible increase in pregnancy rate	Radiation exposure
	Less limited by body habitus	Risk of sensitivity to contrast
		Not available in all facilities
MRI	Better method to assess leiomyomas or uterine anomalies	Expensive
	Not limited by body habitus, less observer dependent, more reproducible	Not available in all facilities

Table 5.2 Reproductive imaging modality decision tree analysis



5.2 Pelvic Ultrasonography

Both transabdominal (TAS) and transvaginal **sonography** (TVS) are safe, noninvasive, and readily available in most office settings. Sonography can provide an easily accessible image of the uterus, ovaries, and other pelvic structures (pelvic kidney, appendix or adnexal masses).

5.2.1 Principles

Ultrasound images are obtained by emitting pulses of high-frequency sound and measuring the echoes that are reflected back to the transducer from interfaces between tissue structures of different impedance. The echoes are then transformed into real-time dynamic images of these structures. Most probes are curvilinear or convex, providing a wider field of view within a compact probe design. Most ultrasound images are obtained using *B-mode* (brightness) or *gray-scale*, displaying images in two dimensions. **Ultrasound** can also be used in the following modes: (1) *M-mode* to analyze cardiac motion, (2) *color flow Doppler ultrasonography* to measure the speed and direction of blood flow, or (3) *three-dimensional (3D) ultrasonography*, in which multiple B-mode images are combined into a 3D image that displays volume. Ultrasound frequencies in gynecology usually range between 3 and 7.5 MHz. Lower frequencies penetrate tissue more deeply with poorer resolution [1]. Conversely, higher frequencies penetrate tissue less deeply but give better resolution. Since ultrasound is a real-time technique, the performing provider can obtain additional information during the exam in regard to focal pain or lack of organ movement, which can be indicative of pelvic pathology.

5.2.2 Technical Considerations

Ultrasound can be performed via transabdominal or transvaginal approach, with the latter being

preferred for gynecologic imaging since the probe is closer to the pelvic organs and allows better resolution of these structures. Large uterine or ovarian masses extending out of the pelvis, however, can be missed on transvaginal scanning; therefore, both approaches may be needed. The transabdominal approach is an option in a virginal patient and requires a full bladder to provide an acoustic window in order to fully delineate the pelvic structures. No prophylactic antibiotics or special analgesics are required for pelvic ultrasonography. A pelvic ultrasound is considered basically risk-free in non-pregnant patients. In pregnant patients, ultrasounds should only be performed if indicated [2]. However no studies have shown any abnormalities in children after prenatal ultrasounds [3].

Transvaginal probes should be disinfected after use and covered to prevent the transmission of infections between patients. Of note, the leakage rate when using condoms as vaginal probe covers was reported to be 0.9–2%, and as high as 8–81% when using commercial probe covers [4]. Agents available for high-level disinfection include glutaraldehyde, stabilized hydrogen peroxide (6%), orthophalaldehyde, peracetic acid, and peracetic acid-hydrogen peroxide [5], but compatibility of the probe and disinfectant should be confirmed per manufacturer's instructions.

Pelvic ultrasound is best performed in the early follicular phase, when the endometrium is thin and endometrial pathology can be better visualized [6]. Heavy bleeding should not be present, because blood clots can be misinterpreted as polyps or adhesions [7]. A small amount of blood, however, can delineate the endometrial–myometrial interface (spontaneous sonohysterogram [8]).

Evaluation of the pelvis should proceed in the following systematic fashion, examining each area with respect to the following parameters:

1. Uterus: Measurement of length, height, and width in longitudinal and transverse axes; size, number, and location of any leiomyomas, position and configuration of the uterus, thickness and appearance of endometrial lining, description of the junctional zone between endometrium and myometrium, any uterine malformations, or cervical abnormalities.
2. Ovaries: Measurement of length, height, and width in longitudinal and transverse axes, number of antral follicles measuring between 2 and 9 mm, size and characteristics of any larger ovarian masses.
3. Posterior cul de sac: Presence of any free fluid.
4. Fallopian tubes: Normal fallopian tubes are rarely seen on pelvic ultrasound. A hypoechoic tubular or tortuous structure is suggestive of a hydrosalpinx [9], particularly if the "waist sign" is observed. The "waist sign" refers to diametrically opposed indentations in the wall of a cystic collection [10],

5.2.3 Limitations

Visualization of pelvic structures can be difficult when using a transabdominal approach on an obese individual. Overlying bowel can also interfere with visualization on both transabdominal and transvaginal ultrasound studies. There can be considerable variability in the quality and diagnostic capability of sonography dependent upon the experience and expertise of the ultrasonographer. Of note, it is often easier for a clinician to detect abnormalities while performing or observing a real-time dynamic scan rather than when reviewing previously acquired static images. Tubal patency cannot be assessed with standard ultrasonography. If endometrial pathology is suspected, *saline infusion sonogram* should be utilized.

5.2.4 Indications

Indications for pelvic ultrasonography include the following: (1) management of pelvic masses, (2) evaluation for ovarian torsion, (3) abnormal uterine bleeding, (4) uterine leiomyomas, (5) pelvic pain, (6) recurrent pregnancy loss, or (7) foreign bodies in the uterus. Ultrasonography is widely used for an infertility evaluation and includes (1) monitoring of follicle maturation (Fig. 5.1), (2) assessment of endometrial thickness (Fig. 5.2), (3) transvaginal oocyte aspiration, (4) ultrasound-guided embryo transfer [11], or (5) detection of hydrosalpinges (Fig. 5.3a, b). Different publications have shown sensitivity of 86% [12] and specificity of 99.6% [13] for detecting a hydrosalpinx on transvaginal ultrasonography. 3D ultrasound can help to distinguish a hydrosalpinx from a complex ovarian cyst, since the entire tube is visualized spatially. Ultrasonography may be also helpful for the

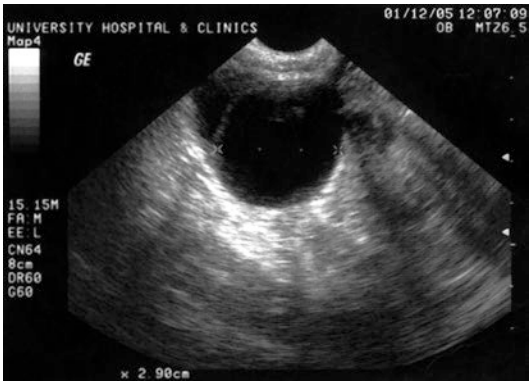


Fig. 5.1 Cystic structure representing a dominant ovarian follicle. Reproduced with permission from Lindheim SR, Uhler ML. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

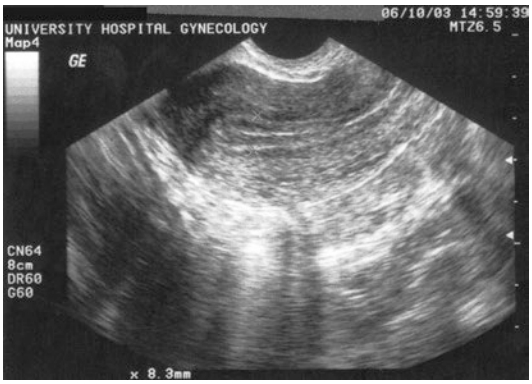


Fig. 5.2 Trilaminar appearance of the endometrial echo. Reproduced with permission from Lindheim SR, Uhler ML. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

diagnosis of adenomyosis. Adenomyosis is suspected when the uterus is globular and bulky, with the myometrium being asymmetrically thickened. The junctional zone between endometrium and myometrium is usually ill-defined and heterogenous, which is caused by dilated endometrial glands in the myometrium.

For the detection and classification of congenital uterine anomalies, a number of studies have demonstrated that 3D sonography is a reasonable alternative to a HSG or MRI procedure [14, 15]. 3D sonography allows one to visualize the external uterine contour as well as the internal morphology in the coronal plane. Bocca et al. [14] performed a prospective blinded study with 101 females who underwent routine HSG and 3D sonography as compared to surgical findings. The authors found 30 congenital anomalies (arcuate, unicornuate, bicornuate, septate uteri as well as uterine didelphys). Of the 30 congenital anomalies, all were correctly identified with 3D sonography, compared to only 10 correctly identified with HSG. Caliskan et al. [16] found that uterine anomalies visualized on 3D sonography are easier to interpret in the luteal phase compared to the follicular phase secondary to increased thickness and echogenicity of the endometrium. Ghi et al. [17] performed a prospective study on 284 nulliparous patients with at least three consecutive miscarriages who underwent 3D sonography. If the 3D sonography demonstrated a normal external and internal uterine contour, patients would undergo an office hysteroscopy. If the 3D sonography was

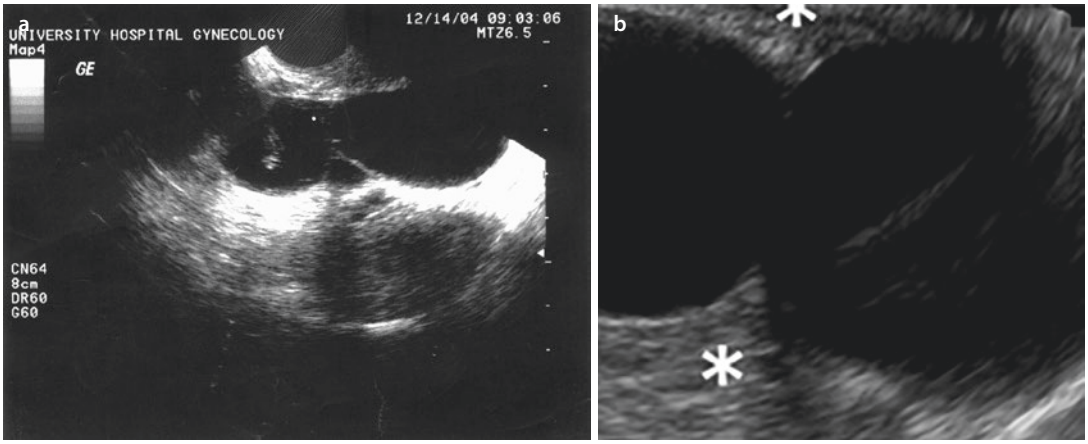


Fig. 5.3 **a** Hydrosalpinx. A hypoechoic tubular structure. Reproduced with permission from Lindheim SR, Uhler ML. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007. **b** A hypoechoic tubular or tortuous structure is suggestive of a hydrosalpinx, particularly if the "waist sign" is observed. The "waist sign" refers to diametrically opposed indentations in the wall of a cystic collection

abnormal, they underwent a concurrent hysteroscopy and laparoscopy. All 230 patients with negative 3D sonography results exhibited a normal uterine cavity at the time of office hysteroscopy. In the group with the abnormal 3D sonography, the presence of a Müllerian anomaly was correct in 52 out of 54 patients. 3D sonography in this study had a positive predictive value of 96.3% and a negative predictive value of 100%.

Currently only limited information is available to compare 3D sonography with MRI in the diagnosis of Müllerian anomalies. Bermejo et al. [15] found a concordance rate (kappa index = 0.880) between the two imaging modalities, however only 65 of the 286 females undergoing 3D ultrasound also had an MRI procedure.

The above studies suggest 3D sonography is an accurate and non-invasive tool to detect congenital uterine anomalies, with the advantages of less cost, lower morbidity, shorter examination time, and the availability to perform this procedure in the office setting.

5.3 Saline Infusion Sonography (SIS) or Sonohysterography (SHG)

5.3.1 Principles

Transvaginal ultrasonography alone has limited usefulness for demonstrating pathology in the endometrial cavity [18]. SIS enhances the ability to visualize lesions projecting into the uterine cavity. During the procedure, the endometrial cavity is filled with saline via a transcervical catheter, as first described by Nannini in 1981 [19]. Deichert et al. reported statistical equivalence among SIS, HSG, and hysteroscopy in regard to the evaluation of intrauterine pathology [20].

5.3.2 Technical Considerations

The SIS procedure should be performed between cycle days five to ten to avoid menstrual blood being misinterpreted as an intracavitary artifact. A follicular phase study ensures a thin endometrial lining, and avoids the possibility of an early pregnancy. Some providers recommend a urine pregnancy test prior to the SIS to decrease the risk of a concurrent pregnancy. If a patient is taking oral contraceptive pills, an SIS can be performed with less concern in regard to an occult conception and can facilitate timing of the procedure. Uterine cramping can occur, which usually responds well to the use of a nonsteroidal anti-inflammatory medication 30 min prior to performing the procedure.

Prior to the SIS procedure, informed consent should be obtained for the following possible sequelae: cramping, uterine bleeding, vasovagal reaction, or infection. Using an open-sided speculum, the cervix is cleansed with an antiseptic, and the SIS catheter is placed through the cervix. Different catheters can be used, including a standard size 5- or 7-Fr double-lumen intrauterine HSG catheter, a more rigid Goldstein sonohysterography catheter (Cook Ob/Gyn, Spencer, IN, USA), or a latex-free urethrane H/S Elliptosphere catheter (Akrad Laboratories, Cranford, NJ, USA), which contains an inflatable balloon (Figs. 5.4 and 5.5). An 8-F pediatric Foley catheter can also be used, but it is more difficult to insert. Next, the speculum is removed and the transvaginal probe replaced. Sterile saline is then

Fig. 5.4 Goldstein sonohysterography catheter (Cook Ob/Gyn, Spencer, IN, USA). Reproduced with permission from Lindheim SR, Uhler ML. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

Fig. 5.5 H/S Elliptosphere catheter with latex-free urethane (Cooper Surgical Inc., Turnbull, CT, USA). Reproduced with permission from Lindheim SR, Uhler ML. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

slowly injected into the uterine cavity, which leads to separation of the anterior and posterior uterine walls. Usually there is no need to insufflate the balloon. The uterus is then scanned in the longitudinal and transverse plane and 3D pictures can be obtained. If one encounters difficulty placing the catheter, a tenaculum can be placed on the cervix for straightening of the cervical canal, and a dilator utilized. If this is unsuccessful, the procedure can be repeated after having the patient take 400 μg of misoprostol orally 12 h prior to the SIS procedure for pre-procedure cervical dilation.

No uniform guidelines exist for antibiotic administration in patients undergoing SIS. For the HSG procedure, the American College of Obstetricians and Gynecologists (ACOG) recommends doxycycline 100 mg orally BID × 5 days if the patient has a history of pelvic inflammatory disease or if the procedure demonstrates dilated tubes [21]. There are no studies assessing the rate of post-SIS pelvic infections. One study reported four cases of pelvic infections after diagnostic or operative hysteroscopy that were successfully treated with antibiotics [22]. The decision to prophylactically treat a patient undergoing an SIS procedure is left to the provider, with some arguing to use the same criteria as outlined by ACOG for the HSG procedure.

5.3.3 Limitations

SIS should not be performed in a patient with documented intrauterine gestation, pelvic infection, or unexplained pelvic tenderness [23]. If a patient has a history of a known hydrosalpinx, some would defer the SIS procedure for the concern of a post-procedure infection. SIS can indirectly assess tubal patency by documentation of fluid in the posterior cul de sac; however, it cannot differentiate between laterality.

5.3.4 Indications

SIS can detect focal intrauterine lesions, such as polyps (Fig. 5.6), submucosal uterine leiomyomas, or endometrial hyperplasia [24]. The incidence of polyps and submucosal leiomyomas in symptomatic premenopausal women is 33% and 21%, respectively [25]. The SIS procedure has been reported to be as accurate as hysteroscopy for the detection of focally growing lesions, with sensitivities for both procedures of approximately 96% [26]. Histologic tissue evaluation is recommended whenever intrauterine pathology is discovered, and blind endometrial biopsy may miss the pathology [27]. SIS in conjunction with 3D ultrasound can also help clarify any uterine malformations, such as septate versus bicornuate uterus, by depicting the outer uterine contour.

Uterine leiomyomas are classified into three categories based on their location within the uterus, according to the European Society of Hysteroscopy Classification of Submucosal Fibroids [28]:

Class 1: Complete submucosal involvement with no myometrial involvement (intracavitary)

Class 2: Submucosal component involving less than half of the myometrium

Class 3: Submucosal involvement with an intramural component of greater than 50%

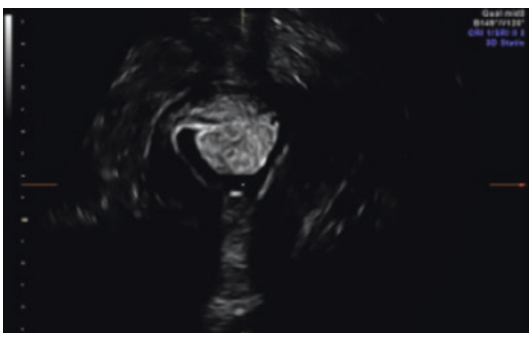


Fig. 5.6 Large endometrial polyp. Image provided by Steven Nakajima, MD, University of Louisville, Louisville, KY

5.4 Hystero-Salpingo Contrastsonography (HyCoSy)

5.4.1 Principles

The HyCoSy can be the most comprehensive tool to evaluate the pelvic organs when compared to transvaginal ultrasound and SIS. In addition to gathering information about the uterus and the ovaries, the HyCoSy can also evaluate patency of the fallopian tubes, which are difficult to visualize on regular ultrasound if they are anatomically normal [29]. The HyCoSy uses contrast media that helps to visualize the structure of the fallopian tubes while transvaginal ultrasound is performed concomitantly. When compared to hysterosalpingogram (HSG), there is no exposure to radiation, and the procedure can be performed in the office rather than in the radiology suite.

5.4.2 Technical considerations

The HyCoSy procedure is normally conducted after performing an SIS procedure. The timing of the procedure, prerequisites, contraindications, and use of antibiotics are the same as for an SIS procedure. After evaluation of the uterine cavity, the intrauterine balloon is inflated with 3 mL of either fluid or air to occlude the lower uterine segment. For this reason, a Goldstein catheter cannot be used for the HyCoSy procedure. Next, a 20 mL syringe filled with both saline and air can be tilted intermittently to infuse 1–3 mL increments of saline followed by air [26]. Alternatively, a syringe with saline and air can be vigorously shaken immediately prior to infusion [30], or a commercial device is available that mixes the saline and air prior to the infusion. The mixture of saline and air can then be seen as "scintillations" travelling from the proximal interstitial portion of the tube to the distal fimbria and ovary [31] (Figs. 5.7 and 5.8). If a tube fails to fill with the air/fluid mixture, it can be advantageous to ask the patient to roll slightly to position the tube superiorly. If no proximal or distal scintillations can be seen, this may represent true obstruction versus spasm in the cornual region. Once the study is completed, the balloon is deflated and all instruments removed from the vagina. The possibility of shoulder pain after the procedure secondary to peritoneal irritation by intraabdominal air should be discussed with the patient. This common side effect usually resolves after 24 h.

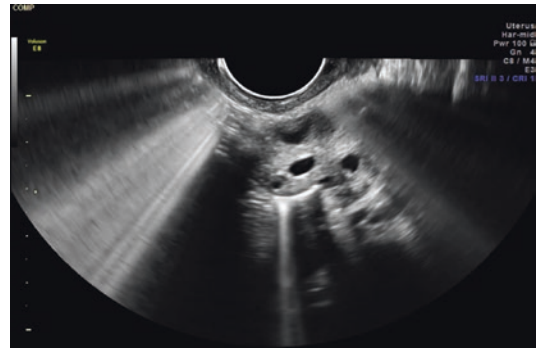


Fig. 5.7 Echogenic contrast identified in the proximal and distal portion of the right fallopian tube. Images provided by Steven Nakajima, MD, University of Louisville, Louisville, KY

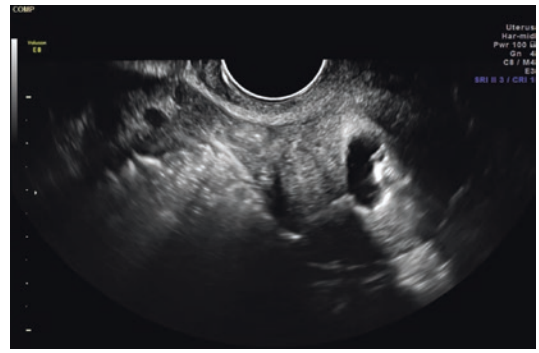


Fig. 5.8 Echogenic contrast identified as scintillations flowing around the left ovary. Images provided by Steven Nakajima, MD, University of Louisville, Louisville, KY

Different contrast media have been used and evaluated, such as Hyskon (Pharmacia Laboratories, Piscataway, NJ, USA [32]) or Echovist-200 (Schering AG, Berlin, Germany), a galactose microparticle/air microbubble suspension that is not FDA-approved in the USA[33]. Fenzl [34] showed in a prospective randomized trial that using contrast media at body temperature caused less pain. To better characterize the fallopian tubes, Exacoustos and colleagues have combined 3D imaging with the HyCoSy procedure [35].

5.4.3 Limitations

Visualization of scintillations in the fallopian tubes may be more difficult in obese patients with a BMI greater than 35 kg/m^2, as well as in case of

distorted pelvic anatomy secondary to large uterine leiomyomas or adnexal masses [36]. Potential causes for false interpretation of HyCoSy findings include (1) missing distal occlusion when echogenic flow is seen in tube but not over the adjacent ovary, (2) presence of a tubal fistula, and (3) false tubal occlusion findings secondary to myometrial spasm at the cornua of the uterus [26].

5.4.4 Indications

Indications for a HyCoSy are usually fertility-related, since this procedure assesses tubal patency as well as evaluates the uterus. Multiple studies have compared the applicability of HyCoSy in regard to tubal patency with the HSG procedure and laparoscopy with chromopertubation. The concordance rate for these studies was between 72% and 86% [33, 37], with a 10% false occlusion rate and 7% false patency rate of the HyCoSy procedure when compared to laparoscopy. Some studies have evaluated pregnancy rates after laparoscopy, HSG, and HyCoSy and found no statistically significant difference [38, 39], thereby not supporting pregnancy enhancement after HyCoSy. Ayinda et al. [40] found no significant difference between HSG and HyCoSy in regard to frequency or severity of pain at different stages up to 28 days post procedure, and concluded that both tests are equally well tolerated, with HyCoSy avoiding pelvic radiation. Based on a retrospective chart review, Luciano et al. suggest HyCoSy as a method to accurately determine tubal occlusion after hysteroscopic sterilization [41].

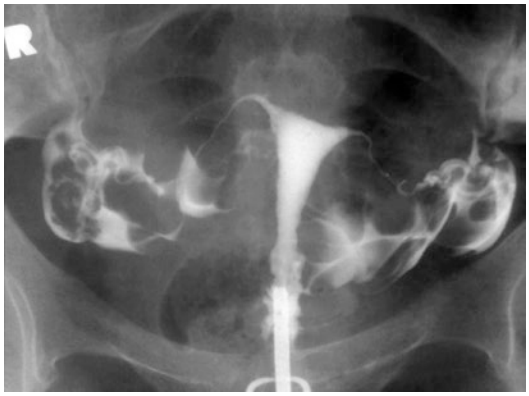


Fig. 5.9 Features of a technically adequate HSG study. The speculum is not obscuring anything. The tenaculum on the cervix has straightened the uterus such that it is perpendicular to the X-ray beam. The tip of the cannula remains below the internal os. Free spill is seen outlining loops of bowel. Reproduced with permission from Goldberg JM. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

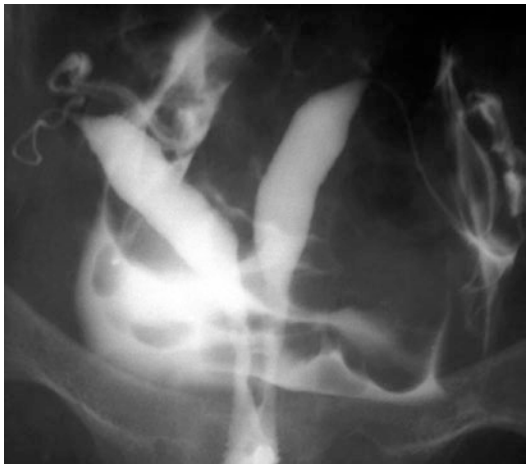


Fig. 5.10 A uterine septum cannot be differentiated from bicornuate uterus by HSG. Reproduced with permission from Goldberg JM. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

5.5 Hysterosalpingogram (HSG)

5.5.1 Principles

The HSG procedure images the uterine cavity and fallopian tubes by injecting radiographic contrast media through the cervix and taking radiographs of the pelvis (Fig. 5.9). The HSG has a high sensitivity but low specificity for the diagnosis of uterine cavity abnormalities and is therefore a good screening test [42]. One limitation of the HSG procedure is the differentiation between a septate and bicornuate uterus, since the external fundal contour is not imaged (Fig. 5.10). This obstacle may be overcome by using the uterine push–pull technique to visualize the fundal contour of the uterus [43]. After tubal patency is verified, the uterus is mobilized by movement of the attached tenaculum, dispersing the contrast over the fundus of the uterus and thereby imaging the external fundal contour.

Two relatively new imaging modalities are available in select centers to visualize the fallopian tubes and uterus: virtual HSG combined with

either CT (computerized tomographic virtual hysterosalpingography, CT-VHSG) or MRI (magnetic-resonance virtual hysterosalpingography, MR-VHSG). These procedures begin with the infusion of a dilute contrast solution into the uterus using a power injector over a 30–60 s interval. The contrast solution is often approximately 10–20 mL (1–5 mL of iodine contrast and 9–15 mL of saline) and it is infused at 0.3 mL/s. After the infusion of contrast a CT or MRI imaging scan is performed. These procedures require specific equipment, are costly, and are geared towards a fertility evaluation as they allow for a complete evaluation of the cervix, uterus, and fallopian tubes in one single examination. Abnormalities can often be detected and include the presence of cervical stenosis, synchiae, polyps, fibroids, or the presence of a hydrosalpinx [44]. For the CT-VHSG, a multidetector computerized tomography (MDCT) machine is required along with at least a 64-detector-row CT scanner. The more rows present, the shorter the study time is. This is helpful specifically for imaging of the fallopian tubes since infused contrast is quickly dispersed from the tubes. Techniques to minimize radiation exposure of the patient need to be applied, and the current of contrast through the fallopian tube needs to be adjusted depending on the patient's BMI. Total CT scan time after contrast injection is approximately 1.5–4.0 s. For the MR-VHSG, a high-field 3-T MR imaging scanner with three-dimensional volumetric time-resolved MR sequences is required in order to allow for the best temporal and spatial resolutions. Usually T1- and T2-weighted images are combined with 3D volume scan to obtain the best image quality. Results are available shortly after completion of the exam, and the pelvic structures can be visualized from different angles after reconstruction of the images. The total exam time for CT-VHSG is approximately 20 min, while an MR-VHSG is approximately 40 min [44].

5.5.2 Technical Considerations

The HSG procedure should be preferably performed by a clinician with clinical experience performing pelvic examinations. The clinician should have a familiarity with both normal female pelvic anatomy and uterine anomalies. It should be performed during the early follicular phase (cycle days five to nine) to minimize artifacts from blood clots, luteinized endometrium, and to minimize the risk of an occult early pregnancy. A urine pregnancy test can be performed prior to the procedure. Patients usually experience uterine cramping during the HSG, which can be improved by taking a nonsteroidal anti-inflammatory drug (NSAID) 30–60 min prior to the procedure.

Water-soluble media such as iothalamate meglumine 30% or 60% usually result in better imaging quality. These media don't obscure fine details and they dissipate quickly, making delayed films (one to 24 h later) unnecessary. An oil-soluble media such as Ethiodol can be associated with oil embolism and granuloma formation. Use of this contrast media, however, may increase post-procedure pregnancy rates [42, 45].

Either a rigid metal cannula or a standard 5- or 7-Fr double-lumen intrauterine HSG catheter can be used. Rigid metal cannulas are inexpensive and reusable, and may allow better manipulation of the uterus. Intrauterine HSG catheters are disposable, and the balloon may obscure the lower uterine segment. It is therefore important to only insufflate the balloon if needed and after images of the uterine cavity and lower uterine segment have been obtained.

After informed consent, an open-sided speculum is placed in the vagina and the cervix is cleansed with an antiseptic. Local anesthetic may be used in the form of a gel or spray, and a tenaculum is gently placed on the cervix for uterine traction. It is important to flush the catheter prior to placement in the uterus in order to avoid introducing air bubbles, which could be mistaken for uterine pathology. If cervical stenosis is present, dilation or administration of 400 μg of misoprostol 12 h prior to the procedure vaginally or orally may be necessary. The HSG procedure is combined with fluoroscopy to ensure correct positioning of the pelvic structures prior to the injection of the contrast. If contrast returns from the cervix, the balloon should be inflated to create a seal. Proximal tubal obstruction is usually caused by tubal spasm, and in 60% of these cases a repeat HSG was normal [46]. Rolling the patient so that the side of cornual occlusion is down will result in its resolution >50% of the time [57].

If the patient has a history of pelvic infection or if hydrosalpinges are noted (Fig. 5.11), antibiotic coverage in the form of doxycycline 100 mg orally twice a day for 5 days is recommended [21].

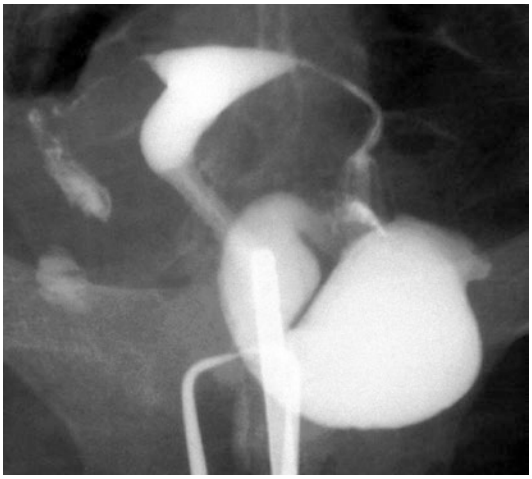


Fig. 5.11 A large left hydrosalpinx. Reproduced with permission from Goldberg JM. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

To perform a CT-VHSG or an MR-VHSG, placement and preparation of the patient is similar to a HSG, except that a plastic cannula is placed on the disinfected cervix and a tenaculum is not necessary. Contrast is then injected slowly at a set speed with the help of a power injector in order to obtain the best images and to minimize patient discomfort. CT-VHSG requires a 15 ml mixture of iodinated contrast and normal saline at a 70% dilution, whereas MR-VHSG is usually performed with a 20 mL mixture of gadolinium, iodinated contrast and normal saline. The risk for complications is low, and most patients report no discomfort during the procedure. The study should not be performed if the patient reports vaginal bleeding or if there is concern for a pelvic infection [44].

Contraindications for performing a HSG are as follows: (1) active pelvic infection, (2) iodine allergy, (3) active uterine bleeding, (4) known or suspected endometrial cancer (for fear of disseminating tumor cells), or (5) pregnancy. If a patient has a known iodine allergy, using a nonionic agent such as Isovue 370 (iopamidol) or Omnipaque (iohexol) may decrease the risk of a reaction. Premedication with prednisone, 50 mg orally 13, 7, and 1 h prior to contrast exposure, and diphenhydramine, 50 mg orally 1 h prior to exposure, may also be considered [47]. There have been reports of the successful use of gadolinium-based contrast (Magnevist) for HSG in patients who cannot be exposed to iodinated contrast [48]. Some misperceptions exist regarding the history of an "iodine allergy," which is not the same as an allergy to iodinated contrast media. "Iodine allergy" may refer to an allergy to topical iodine or to shellfish, and these allergies are not the same as being allergic to iodinated contrast and do not necessarily mean that a patient will react to contrast. The American College of Radiology (ARC) 2011 HSG practice guideline [49] states that a known prior allergic or idiosyncratic reaction to iodinated contrast is a relative contraindication to the procedure and may require premedication as described above [47]. "Non-ionic" contrast does not mean that it is not iodinated. "Non-ionic" contrast has a lower osmolality than ionic contrast, and the risk of an allergic reaction is lower with non-ionic agents.

Risks of a HSG include a vasovagal reaction, pelvic infection, radiation exposure, or possibly uterine perforation. Granuloma formation and oil embolism may occur if oil-soluble contrast material is used. A vasovagal reaction with lightheadedness, pallor, sweating, bradycardia, and hypotension occurs in less than 5% of patients [50] and usually resolves while the patient stays in the supine position. A study by Pittaway et al. reported that 1.4% of patients who had a HSG performed developed pelvic inflammatory disease. All of these patients had tubal dilation [51]. Another study by Stumpf et al. [52] found five risk factors for developing a post-HSG infection, which included (1) history of infertility, (2) previous pelvic surgery for an infection, (3) previous pelvic inflammatory infection, (4) adnexal tenderness at the time of the procedure, and (5) adnexal mass. They suggested that high-risk patients based on these criteria should avoid a HSG and proceed instead with a laparoscopy.

The radiation dose during a HSG depends on a number of factors, including (1) the patient's size, (2) position of the ovaries, (3) the technical equipment used, (4) the distance between the ovaries and the fluoroscopy unit, (5) the duration of fluoroscopy, (6) the number of images acquired, and (7) the degree of image magnification [53]. Only the minimum number of images should be acquired. Modern digitally enhanced fluoroscopy results in a much lower dose of radiation than old fashioned fluoroscopy. Capturing images does not increase the dose since it is only recording a video image. The average gonadal radiation dose during

one completed HSG procedure is estimated to be at most 5 mGy [54], and is considered to be within the margins of safety [55]. Another study by Perisinakis et al. [56] quoted the risk for embryologic anomaly in future pregnancy as 27×10^{-6} and the risk for a fatal cancer as 145×10^{-6}.

Granulomas can occur as a foreign-body reaction in the uterus or tubes in response to oil-soluble contrast and may persist for years. The effects of granulomas on future fertility are unknown [53]. Granulomas rarely form in the normal tube. Therefore, water-soluble media should be used in patients at risk for distal obstruction. An oil embolism can form when oil-soluble contrast intravasates into myometrial veins and lymphatics and is then transported to the lungs via the uterine and ovarian veins [57]. Risk factors for intravasation are tubal obstruction, excessive injection pressure, recent uterine surgery, misplaced cannula, or uterine malformations [53]. If the patient complains of chest pain, cough, light-headedness, or headache, the procedure should be terminated and the patient assessed for contrast intravasation. Overall, there seems to be a provider-directed movement to use predominantly water-soluble contrast media. In February 2010, the manufacturing of the oil-based contrast Ethiodol® was discontinued. In January 2014, a new product called Lipiodol® (ethyl esters of iodized fatty acids of poppy seed oil) was temporarily FDA-approved, but a nationwide shortage of this product is reported as of June 2016.

5.5.3 Limitations

Tubal spasm, as mentioned above, can give the false impression of tubal obstruction.

5.5.4 Indications

The HSG procedure is usually obtained as a diagnostic test of tubal patency in the setting of infertility. Women at risk for tubal obstruction are those with a history of pelvic infection, pelvic surgery, or endometriosis. The HSG procedure is also being used to document tubal occlusion three months after hysteroscopic placement of a tubal occlusion device for sterilization purposes.

5.6 Magnetic Resonance Imaging (MRI)

5.6.1 Principles

The addition of **saline infusion hysterosonography** and 3D imaging has improved the precision of gynecologic ultrasonography, and it remains the mainstay of reproductive imaging. There are instances, however, in which ultrasound is unclear, inconclusive or equivocal. MRI can be used in those situations where the clinical diagnosis is in question and treatment would be affected. MRI technology applies external magnetic fields to align the inherent small magnetic fields of water protons. Radiofrequency electromagnetic pulses are then applied to temporarily alter this alignment. Radiofrequency energy is emitted by the protons as they resume their previous state of alignment. Different types of tissue will recover their original alignment with the external magnetic field at different rates with different time constants. The most common types of images to be encountered in a clinical pelvic MRI are T1- and T2-weighted images. On T1-weighted images, water is darker (low signal intensity) than fat, which appears as bright white (high signal intensity). On T2-weighted images, both fat and fluid are typically of high signal intensity. Some T2-weighted sequences are performed with fat-suppression techniques that cause the fat to be of low signal, thereby accentuating the remaining bright fluid signal [58]. On T1-weighted images, fluid that is of high signal intensity is likely to be hemorrhagic or proteinaceous [59]. MRI has the advantages of multiplanar capabilities, less limitation by uterine and/or patient size, less operator dependence relative to sonography, and no exposure to ionizing radiation.

5.6.2 Technical Considerations

Pelvic MRI is performed per standard radiology protocols. It is best performed in the proliferative phase after menstruation has ceased, to prevent any artifacts from blood, and to minimize the chance of an occult pregnancy. Patients should fast for 4–6 h to minimize bowel activity and asked to void prior to the procedure. An antiperistalsis medication can be given to prevent any bowel-motion artifacts.

5.6.3 Limitations

Patients with pacemakers and other medical or surgical implants should not undergo an MRI procedure. Claustrophobia and patient discomfort are additional concerns that may limit the use of this procedure. A pregnant patient should not be subjected to MRI unless absolutely necessary and specifically not in the first trimester. Nonetheless, no MRI-associated fetal malformations have been noted to date [60]. Gadolinium contrast use in pregnancy should be avoided since it crosses the placenta and fetal effects are unknown. Gadolinium use is contraindicated in patients with known hypersensitivity [61]. Caution should be exercised if gadolinium is used in women with severe renal failure (stage IV or V) due to the increased risk of subsequent nephrogenic systemic fibrosis [62].

5.6.4 Indications

The MRI procedure is an excellent modality for imaging the pelvic organs when ultrasonography is not adequate or definitive. It is especially good for characterizing congenital (Müllerian) and acquired anomalies of the reproductive tract. Congenital anomalies include uterine agenesis and the presence of an arcuate, unicornuate, septate, or bicornuate uterus. The most widely accepted classification of Müllerian anomalies was set forth in 1988 by the American Fertility Society (now American Society for Reproductive Medicine), although subtle variations have been found in clinical practice [63]. In a meta-analysis by Chan et al. the reported prevalence of congenital uterine anomalies is about 5.5% in an unselected population, but in those with recurrent pregnancy loss the rate increased to approximately 13.3%. An arcuate uterus, which is of no clinical significance, is found in 3.9% of all women, whereas a septate uterus is found in 2.3% of an unselected population. In contrast, a septate uterus was present in 5.3% and 15.4% of women with recurrent pregnancy loss alone and combined with infertility, respectively. Unification defects, including unicornuate, bicornuate, and uterine didelphys, are found in 0.4%, 0.1%, and 0.3% of the unselected population and are only mildly elevated in the infertile woman [64].

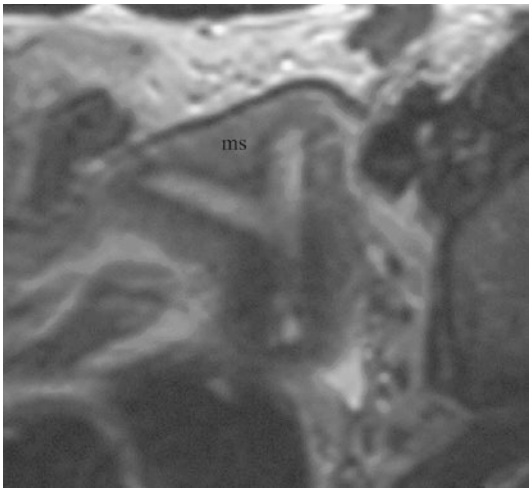


Fig. 5.12 Septate uterus. This is best appreciated on oblique coronal T2-weighted image through the uterine canal demonstrating myometrial septum (ms). Reproduced with permission from Magen A, Veniero JC. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

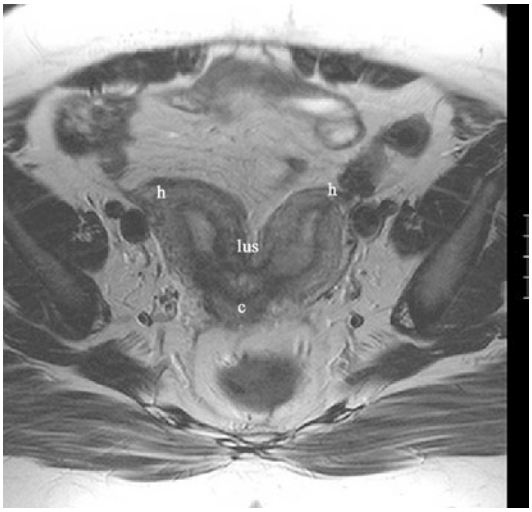


Fig. 5.13 Bicornuate uterus. Coronal image through the plane of the uterus demonstrating the large gap between the two uterine horns (h), which join at the lower uterine segment (lus), and the single cervix (c). Reproduced with permission from Magen A, Veniero JC. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

The MRI procedure can definitely distinguish between a septate (Fig. 5.12) and bicornuate uterus (Fig. 5.13). Numerous studies have examined the sensitivity and specificity of MRI and ultrasound as compared to surgical findings

in patients with suspected Müllerian anomalies. In a 1992 study of twenty-four adult women with uterine anomalies, transvaginal ultrasound had a diagnostic accuracy of 92%, whereas the accuracy of MRI was 100% [65]. In the pediatric and adolescent population reported by Santos et al., MRI findings were consistent with surgical findings in 90.9% of cases, whereas transabdominal ultrasound diagnosis was correct in only 59.1% [66]. With regard to acquired anomalies, MRI can be helpful in the diagnosis of adenomyosis and in clarifying the exact size, location, and number of leiomyomas (Fig. 5.14).

Multiple prospective studies have examined the diagnostic accuracy of both transvaginal ultrasound and pelvic MRI for adenomyosis (Fig. 5.15), with most showing a non-significant difference between the two [67, 68]. Overall, the sensitivity and specificity of MRI are estimated at 88–93% and 66–91%, respectively, while that of transvaginal ultrasound are estimated at 53–89% and 50–98%, respectively [69]. One historical study by Ascher et al. in 1994 compared transvaginal ultrasound and MRI with regard to accuracy in diagnosis of adenomyosis, and found a significant benefit with the usage of MRI; sonographic technological ability, however, has greatly improved in the intervening time period, and the authors did not report sensitivity or specificity for either modality [70]. Some instances in which the MRI procedure would likely be superior include subject obesity, which can severely limit ultrasound capability, and when concomitant leiomyomas and adenomyosis are present [67].

In our practice, characteristics of uterine fibroids are visualized with an MRI procedure prior to a planned myomectomy so as to determine the minimum number of uterine incisions, surgical efficiency, and to minimize the chance of neglecting to remove an occult leiomyoma. Some have proposed the usage of MRI to assess ovarian volume and antral follicle counts in obese women with polycystic ovarian syndrome (PCOS), but the issues of cost and patient inconvenience are definite drawbacks to widespread use of MRI for the diagnosis of PCOS [71].

Endometriomas are best seen on T1-weighted fat-suppression MRI images, with a sensitivity of 90% and specificity of 98% [72]. T1 images show a

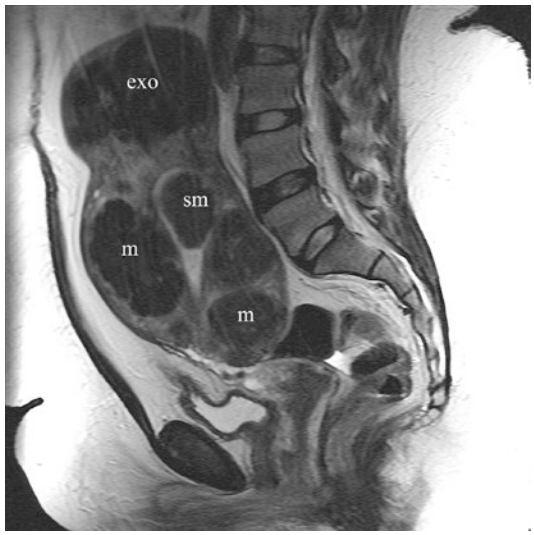


Fig. 5.14 Fibroids, T2-weighted sagittal **a** and axial **b** images of multiple uterine fibroids of low signal relative to the myometrium throughout the uterus in multiple locations, including submucosal (sm), myometrial (m), and exophytic (exo). Reproduced with permission from Magen A, Veniero JC. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

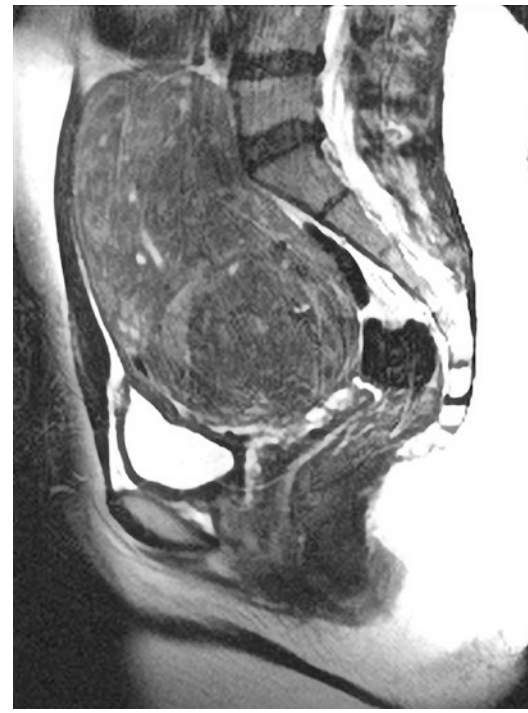


Fig. 5.15 Diffuse adenomyosis. Sagittal T2-weighted image. Reproduced with permission from Magen A, Veniero JC. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

hyper-intense ovarian cyst, and endometriomas appear hypo-intense on T2-weighted images. Peritoneal implants are usually hyper-intense on both T1- and T2-weighted images. Although laparoscopy remains the gold standard for detection of endometriosis, Ha and colleagues proposed that fat-suppression T-1 weighted MRI images could be used to noninvasively detect peritoneal implants with an accuracy of 77% and sensitivity of 61% [73]. DeVenecia and Ascher report that MRI can detect peritoneal implants as well as deep pelvic endometriosis with implants more than five mm below the peritoneum [74].

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Amenorrhea

Melissa F. Wellons, Kaitlyn M. Weeber, and Robert W. Rebar

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6.1 Introduction

This chapter serves as an overview of the presentation, initial workup, and most common etiologies of primary and secondary amenorrhea. In this brief introduction, it cannot be comprehensive, and additional sources may be consulted for more detailed information regarding management.

In the USA, approximately 4% of women will experience secondary amenorrhea, while less than 0.1% of women will experience primary amenorrhea [1]. The differential diagnosis for patients with amenorrhea is broad, and the purpose of this chapter is to give the reader a systematic guide for the evaluation of such patients.

Clinical Case

An 18-year-old gravida 0 college freshman presents to the Student Health Service complaining of amenorrhea since beginning college 3 months ago. She underwent menarche at age 13 after appropriate development of secondary sex characteristics. Her menses occurred regularly at approximately 30-day intervals until 4 months ago.

She relates that she is from a small town in Michigan's Upper Peninsula and had never left her family before. Her boyfriend of 2 years went to an Eastern college. The couple was sexually active and she states that she always used condoms for contraception. She admits to being unhappy and homesick in her new setting and has lost 10 lbs. since leaving home. She is on no medications, has no allergies, and has no significant past medical history.

Physical examination reveals her height as 5′-6″ and her weight as 120 lbs (BMI 19.4). Vital signs are normal. She has Tanner stage 5 breasts and pubic and axillary hair development. Pelvic examination shows a good estrogen effect of the vagina and cervix. Pregnancy test is negative. Other laboratory tests are negative.

6.2 Diagnosing Amenorrhea

6.2.1 History

The initial workup of amenorrhea should include a detailed history with particular attention to last menstrual period and sexual history, if applicable, recent changes in physical and emotional stressors that could lead to hypothalamic dysfunction, a detailed review of current and recent medications that may be gonadotoxic, and specific evidence of hormonal dysfunction, including symptoms of *hyperandrogenism*, *hyperprolactinemia*, as well as *hyper-* and *hypothyroidism*. A contraceptive history should be gathered, as several modern means of contraception are prone to iatrogenic amenorrhea, such as methoxyprogesterone acetate (Depo-Provera®). Associated symptoms of systemic diseases, such as the weight gain associated with both hypothyroidism and Cushing syndrome, may also come to light in a detailed history.

6.2.2 Physical Examination

A detailed physical examination should focus on evidence of biologically active reproductive hormones. This includes a gynecologic examination and *Tanner staging* to measure pubertal development, as well as measurement of height, arm span, body mass index, signs of hyperandrogenism, and skin manifestations of endocrine disorders. Arguably the most important single feature in the evaluation of primary amenorrhea is the presence or absence of any evidence of pubertal development. Breast development indicates the presence of biologically active estrogen, and the evidence of any terminal pubic and axillary hair indicates the presence of biologically active androgen. Gynecologic examination will usually reveal the presence of genital abnormalities, including obstructive processes such as a transverse hymen or external cervical stenosis, or vaginal atrophy

associated with hypoestrogenemia. The bilaterally enlarged ovaries sometimes present in women with PCOS often can be detected during bimanual examination.

The clinician should be alert to subtle physical signs, such as those in patients with PCOS. Patients with PCOS will often, but not always, be overweight with increased hair on the upper lip, chin, chest, and inner thighs. In particularly severe cases, acanthosis nigricans may be present. Ovarian and adrenal tumors can also produce sudden and dramatic hirsutism. Short stature and the stigmata of Turner syndrome can suggest primary ovarian insufficiency with a genetic basis. Galactorrhea suggests hyperprolactinemia, although only one-third of women with elevated prolactin will have this finding. When galactorrhea is present, the examiner should note whether it is unilateral or bilateral, constant or intermittent. Cushing syndrome is often associated with central obesity, "moon facies," a ruddy complexion, abdominal striae, and a "buffalo hump," as well as hypertension and insulin resistance.

6.2.3 Laboratory Studies

First-line laboratory tests that are relatively quickly and inexpensively obtained include a pregnancy test, followed by serum follicle-stimulating hormone (FSH), serum prolactin, and serum thyroid-stimulating hormone (TSH). (See Fig. 6.1 for a flow diagram for the evaluation of amenorrhea.) FSH and LH typically trend together and generally need not both be obtained as part of the initial workup. Elevated levels of TSH or prolactin are indications that one should evaluate the patient further for hypothyroidism or pituitary adenoma, respectively. Additionally, a

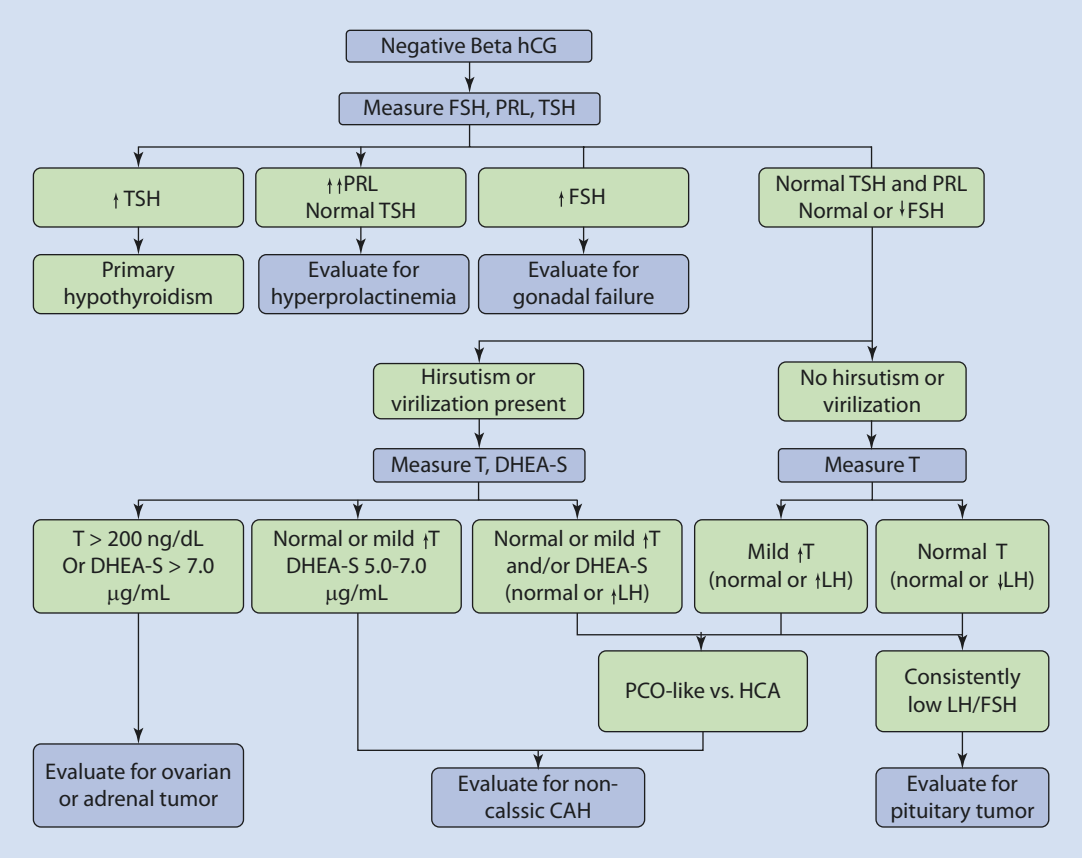

Fig. 6.1 Evaluation of amenorrhea

karyotype is warranted in any young woman with elevated levels of FSH. Serum androgens should be measured if a patient has signs or symptoms of hyperandrogenism, and both a serum 17α-hydroxyprogesterone level and a karyotype should be obtained in the case of sexual ambiguity. Although many reproductive endocrinologists recommend measuring free testosterone levels rather than total testosterone levels, free testosterone levels are calculated and are more inaccurate than are measurements of total testosterone (and commercial laboratories typically measure even total testosterone poorly). A *progestin challenge* test can be conducted to evaluate estrogen production and ovarian function, but both false positives and false negatives are common; it is rarely, if ever, necessary in the contemporary evaluation of the patient with amenorrhea. More dangerous etiologies, for example intracranial masses or tumors and ovarian or adrenal tumors, should be ruled out by appropriate imaging studies.

6.2.4 Imaging

Abdominal ultrasonography can be used to determine the presence or absence of a uterus. Magnetic resonance imaging (MRI) of the pelvis is probably the most effective imaging method for characterizing congenital anomalies if one is suspected on the basis of examination and vaginal ultrasound. Patients without secondary sexual development should undergo radiographic determination of bone age, generally by evaluating the bones of the non-dominant hand. In patients with persistently elevated prolactin levels and no evidence of primary hypothyroidism, an MRI of the pituitary gland is indicated.

6.3 Etiologies of Primary Amenorrhea

While it is true that virtually any disorder that leads to secondary amenorrhea may also cause primary amenorrhea, certain disorders more commonly present as primary amenorrhea (Table 6.1). The four most common etiologies of primary amenorrhea are reported to be gonadal dysgenesis, Müllerian agenesis, hypothalamic disorders, and constitutional delay of puberty [4]. Less common causes include androgen insensitivity syndrome, inborn defects in gonadotropin secretion or response, and outflow obstructions of the genital tract, such as imperforate hymen and transverse vaginal septum.

Table 6.1 Classification of amenorrhea, both primary and secondary, and primary ovarian insufficiency [2]

Anatomic defects (outflow tract)	Müllerian agenesis (Mayer–Rokitansky–Kuster–Hauser syndrome)
	Complete androgen resistance (testicular feminization)
	Intrauterine synechiae (Asherman syndrome)
	Imperforate hymen
	Transverse vaginal septum
	Cervical agenesis—isolated
	Cervical stenosis—iatrogenic
	Vaginal agenesis—isolated
	Endometrial hypoplasia or aplasia—congenital

Table 6.1 (continued)

Primary hypogonadism	Gonadal dysgenesis	Abnormal karyotype	Turner syndrome 45,X	
			Mosaicism	
		Normal karyotype	Pure gonadal dysgenesis	46,XX
				46,XY (Swyer syndrome)
	Gonadal agenesis			
	Enzymatic deficiency	17α-Hydroxylase deficiency		
		17,20-Lyase deficiency		
		Aromatase deficiency		
	Primary ovarian insufficiency (see also Table 6.2)	X Chromosomal causes		
		Mutations associated with a 46,XY karyotype		
		Autosomal causes		
		Environmental insults		
		Immune disturbances		
		Idiopathic causes		
Hypothalamic causes	Dysfunctional	Stress, Exercise, or Nutrition-related		
		Pseudocyesis		
	Other disorders	Isolated gonadotropin deficiency	Kallmann syndrome	
			Idiopathic hypogonadotropic hypogonadism (IHH)	
	Infection			
	Tuberculosis			
	Syphilis			
	Encephalitis/meningitis			
	Sarcoidosis			
	Chronic debilitating disease			
	Tumors	Craniopharyngioma		
		Germinoma		
		Hamartoma		
		Teratoma		
		Endodermal sinus tumor		
		Metastatic carcinoma		
	Proliferative	Langerhans cell histiocytosis		

(continued)

Table 6.1 (continued)

Pituitary causes	Tumors	Prolactinomas	
		Other hormone-secreting pituitary tumor (corticotropin, thyrotropin-stimulating hormone, growth hormone, gonadotrophin)	Mutations of FSH or LH receptor
			Fragile X syndrome
		Autoimmune disease	
		Galactosemia	
Other endocrine gland disorders	Adrenal disease	Adult-onset adrenal hyperplasia	
		Cushing syndrome	
	Thyroid disease	Hypothyroidism	
		Hyperthyroidism	
	Ovarian tumors	Granulosa-theca cell tumors	
		Brenner tumors	
		Cystic teratomas	
		Mucinous/serous cystadenomas	
		Krukenberg tumors	
		Nonfunctional tumors (craniopharyngioma)	
		Metastatic carcinoma	
	Space-occupying lesions	Empty sella	
		Arterial aneurysm	
	Necrosis	Sheehan syndrome	
		Panhypopituitarism	
	Inflammatory/infiltrative	Sarcoidosis	
		Hemochromatosis	
		Lymphocytic hypophysitis	
	Gonadotropin mutations (FSH)		
Multifactorial causes	Polycystic ovary syndrome		

6.3.1 Gonadal Dysgenesis

The term "gonadal dysgenesis" refers to a number of disorders in which the gonads have not formed normally. This condition can occur in individuals with normal karyotypes as well as in a variety of abnormal or mosaic states. Gonadal dysgenesis accounts for almost half of all cases of primary amenorrhea. Gonadal dysgenesis with the stigmata of Turner syndrome is the most common variation, which has a wide spectrum of genotypes (most commonly 45,X but including individuals who may have a portion of a Y chromosome as well) and phenotypes. Individuals with gonadal dysgenesis may develop hypothyroidism and also commonly develop hypertension and glucose intolerance. Swyer syndrome (46,XY) is also associated with gonadal dysgenesis, and 46,XX

gonadal dysgenesis may occur as well; in both cases, the individual presents as a normal appearing but sexually immature female.

6.3.2 Müllerian Agenesis

Müllerian agenesis (Mayer–Rokitansky–Kuster–Hauser syndrome) is a condition in which all or part of the uterus and vagina are absent with blind vaginal pouch in the presence of otherwise normal female sexual characteristics and a normal 46,XX karyotype (which generally need not be assessed because the diagnosis is evident on examination). This condition accounts for approximately 10% of cases associated with primary amenorrhea and occurs in 1 in 4000–5000 births; it is autosomal dominant with incomplete penetrance and variable expressivity [5, 6]. A karyotype can be performed to rule out androgen insensitivity, but individuals with Müllerian agenesis have completely developed secondary sexual characteristics whereas those with androgen insensitivity generally have only Tanner stage 3 breast development and very little, if any, pubic and axillary hair. Serum FSH, LH, estradiol, TSH, prolactin, and testosterone will be within normal limits barring iatrogenic effects of hormonal therapy. Pelvic ultrasound shows variable absence of Müllerian structures, and follow-up MRI of the abdomen and pelvis can reveal associated renal abnormalities, found in 30% of patients [1]. Other associated findings include skeletal abnormalities of the spine, syndactyly, and auditory deafness.

6.3.3 Hypothalamic Disorders

Hypothalamic disorders that may cause amenorrhea include emotional/physical stress, intense exercise, malnutrition or a chronic disease state, as well as primary or secondary gonadotropin deficiency, and a wide variety of rare tumors and diseases (Table 6.1). Patients with hypothalamic amenorrhea may present with the absence of secondary sexual characteristics or following normal puberty. Circulating levels of FSH, LH, and estradiol levels are all low. In a patient with a history concerning for emotional or physical stress, malnutrition, or a chronic disease state, growth charts can be very illustrative when combined with low to normal FSH and low estradiol concentrations. Dual energy X-ray absorptiometry (DEXA) scan will reveal low bone density when compared to age-matched controls.

Gonadotropin-releasing hormone deficiency presents with the delayed development of secondary sexual characteristics, but most commonly with associated anosmia and sometimes color blindness due to an absent olfactory bulb in 50% of cases, in which case it is termed Kallmann syndrome. Kallmann syndrome can be difficult to distinguish from both constitutional delay (discussed subsequently) and other forms of hypothalamic amenorrhea in which there is an environmental stressor. Other forms of isolated hypogonadotropic hypogonadism are associated with GnRHR (GnRH receptor) inactivating mutations [7, 8]. There actually is a whole spectrum of midline abnormalities associated with GnRH deficiency and hypothalamic amenorrhea, with absence of the septum pellucidum representing the most extreme example.

Isolated gonadotropin deficiency is characterized by decreased or absent endogenous gonadotropin-releasing hormone (GnRH) secretion, resulting in very low to undetectable levels of LH and FSH, along with incomplete development of secondary sexual characteristics and primary amenorrhea. These characteristics may be accompanied by eunuchoid features, anosmia, and, more rarely, color blindness (and again is termed Kallmann syndrome). Abnormalities of GnRH receptors have also been found, but are difficult to distinguish from isolated gonadotropic deficiency. Abnormalities of the LH receptor in 46,XX females result in normal female sexual development and primary amenorrhea [9]. Serum LH may be normal or increased, and FSH will be normal, as will follicular phase estradiol levels. Progesterone will be low. The uterus in patients with LH receptor abnormalities is small, and the ovaries are consistent with the absence of ovulation.

6.3.4 Constitutional Delay of Puberty

Constitutional delay is the single most common cause of delayed puberty in both genders and is defined as the onset of otherwise normal puberty 2.5 standard deviations later than the mean age of

pubertal onset (breast development by 13 years in girls and testicular development by 14 years in boys). Patients with constitutional delay frequently experience concomitant delay in adrenarche and pubarche. Fifty to 75% of patients have a family history of delayed puberty followed by normal pubertal development, as well as short stature [10]. This is a diagnosis of exclusion.

6.3.5 Androgen Insensitivity Syndrome

Although an abnormality of sexual differentiation, androgen insensitivity syndrome is identified in as many as 5% of all patients presenting with primary amenorrhea [11]. The disorder is due to the inability of biologically active testosterone to act normally in cells, generally because of the absence of the androgen receptor but sometimes because of a defect in the post-receptor action of androgens. Patients with androgen insensitivity commonly present with distinctive physical characteristics, including a blind vagina, eunuchoid habitus, breasts that have matured only to Tanner stage 3, and small nipples with pale areolae. Pubic and axillary hair is generally scant or absent. There may be fullness in the inguinal area if the normal testicles are located there rather than intra-abdominally. The diagnosis can be confirmed by determining that serum testosterone levels are within or above the range normally found in males and by the presence of a 46,XY karyotype. The gonads, which are histologically normal testes, should be removed after the age of sexual maturity to eliminate the 30% risk of gonadal tumors later in life [1]; estrogen should be provided exogenously. Such individuals first may be identified after straddle injuries in childhood associated with intense pain due to trauma to the sometimes present inguinal testes.

6.3.6 Outflow Obstructions of the Genital Tract

Disorders of the genital tract encompass abnormalities of the Müllerian system as well as abnormalities of the external genitalia. Genital tract disorders will be found in 15% of adolescents who present with normal adolescent development and primary amenorrhea. Common disorders of the genital tract include Müllerian agenesis (see above), imperforate hymen, and transverse vaginal septum. Imperforate hymen is the most frequent obstructive female genital tract anomaly, with an estimated frequency of approximately 0.1%. Transverse vaginal septum is less common, occurring in fewer than one in 20,000 females. Patients with these abnormalities often present with adult secondary sexual characteristics, cyclic pelvic pain, and lack of menses. A bulging hymen with hematocolpos, evidence of an imperforate hymen, may be detected on pelvic exam. MRI of the pelvis may be used to detect transverse vaginal septum and is also more sensitive than ultrasound when excluding other structural abnormalities [1, 12]. If a normal uterus and fallopian tubes are present, these individuals will develop endometriosis as well because of the obstructed outflow tract.

6.4 Etiologies of Secondary Amenorrhea

In secondary amenorrhea, menstruation begins at the appropriate age, but later stops for reasons other than pregnancy, lactation, or menopause. To arrive at this diagnosis, the length of amenorrhea should be equal to at least three of the previous cycle intervals, or 6 months, although patients with *oligomenorrhea* often have similar underlying pathology. Three to 5% of women of reproductive age are affected by secondary amenorrhea [13]. Secondary amenorrhea is more common in those whose weight is below or above the normal range, with hypothalamic amenorrhea and the polycystic ovary syndrome being the most common causes of secondary amenorrhea, excluding pregnancy [14]. Two more common causes of secondary amenorrhea include pituitary disorders and primary ovarian insufficiency.

6.4.1 Hypothalamic

Hypothalamic forms of amenorrhea result from diminished GnRH input to the pituitary gland and commonly occur in women stressed mentally, emotionally, or physically as well as in those who are nutritionally deficient. Often a combination of these stressors is present, resulting in

anovulation. Menstrual cycle disturbances are common among competitive athletes, especially in those sports that encourage a low body weight. Menstrual irregularities appear to be greatest in ballet dancers (6–43%) and middle- and long-distance runners (24–26%). Hypothalamic amenorrhea is also common in women who have experienced a profound stress, such as rape, incest, or loss of someone particularly close. Severe eating disorders such as bulimia and anorexia nervosa can disrupt menstrual function in a similar manner. Clinicians should screen for stressors by reviewing the patient's lifestyle, including diet, exercise, and drug use [8, 15].

Patients with hypothalamic amenorrhea typically have a history of normal menarche and regular menstruation, but several cases of primary amenorrhea have been reported. The physical examination should focus on identifying thyroid dysfunction, galactorrhea (suggestive of central lesion), and evidence of hyperandrogenemia such as acne and hirsutism (suggestive of an androgen-secreting tumor or PCOS). Oral examination may reveal the distinctive eroded "moth-eaten" dentition and enlarged salivary glands of a bulimic patient. The results of the pelvic examination should be normal except for a thinned vaginal mucosa or absent cervical mucus, which are characteristics of hypoestrogenism.

The simplest treatment often includes counseling as well as estrogen replacement. Oral contraceptives can be used, but affected women should be told that they may be amenorrheic when the estrogen is discontinued, and infertility may be an issue. Estrogen is warranted in these women due to their increased risk of accelerated bone loss, although simultaneous relative hypercortisolism may limit the efficacy of estrogen therapy in preventing this bone loss. [16] Because ovulation may precede the first menses, these women sometimes become pregnant unexpectedly if contraception is not utilized.

Post-Contraception Amenorrhea

Modern low-dose oral contraceptives do not affect fertility long term. However, as noted, women with amenorrhea prior to the administration of oral contraceptives may still be amenorrheic when contraceptives are discontinued. Amenorrhea following contraceptive use is generally due to a preexisting cause, except when there is a history of depot medroxyprogesterone acetate (e.g., Depo-Provera®). Return to ovulation or baseline fertility upon discontinuation of medroxyprogesterone is reported to range from 7 to 10 months after last injection and may rarely be even longer [17, 18].

6.4.2 Hyperandrogenic States

Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is one of the most common causes of ovulatory dysfunction. According to the Rotterdam criteria, the overall prevalence of PCOS is 16.6%, with up to 33.3% prevalence in women under 30 years old [19]. As originally described, large, pale polycystic ovaries with thickened capsules were found in women with amenorrhea (usually secondary), hirsutism, and "sterility" [20]. Over time it was recognized that PCOS was heterogeneous, with a wide clinical spectrum that include gradual onset hirsutism, infertility, amenorrhea, obesity, and irregular menses. Defined early as "LH-dependent ovarian hyperandrogenism" commonly beginning around puberty, the definition was broadened by the Rotterdam Consensus Conference in 2004 to include the presence of two of the three following features after exclusion of other etiologies: (1) hyperandrogenism (clinical and/or biochemical), (2) oligo- and/or anovulation, and (3) polycystic ovaries. This last (and now most frequently used) definition has resulted in inclusion of many more women into the "PCOS spectrum" and may well contribute to confusion about the pathophysiology. Using this definition, PCOS is now regarded as the most common endocrinopathy in women.

Common laboratory features include elevated levels of LH (compared to those found in the normal follicular phase) in many women with the ratio of LH:FSH > 2:1, elevated levels of testosterone and virtually any other ovarian androgen measured, and decreased levels of sex hormone-binding globulin [21]. More recently, it has become recognized that insulin resistance and lipid and lipoprotein abnormalities are common in PCOS as well [22]. These laboratory abnormalities may put women with PCOS at higher risk of cardiovascular disease and metabolic abnormalities that shorten lifespan, but this remains to be established [23]. In fact, it appears that the severity of PCOS decreases toward menopause, as documented by longitudinal studies [24].

Management of PCOS is directed at treating the primary complaint, whether it is hirsutism, irregular menses or amenorrhea, infertility, or glucose intolerance. Commonly utilized therapies include oral contraceptives and spironolactone for the irregular menses and hyperandrogenism. Ovulation induction with clomiphene citrate or an aromatase inhibitor such as letrozole is often needed for women desiring pregnancy. Obese individuals should be encouraged to lose weight because signs and symptoms are not as severe in normal-weight women as they are in those who are overweight [1]. Metformin is commonly provided to women with glucose intolerance. It is known that adverse pregnancy outcomes, including pregnancy-induced hypertension and preeclampsia, gestational diabetes, pre-term birth, and perinatal mortality, are increased in women with PCOS [25].

Other Hyperandrogenic States

Other hypoandrogenic states, such as ovarian and adrenal tumors, mimic the symptoms of PCOS, although the onset of symptoms is generally more rapid, and can also result in amenorrhea. Similarly, Cushing syndrome can cause amenorrhea secondary to increased androgens (or to hypothalamic suppression of GnRH secretion). Another cause is adult-onset congenital adrenal hyperplasia. The ovary apparently can respond to increased androgens in only limited ways, and polycystic ovaries are invariably present in all of these disorders. To rule out these conditions, the patient's serum androgens should be measured, including total testosterone, dehydroepiandosterone sulfate (DHEA-S), and 17-hydroxyprogesterone. Imaging of the ovaries and of the adrenal glands may be indicated in some cases.

6.4.3 Pituitary Disorders

Disorders of the Anterior Pituitary Gland

Small pituitary tumors often present as irregular or absent menses or galactorrhea due to the impairment of prolactin suppression or GnRH regulation. Large pituitary tumors may manifest as headaches and compression of the optic chiasm with bitemporal hemianopsia related to their growth in a confined anatomical space.

Prolactin-Secreting Adenomas and Hyperprolactinemia

Prolactin-secreting adenomas are the most common pituitary tumors, with hyperprolactinemia being the most common cause of pituitary-associated amenorrhea [8]. As many as one-third of patients with secondary amenorrhea will have a prolactinoma [26, 27]. Hyperprolactinemia is associated with decreased estradiol concentrations as well as amenorrhea or oligomenorrhea, galactorrhea, headaches, and infertility. About one-third of women with amenorrhea will have elevated prolactin levels, one-third of women with galactorrhea and elevated prolactin levels will have normal menstrual cycles, and one-third of women will have high prolactin levels without galactorrhea [28]. In patients presenting with hyperprolactinemia, the prevalence of a pituitary tumor is 50–60%. [29, 30]. Whenever serum prolactin levels are consistently elevated, MRI or CT scanning of the pituitary should be performed [26]. The so-called non-functioning pituitary tumors commonly secrete the alpha subunit common to LH, FSH, and TSH, and may present only with amenorrhea. Other causes of hyperprolactinemia include pituitary stalk disruption, primary hypothyroidism, renal failure, and chest wall injury. Prolactin-secreting pituitary tumors less than 10 mm in diameter rarely increase in size or cause "pressure" symptoms; these can be treated medically with dopamine agonists such as cabergoline or merely managed expectantly. There is no evidence that estrogen-containing oral contraceptives cause tumor growth, and these agents may be administered to those women who desire contraception. Either a dopamine agonist or an estrogen is warranted to prevent the osteoporosis and other signs and symptoms of estrogen deficiency that typically accompany the hyperprolactinemia.

Postpartum Pituitary Necrosis (Sheehan Syndrome)

Postpartum pituitary necrosis can be a life-threatening condition usually preceded by a history of severe obstetrical hemorrhage with hypotension, circulatory collapse, and shock [31]. It is known that diminished perfusion to the pituitary gland must be present for a considerable number of hours and most of the pituitary gland must undergo necrosis for Sheehan syndrome to

result. Patients often experience nausea, vomiting, slowed mental function, postural hypotension, and adrenal crisis [12]. These women often present for their first postpartum visit complaining of inability to lactate and profound malaise. MRI of the brain may show an empty or CSF-filled sella turcica or a small pituitary gland.

Pituitary Apoplexy

Pituitary apoplexy is a serious condition characterized by an acute infarction of the pituitary gland. Patients experience a sudden onset of a severe retro-orbital headache and visual disturbances that may be accompanied by lethargy or a loss of consciousness.

Cushing Disease and Syndrome

Amenorrhea and galactorrhea are usually encountered with pituitary prolactinomas, but these symptoms may also precede adrenocorticotropic hormone (ACTH) or growth hormone–secreting tumors. If the patient presents with clinical symptoms of excessive glucocorticoid, suggesting Cushing disease, a corticotropin serum level test, a midnight salivary cortisol level, and/or a 24 h urine collection for free cortisol may be indicated. The term Cushing Disease is used for individuals who have a corticotropin-secreting pituitary tumor, whereas Cushing Syndrome refers to hypercortisolism of extrapituitary origin (typically iatrogenic or due to a pulmonary carcinoma).

Post-irradiation Hypopituitarism

Exposure to therapeutic radiation sources for treatment of midline central nervous system tumors can place patients at increased risk for delayed development of hypopituitarism. Common symptoms include vaginal dryness, decreased libido, fatigue, weight gain, and vasomotor symptoms [12]. This disorder usually arises decades after the irradiation.

6.4.4 Disorders of the Genital Tract

Intrauterine adhesions (i.e., Asherman syndrome) account for 7% of cases of secondary amenorrhea, with the incidence rising secondary to the increasing use of hysteroscopy and appropriate diagnosis [32]. Patients with Asherman syndrome have been found to have higher rates of endometriosis. Another infrequent cause is an outflow obstruction secondary to cervical stenosis. This usually results from treatment of cervical dysplasia with modalities such as cryosurgery, electrocautery, or cold knife cone biopsy.

6.4.5 Primary ovarian insufficiency

Among all women, 10% may become menopausal by age 45, and these women may have experienced an accelerated decline of fertility in their young life and before women who experience menopause at a more average age (i.e., ~age 50–51). These patients may be considered to have "early ovarian aging" in spite of having menstrual function. Although the absence of ovarian function before the age of 40 ("primary ovarian insufficiency" sometimes also referred to as premature ovarian failure or "premature menopause") can be the result of a "normal" physiologic process at an unusually young age, it is sometimes due to an identifiable underlying pathology (◘ Table 6.2). Regardless of etiology, the clinical results are hypoestrogenemia and decreased fertility.

Primary ovarian insufficiency (POI) accounts for 4–18% of cases of secondary amenorrhea, with the primary mechanisms being follicular dysfunction or depletion. Ovarian function can be unpredictable, and 5–10% of women conceive and deliver a child after receiving a diagnosis of POI [33]. Although 90% of cases are idiopathic, it is likely that many cases of POI result from genetic mutations [34] and abnormalities in sex chromosomes (such as fragile X syndrome); 10–15% of patients have a first-degree family history of secondary amenorrhea. For patients <30 years of age presenting with amenorrhea, a karyotype should be obtained to rule out chromosome abnormalities, because conditions in which a portion of the Y chromosome is present are associated with an increased risk of ovarian malignancies.

There is a growing understanding of the relationship between POI and underlying or associated autoimmune diseases. Five percent of cases of POI are caused by underlying autoimmune disease, with 60–80% being adrenal in origin [35]. Additionally, up to 30% of women with primary ovarian insufficiency have an autoimmune abnormality, the most common of which are autoimmune thyroiditis resulting in hypothyroidism, pernicious anemia, type 1 diabetes mellitus, and

Table 6.2 Causes of primary ovarian insufficiency [3]

X Chromosomal causes	Structural alterations or mutations in or absence of an X chromosome	With the stigmata of Turner syndrome (45,X or mosaic)	
		Without the stigmata of Turner syndrome	Mutations in premature ovarian failure 1 (Xq26-q28)
			Mutations in premature ovarian failure1 in association with Fragile X premutation (Xq27.3)
			Mutations in premature ovarian failure 2A (Xq22)
			Mutations in premature ovarian failure 2B (Xq21)
			Mutations in premature ovarian failure 4 in association with mutations in bone morphogenetic protein 15 (Xp11.2
	Trisomy X with or without mosaicism		
Mutations associated with a 46,XY karyotype	Mutations in Xp22.11-p21.2 (Swyer syndrome)		
	Mutations in 5 cen		
Autosomal causes	Mutations involving enzymes important for reproduction	Galactosemia (galactose-1-phosphate uridyltransferase deficiency) (9p13)	
		17α–Hydroxylase deficiency (CPY17A1) (10q24.3)	
	Mutations involving reproductive hormones, their receptors, and action	Mutations of luteinizing hormone or follicle-stimulating hormone or both rendering them biologically inactive (theoretical)	
		Mutations of inhibin (theoretical)	
		Receptor mutations	Follicle-stimulating hormone receptor (2p21-p16)
			Luteinizing hormone/human chorionic gonadotropin receptor (2p21)
		Mutations in the hormone action pathways	
	Other mutations	Blepharophimosis, ptosis, and epicanthus inversus, type 1 (BPES) (premature ovarian failure 3) (3q23)	
		Premature ovarian failure 5 (newborn ovary homeobox) (7q35)	
		Autoimmune polyendocrine syndrome, type 1(APS1) (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, APECED) (autoimmune regulator gene, AIRE) (21q22.3)	
		Vanishing white matter leukodystrophy with ovarian failure (genes encoding the translation initiation factor E1F2B) (14q24, Chr 12, 1p34.1, 3q27, 2p23.3)	
		Congenital disorders of glycosylation, type 1a (CDG1a) (genes encoding phosphomannomutase-2, PMM2) (16p13.3-p13.2)	

(continued)

Table 6.2 (continued)

Environmental insults	Chemotherapeutic (especially alkylating) agents
	Ionizing radiation
	Viral infection (documented for mumps)
	Surgical injury or extirpation
Immune disturbances	In association with other autoimmune diseases
	Isolated
	In association with congenital thymic aplasia
Idiopathic causes	

myasthenia gravis [35–39]. In 10–60% of cases, Addison disease patients also may have autoimmune ovarian insufficiency, with POI often preceding symptoms of adrenal insufficiency by 8–14 years [40]. Patients with POI should be screened for adrenal disease with evaluation for anti-adrenal antibodies, as 50% of women with autoimmune POI will develop adrenal insufficiency. If these are positive, then more sophisticated testing, such as a corticotropin stimulation test, is in order (a fasting morning serum cortisol is not sufficiently sensitive). In order to exclude other autoimmune disorders, patients with unexplained primary ovarian insufficiency should undergo more complete biochemical evaluation, including measurement of serum calcium, phosphorus, fasting glucose, adrenal antibodies to 21-hydroxylase enzyme, free T4, TSH, and thyroid antibodies [41].

Early menopause has been associated with an increase in all-cause mortality in multiple large well-designed studies, although the evidence supporting associations between POI and cancer, cardiovascular disease, and cerebrovascular disease is mixed. [42]

Other Causes of Primary Ovarian Insufficiency

Although elevated serum FSH levels are virtually synonymous with ovarian disorders, there are less common conditions that can raise FSH but that are associated not with a primary ovarian problem but a central problem. These are pituitary adenomas that secrete FSH, abnormalities of the FSH receptor precluding normal FSH action, and defects in specific enzymes such as 17-hydroxylase (P450c17) (a form of congenital adrenal hyperplasia) and galactose-1-phosphate uridyl transferase (galactosemia). In closing, tt should be emphasized again that a variety of autoimmune (AI) disorders are associated with POF, including AI hypothyroidism, adrenal insufficiency, type 1 diabetes mellitus, pernicious anemia, and hypoparathyroidism [43].

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Polycystic Ovary Syndrome

Erika B. Johnston-MacAnanny and Sarah L. Berga

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7.1 Introduction

Polycystic ovary syndrome (PCOS) remains an enigmatic condition that classically presents as oligomenorrhea in the context of hyperandrogenism and obesity. The metabolic features include insulin resistance, dyslipidemia, and obesity. The widespread availability of ultrasonography has fostered the notion that there is a classical ovarian morphology with increased ovarian size and stroma and a cortical ring of follicles often referred to as a "pearl necklace". The advent of anti-Müllerian hormone (AMH) assay has confirmed an increase in oocyte count. Importantly, the presentation and recognition of PCOS changes with age due to oocyte depletion. Postmenopausal persistence of ovarian stromal and theca leads to a related condition referred to as hyperthecosis.

Clinical Case

A 25-year-old woman with irregular periods since puberty wishes to discuss her weight problem and its potential impact on her future fertility. She is not interested in fertility at this moment. She has tried different methods for weight loss unsuccessfully. She has some increased facial and body hair that has slightly increased with time. She is presently in a relationship and wants contraception. Her body mass index reveals a body mass index (BMI) of 31.

7.2 Diagnostic Criteria

Multiple professional organizations offer criteria for the diagnosis of PCOS. Indeed, a recent NIH consensus conference suggesting that the syndrome should be renamed lead to a debate that continues without resolution about the criteria for the diagnosis of PCOS. The 1990 NIH conference on PCOS suggested diagnostic criteria of hyperandrogenism and/or hyperandrogenemia, chronic anovulation, and exclusion of other known disorders [1]. In 2003, a PCOS consensus workshop in Rotterdam sponsored by the European Society of Human Reproduction and Embryology (ESHRE)/American Society of Reproductive Medicine (ASRM) revised the diagnostic criteria for PCOS [2]. The revised criteria state that PCOS remains a diagnosis of exclusion, but that two out of the following three criteria must be present: (1) oligo- or anovulation, (2) hyperandrogenism and/or hyperandrogenemia, and (3) polycystic ovaries. A polycystic ovary is defined as having 12 or more follicles in one ovary measuring 2–9 mm in diameter, and/or increased ovarian volume of greater than 10 mL, which is the maximum size of a normal ovary (Fig. 7.1). Administration of oral contraceptive pills and the presence of follicles >10 mm modify ovarian morphology; thus, the definition of a polycystic ovary does not apply to these clinical scenarios.

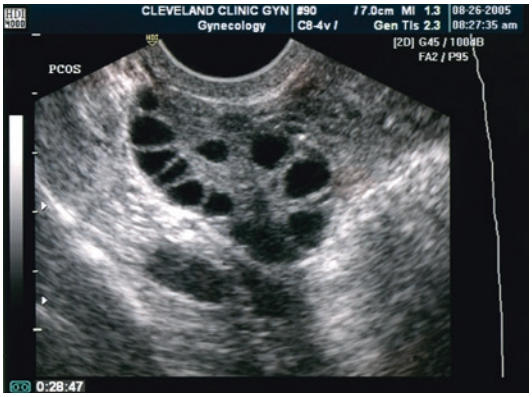

Fig. 7.1 This is the classical image of PCOS with an enlarged ovary containing an increased number of small follicles around the periphery of the cortex, resembling a string of pearls, along with a bright echogenic stroma

The differential diagnosis should include other causes of hyperandrogenism and oligomenorrhea such as nonclassical congenital adrenal hyperplasia (CAH), hypothalamic hypogonadism, Cushing's syndrome and disease, hyperprolactinemia, thyroid disease, acromegaly, androgen-secreting neoplasms of the ovary or adrenal gland, and exogenous steroid use. The ontogeny of androgen profiles has been described in a Nordic multicenter collaborative study. Women with PCOS also had elevated serum androgen levels after menopause. In the absence of high sensitivity and high specificity testosterone assays, the best predictive hormone was androstenedione [3].

7.3 Prevalence

The prevalence of PCOS is estimated to be 4–12% of reproductive-age women. The largest US study on PCOS prevalence was published in 1998 [4]. Out of 277 women included in the study, 4.0% had PCOS as defined by the 1990 NIH criteria. The prevalence was 4.7% for white women and 3.4% for black women. The inclusion of polycystic ovaries in the 2003 Rotterdam criteria calls for reevaluation of the prevalence of PCOS, as 21–23% of normal women have polycystic-appearing ovaries on ultrasound.

7.4 Clinical Case

7.4.1 Hyperandrogenism

Clinical manifestations of hyperandrogenemia include hirsutism, acne, and male pattern alopecia. Hirsutism is defined as the growth of coarse, pigmented hairs in androgen-dependent areas such as the face, chest, back, and lower abdomen. Approximately 80% of hirsute patients will have PCOS [5]. The modified Ferriman–Gallwey scoring system can be used for clinical assessment of hirsutism. This system, which was originally used in the United Kingdom for a population of presumably Caucasian women, scores hair growth in nine body areas from 0 (absence of terminal hairs) to 4 (extensive terminal hair growth) [6]. Other hyperandrogenic manifestations commonly found in PCOS patients include acne and alopecia [7, 8]. Acne is a result of androgen stimulation of the pilosebaceous unit with increased skin oiliness [7].

7.4.2 Obesity

Obesity is very common in PCOS, with the android pattern present in approximately 44% of women with PCOS [9]. This central obesity is more characteristic of PCOS, as these patients have an increased waist-to-hip ratio compared to obese women without PCOS [10]. Hyperinsulinemia may stimulate central adiposity, which, in turn, exacerbates underlying or latent insulin resistance [11].

7.4.3 Insulin Resistance, Diabetes, and Acanthosis Nigricans

Insulin resistance and diabetes are important health concerns commonly seen in association with polycystic ovarian syndrome and will be discussed at length later in this chapter. Acanthosis nigricans is a dermatological condition of hyperkeratosis and increased skin pigmentation with raised, symmetrical, darkened, velvety plaques that commonly appear on the nape of the neck. It can also be found in the axilla, groin, and other intertriginous areas of the body. Elevated insulin has a mitogenic effect on basal cells of the epidermis, making acanthosis nigricans a relatively specific clinical marker of insulin resistance [12].

7.4.4 Irregular Menses and Infertility

Some of the menstrual abnormalities seen with chronic anovulation include secondary amenorrhea, oligomenorrhea, and dysfunctional uterine bleeding. Menarche typically begins at a normal or early age, but the menstrual irregularities often seen in adolescents may never resolve for the PCOS patient. The irregular menses may be masked in PCOS patients if they are on oral contraceptives. PCOS is the most common cause of anovulatory infertility, which often serves as the impetus for the patient to seek medical attention. Patients may report false-positive urinary ovulation predictor tests due to chronic elevation in luteinizing hormone (LH).

7.4.5 Miscarriage

The risk of a first-trimester spontaneous abortion is reported to be significantly higher for patients with PCOS. The spontaneous abortion rate in PCOS is reported to be 30% [13]. In comparison, retrospective studies find the risk of spontaneous abortion to be 5–14% for normal women [14, 15]. Of patients with recurrent miscarriage, 36–82% have polycystic ovaries [13, 16, 17].

Several explanations have been offered. For example, Homburg et al. demonstrated that high concentrations of LH during the follicular phase in women with polycystic ovaries have a deleterious effect on rates of conception and are associated with early pregnancy loss [18].

7.5 Pathogenesis

7.5.1 Altered Gonadotropin Secretion

One of the well-described features of PCOS is an increase in LH and relative decrease in follicle-stimulating hormone (FSH) [19]. The relative decrease in FSH is the chief cause of anovulation. The pulsatile secretion of LH from the pituitary is increased in amplitude and frequency [20]. In addition, the pituitary has a greater LH response to gonadotropin-releasing hormone (GnRH) compared with normal women [20, 21].

The pulsatile secretion of GnRH cannot be studied in humans, so it must be inferred by detecting peripheral LH patterns. A study of PCOS women by Berga et al. found increased pulse frequency and amplitude for LH and α (alpha)-subunit, providing evidence for aberrant increases in GnRH pulse frequency (Fig. 7.2) [20]. Elevated LH is not caused by altered pituitary sensitivity to GnRH, as GnRH receptor blockade resulted in similar LH decreases in PCOS and normal women [22]. These findings suggest a derangement of the hypothalamic–pituitary axis, which appears to play a major role, because many of the cardinal features of PCOS can be traced to alterations in gonadotropins.

7.5.2 Neuroanatomical Considerations

The GnRH pulse generator refers to the synchronized pulsatile secretion of GnRH from neurons that are widely distributed in the medial basal hypothalamus. Knobil and associates conducted experiments with the Rhesus monkey to establish that the GnRH system exhibits rhythmic electrical behavior in the arcuate nucleus of the

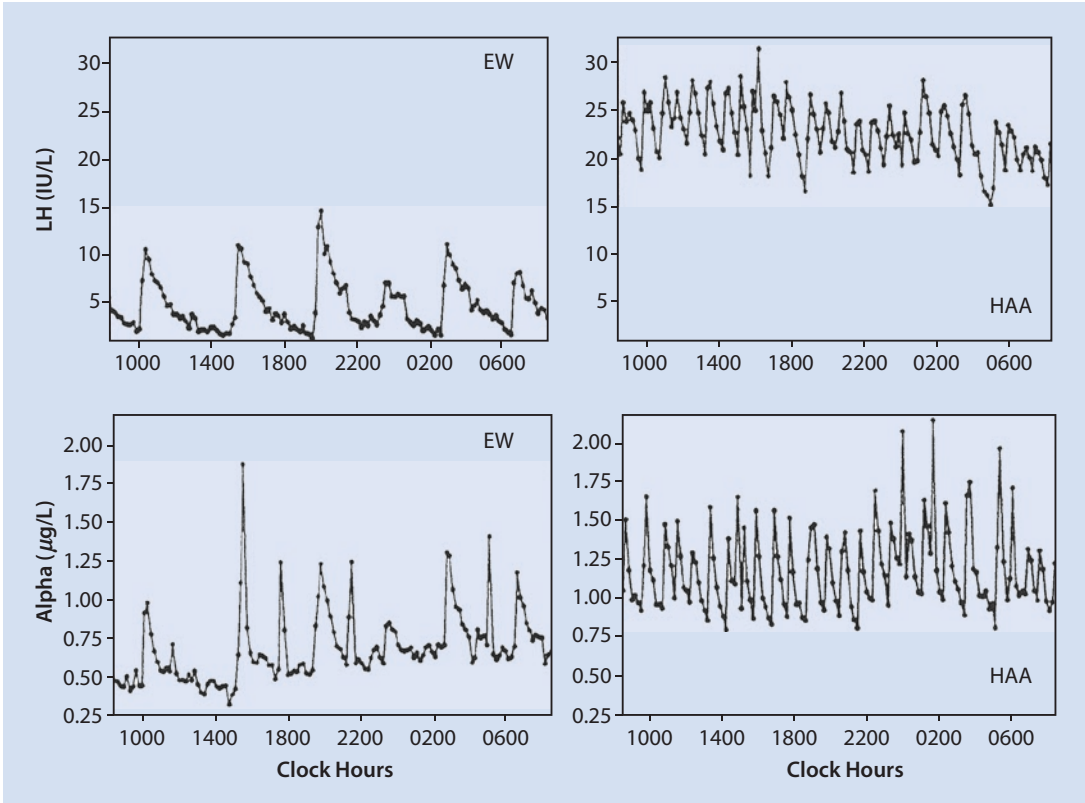

Fig. 7.2 Twenty-four hour concentration profiles of LH (*top*) and α(alpha)-subunit (*bottom*) in an eumenorrheic woman (EW) (*left*), studied in the follicular phase (day 2) and in a woman with hyperandrogenic anovulation (HAA)/PCOS (*right*). Reprinted with permission from Berga S, Guzick D, Winters S. Increased luteinizing hormone and alpha-subunit secretion in women with hyperandrogenic anovulation. J Clin Endocrinol Metab 1993; 77(4):895–901. Copyright 1993, The Endocrine Society

medial basal hypothalamus [23]. There was remarkable synchrony between pulses of GnRH in the portal blood and LH pulses in peripheral blood. This phenomenon was later studied in isolated human medial basal hypothalamus, where GnRH pulses were found to occur at a frequency of 60–100 min [24].

The secretion of GnRH into the portal vasculature also appears to be regulated by dynamic remodeling of GnRH neurovascular junctions. Morphological plasticity of the median eminence during the menstrual cycle has been demonstrated, where the maximal number of GnRH neuro-vasculature junctions are found during the LH surge [25].

7.5.3 GnRH Neuroregulation in PCOS

The GnRH pulse generator in PCOS patients is intrinsically faster, and the frequency is less likely to be suppressed with continuous estrogen and progesterone treatment [26].

Increased central adrenergic tone has been implicated as a cause of the aberrations of GnRH and gonadotropin secretion in PCOS. One possible mechanism is the increase in local blood flow and permeability of the portal vascular system, permitting the entry of increased amounts of GnRH [27]. Dopamine injection into the third ventricle led to a rapid increase in GnRH and prolactin inhibitory factor in portal blood, suggesting dopamine-mediated regulation of GnRH and prolactin inhibitory factor [28]. The identification of β(beta)-1-adrenergic and D1-dopaminergic receptors on GT-1 GnRH neurons provides a mechanism by which norepinephrine and dopamine could regulate gonadotropin release via direct synapses on GnRH neurons [29].

The role of insulin-like growth factor 1 (IGF-1) in modulation of GnRH cells has also been investigated. IGF-1 regulates growth, differentiation, survival, and reproductive function. The IGF receptor is a tyrosine kinase receptor located in the periphery and CNS, including the median eminence [30]. In PCOS women, an increased ratio of IGF-1 to their binding proteins correlated significantly with increased concentrations of circulating LH [21]. These findings suggest that IGF-1 can modulate GnRH neurons by inducing gene expression, resulting in more circulating LH.

7.5.4 Hyperandrogenemia

Circulating androgens are elevated in PCOS, with contributions from the ovary and adrenal glands. The elevated androgens can only be partially suppressed with combined oral contraceptive (COC) therapy. Daniels and Berga treated PCOS women with 3 weeks of COCs and found that androstenedione levels remained significantly higher compared to treated controls [26]. Pulse frequency of LH was suppressed in both PCOS women and controls, but the frequency remained significantly higher in PCOS patients (Fig. 7.3). This suggests there is reduced sensitivity of the GnRH pulse generator to suppression by sex steroids. The authors also suggest that GnRH drive in PCOS women may be intrinsically and irreversibly faster than in eumenorrheic women.

7.5.5 Theca Cell Function

Ovarian hyperandrogenism is driven by LH acting on theca cells, and the effect is amplified by the increased sensitivity of PCOS theca cells to LH [31]. Hyperandrogenism may also result from dysregulation of the androgen-producing enzyme P450c17, which has 17 α (alpha)-hydroxylase and 17,20-lyase activities. In contrast, in vivo studies do not find significant increases in androgen secretion in women with PCOS or normal women, despite considerable increases in insulin levels. A role for insulin is strongly suggested by the observation that reduction of hyperinsulinemia is associated with decreases of serum androgens. Treatment of PCOS patients with metformin, which reduces hepatic glucose production and secondarily lowers insulin, has been shown to decrease levels of testosterone, DHEAS, and androstenedione [32].

7.5.6 Adrenal Function

Excess adrenal androgen production is seen in PCOS women, with a 48–64% increase in DHEAS and 11β (beta)-hydroxyandrostenedione. The underlying cause of elevated adrenal androgens is yet to be elucidated, but PCOS women do not

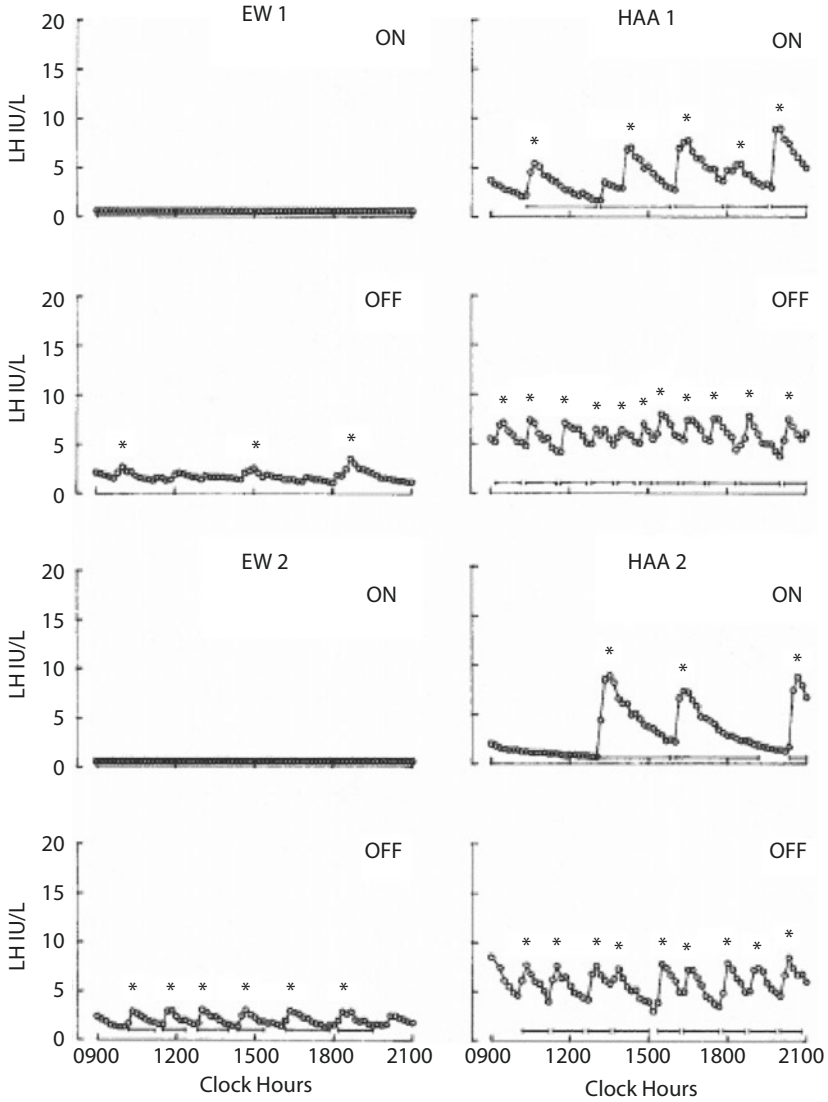

Fig. 7.3 Representative 12-h pulse patterns in two women with polycystic ovary syndrome/HAA are shown on the *right side* and those from eumenorrheic women on the *left*. "ON" means subjects were studied on day 21 of a combined oral contraceptive containing 35 μg of ethinyl estradiol and 1 mg of norethindrone. "OFF" refers to day 7 following cessation of the combined oral contraceptive. Reprinted with permission from Daniels T, Berga S. Resistance of gonadotropin releasing hormone drive to sex steroid-induced suppression in hyperandrogenic anovulation. J Clin Endocrinol Metab 1997; 82(12):4179–4183. Copyright 1997, The Endocrine Society

have increased adrenocorticotropic hormone (ACTH) levels [33]. Increased adrenal androgen production in PCOS is likely caused by either altered adrenal responsiveness to ACTH or abnormal adrenal stimulation by factors other than ACTH.

7.5.7 Anovulation

The cause of anovulation in PCOS patients has yet to be clarified. However, several observations in granulosa function have been described that may give insight into this process.

7.5.8 Granulosa Cell Function

FSH levels are characteristically low in PCOS women, resulting in arrested follicular development. Insufficient granulosa cell aromatase activity was the basis of earlier studies that tried to explain poor follicular development, as follicular fluid estradiol concentrations were thought to be low. To the contrary, more recent studies found that PCOS granulosa cells are hyperresponsive to FSH in vitro, and estradiol concentrations from PCO follicles and normal follicles are no different [34]. A dose response study in PCOS women demonstrated a significantly greater capacity for estradiol production in response to recombinant human FSH compared with normal women [35]. The incremental response of serum estradiol was almost two times greater and considerably accelerated compared with that found in normal women.

7.5.9 Insulin Resistance

Although 50–70% of PCOS patients have insulin resistance [36], it is not one of the diagnostic criteria of PCOS. The topic deservedly receives much attention, as many of the clinical signs and symptoms of PCOS may be attributed to excess insulin exposure. The precise molecular basis for insulin resistance is unknown, but it appears to be a postreceptor defect [37]. There is tissue specificity of insulin resistance in PCOS: muscle and adipose tissue are resistant, while the ovaries, adrenals, liver, skin, and hair remain sensitive. The resistance to insulin in skeletal muscle and adipose tissue leads to a metabolic compromise of insulin function and glucose homeostasis, but there is preservation of the mitogenic and steroidogenic function in other tissues. The effect of hyperinsulinemia on the sensitive organs results in downstream effects seen in PCOS, such as hirsutism [5], acanthosis nigricans [12], obesity [11], stimulation of androgen synthesis, increase in bioavailable androgens via decreased sex hormone-binding globulin (SHBG) [38], and, potentially, modulation of LH secretion.

In 1992, Hales and Barker proposed the concept that the environmental influence of undernutrition in early life increased the risk of type 2 diabetes in adulthood [39]. They discovered a relationship between low birth weight and type 2 diabetes in men from England. In the "thrifty phenotype hypothesis," malnutrition serves as a fetal and infant insult that results in a state of nutritional thrift. The adaptations result in postnatal metabolic changes that prepare the individual for survival under poor nutritional conditions. The adaptations become detrimental when the postnatal environment changes to one of an overabundance of nutrients, resulting in obesity and diabetes.

Insulin resistance is a component of the World Health Organization (WHO) definition of the metabolic syndrome, which is a cluster of risk factors for cardiovascular disease [40]. The WHO defines the metabolic syndrome as the presence of glucose intolerance or insulin resistance, with at least two of the following: hypertension, dyslipidemia, obesity, and microalbuminuria. Women with PCOS are 4.4 times more likely to have the metabolic syndrome, so it becomes prudent to screen these patients, especially in those with insulin resistance [41].

Lipid abnormalities are also more prevalent in PCOS patients. There can be a significant increase in total cholesterol, LDL cholesterol, and triglycerides, and a decrease in HDL cholesterol compared to weight-matched controls [42]. The dyslipidemia, impaired glucose intolerance, central obesity, hyperandrogenism, and hypertension seen in PCOS patients greatly increase the risk for cardiovascular disease. Based on this risk profile, women with PCOS have a sevenfold increased risk of myocardial infarction [43].

7.5.10 Laboratory Evaluation

In addition to confirming elevations of androgens, the laboratory evaluation of PCOS should have the objective of excluding other causes of hyperandrogenic anovulation. Androgen-producing tumors of the ovary and adrenals must be excluded. The adrenal glands contribute 98% of circulating DHEAS, while both the ovaries and adrenals contribute equal amounts of circulating testosterone and androstenedione. If total testosterone is greater than 200 ng/dL or DHEAS is greater than 7000 ng/dL, MRI is warranted to identify the hormone-secreting lesion. Measuring 17 α (alpha)-hydroxyprogesterone will screen for 21-hydroxylase deficiency, the most common enzyme deficiency in

nonclassical CAH. A 17-hydroxyprogesterone level of greater than 3 ng/mL is defined as elevated and should be followed by an ACTH stimulation test, using 250 μg of synthetic ACTH given intravenously following an overnight fast. A 1-h increase of 17 α (alpha)-hydroxyprogesterone of more than 10 ng/mL is indicative of an enzyme defect in 21-hydroxylase.

Cushing's syndrome may masquerade as PCOS. Those who have additional signs of Cushing's syndrome, such as a moon facies, buffalo hump, abdominal striae, easy bruising, and proximal myopathy, should undergo screening with a 24-h urinary-free cortisol. In the work-up for anovulation, exclusion of prolactinoma should be performed. It is not uncommon to detect mild elevations in prolactin levels in PCOS patients. Thyroid-stimulating hormone (TSH) should be evaluated. LH, FSH, and estradiol levels should be obtained to exclude hypothalamic amenorrhea or premature ovarian failure.

7.5.11 Diabetes Screen/Evaluation of Insulin Resistance

The 2003 Rotterdam PCOS consensus group recommends a 2-h oral glucose tolerance test (OGTT) for obese PCOS patients and nonobese PCOS patients with risk factors for insulin resistance, such as family history of diabetes [2]. Defining insulin resistance is difficult, because the concept is nebulous with no universally accepted diagnostic strategy. The WHO defines insulin resistance as the lowest quartile of measures of insulin sensitivity. Women with PCOS are at significantly increased risk for impaired glucose tolerance and type 2 diabetes compared to age-, weight-, and ethnicity-matched controls [44]. If either the fasting glucose is 126 mg/dL or more, or the 2-h level is 200 mg/dL or more, diabetes is detected and should be confirmed with a repeat test. Impaired fasting glucose is defined as a glucose level between 100 and 126 mg/dL. Impaired glucose tolerance is defined as a 2-h glucose level between 140 and 200 mg/dL. It is also reasonable to obtain a fasting lipid profile in women suspected of having risk factors for cardiovascular disease. HgbA1C has been recently advocated as an accurate screening tool in evaluation of insulin resistance and diabetes in women with PCOS [45].

7.5.12 Treatment

There are many considerations when deciding on therapy for PCOS (Fig. 7.4). Identification of patient concerns is necessary when prioritizing goals and formulating a treatment plan. A combination of therapies may be warranted, and the practitioner should appropriately counsel the patient on the treatment expectations. Amelioration of long-term health risks should be emphasized, regardless of the primary complaints of the patient.

7.5.13 Weight Loss

Weight reduction should be a major component of any treatment plan for the overweight patient (BMI >25). Any sustained improvement in weight should involve diet and exercise, and consultation with a nutritionist may be helpful for those with difficulty achieving weight reduction. Obese PCOS patients who achieve weight loss will have an increase in SHBG, decrease in free testosterone, and improvement in fasting insulin levels [46].

7.5.14 Oral Contraceptives

Combination oral contraceptives have been the mainstay of PCOS management for the patient not interested in conception. Contraceptives suppress pituitary LH and consequently reduce ovarian androgen secretion, increase SHBG, and reduce free testosterone, while regulating menses and reducing the risk of endometrial hyperplasia or malignancy. However, there may be mild attenuation of insulin sensitivity.

Korytkowski et al. have shown that short-term use of COCs in PCOS women results in a small decrease in insulin sensitivity and no change in the baseline elevation in triglyceride levels [47]. However, in normal women, COCs were shown to have an even more pronounced decline in insulin sensitivity, along with a significant elevation in triglyceride levels. The long-term effects of COCs upon insulin sensitivity and lipoprotein profiles have not been well documented. PCOS women are at greater risk for the development of diabetes and cardiovascular disease; thus, further investigation into the safety of long-term hormonal therapy is needed.

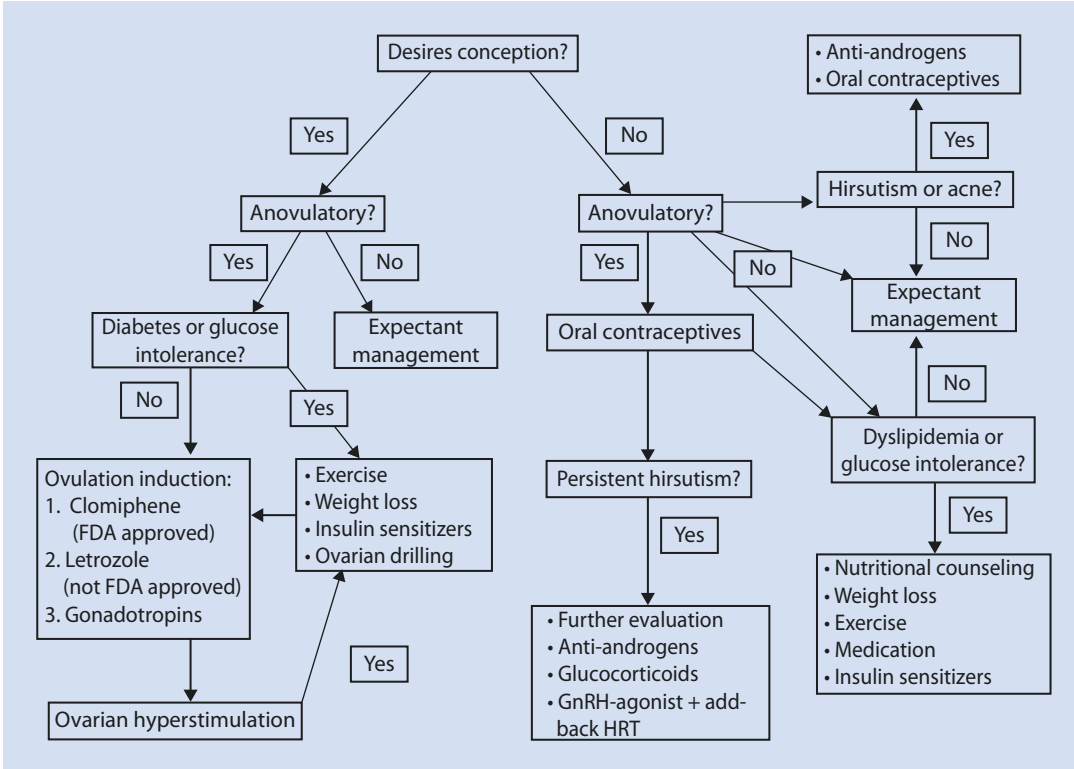

Fig. 7.4 Polycystic ovary syndrome treatment algorithm. Reprinted from Am J Obstet Gynecol, 179/6S, Berga S, The obstetrician-gynecologist's role in the practical management of polycystic ovary syndrome, 109S–113S, Copyright 1998, with permission from Elsevier. Letrozole can also be used for ovulation induction although it is not FDA approved

7.5.15 Hair Removal

In women with significant hirsutism, removal of unwanted hair, especially on the face, chest, and abdomen, is often an important concern. Shaving, plucking, waxing, and depilatories are the most common approaches used for temporary removal. These approaches do not induce coarser or faster hair growth, but must be repeated at frequent intervals.

Electrolysis is probably the most commonly used technique for permanent hair removal, wherein a fine needle is inserted into each hair follicle and an electrical current is applied. Hair follicles must be treated individually and several treatments may be needed to destroy the follicle. Usually, repeated treatments are required over a 12- to 18-month period. Possible side effects include pain, infection, hyper- or hypopigmentation, and keloid formation in susceptible women. Laser hair removal techniques, and the related intense pulsed light devices, are other options for hair removal. These techniques work by emitting light at various wavelengths, energy output, and pulse widths that are selectively absorbed by darker structures. For this reason, laser hair removal works best for light-skinned people with dark hair. As with electrolysis, laser treatments for hair removal must be repeated.

7.5.16 Topical Eflornithine Hydrochloride

Eflornithine hydrochloride (Vaniqa, Bristol-Myers Squibb, New York, NY) is a prescription-only topical cream approved by the FDA for reducing and inhibiting the growth of unwanted facial hair. This drug works by irreversibly inhibiting ornithine decarboxylase, an enzyme that facilitates cell division in hair follicles. The cream is applied twice a day to areas of unwanted facial hair, and <1% is absorbed systemically. It is designated by the FDA as a Pregnancy Category C drug.

Noticeable results are usually observed after 4–8 weeks of therapy. Application must be continued for as long as inhibition of hair growth is desired, although facial hair growth is reduced for up to 8 weeks after discontinuation.

7.5.17 Antiandrogens

Antiandrogens are commonly used as an adjunct to oral contraceptive therapy for treatment of hirsutism, but they have also been found to improve ovulation and restore regular menses. It is important to remember that all antiandrogens are teratogenic and pose a risk of feminizing a male fetus, and thus should be used along with an effective form of contraception.

Spironolactone is an aldosterone antagonist, and it is the most commonly used adjunctive agent in the treatment of hirsutism. It competes for testosterone binding sites on the pilosebaceous unit, inhibits 5α (alpha)-reductase, and inhibits androgen production by interfering with cytochrome P450 [48]. The potassium-sparing effect warrants judicious use in the patient on potassium supplementation or preexisting hypertension.

Flutamide is a nonsteroidal antiandrogen that competes for the androgen receptor. Anovulatory PCOS patients treated with flutamide experienced resumption of ovulation with restoration of normal ovarian appearance with one dominant follicle [49]. This study also reported a reduction in plasma levels of LH, androstenedione, and testosterone. Liver toxicity is a rare but potentially serious side effect of flutamide.

Finasteride is a potent inhibitor of 5α (alpha)-reductase used for the treatment of prostatic hyperplasia with promising results as a treatment for hirsutism. All antiandrogens should be used along with a form of contraception, because they are teratogenic and pose a risk of feminizing a male fetus.

7.5.18 Insulin-Sensitizing Agents

Insulin-sensitizing agents have been shown to improve endocrine and reproductive abnormalities in PCOS. Metformin is the most thoroughly investigated insulin-lowering agent used in PCOS. It is a biguanide that primarily works by suppressing hepatic gluconeogenesis and, to a lesser degree, increases peripheral insulin sensitivity [50]. Thiazolidinediones (TZDs) are peroxisome proliferator activating receptor [PPAR-γ(gamma)] agonists that improve peripheral insulin sensitivity but do not appear to have an effect on hepatic glucose production [50]. This class of medications includes troglitazone, pioglitazone, and rosiglitazone.

Troglitazone is the oldest but was removed from the market in 2000 owing to hepatotoxicity. Rosiglitazone and pioglitazone are still available and appear to be safer. The role of insulin-sensitizing agents is still an area of active investigation.

Many studies have demonstrated the positive effects of metformin on the reproductive axis of PCOS patients, with one of the most comprehensive studies recently demonstrating a dramatic improvement after 6 months of treatment. Metformin administration to nonobese hyperandrogenic PCOS patients resulted in a reduction of (1) LH pulse amplitude; (2) androstenedione levels; (3) testosterone levels; (4) ovarian volume; and (5) Ferriman–Gallwey scores. Menstrual cyclicity was also improved in most patients [51]. The investigators did not determine if metformin increased the likelihood of ovulation or if FSH levels rose. Similarly, troglitazone-treated PCOS patients demonstrated improved ovulation, decreased hirsutism, decreased free testosterone, and increased SHBG [52].

Insulin-sensitizing agents have a favorable effect on hyperandrogenism by reducing LH secretion, thereby removing the main stimulus for pathologic ovarian and adrenal androgen production. The reduction in insulin levels elevates hepatic SHBG production, decreasing free androgen levels. The concurrent improvement in hyperinsulinemia and hyperandrogenemia conferred by the use of insulin-sensitizing agents may ameliorate hirsutism.

The improvement in ovulation and menstrual cyclicity in patients treated with insulin-sensitizing agents suggests improved fertility. Indeed, spontaneous and clomid-induced ovulation rates in metformin-treated women with PCOS are increased [53]. Spontaneous ovulation occurred in 34% of those treated with 500 mg of metformin three times daily compared to only 4% in the placebo group. Clomid-induced ovulation occurred in 90% of women who received metformin compared to 8% who received placebo. For those who are clomiphene-resistant, significant improvements in ovulation and pregnancy rates

were reported in a randomized, double-blind, placebo-controlled trial for women pretreated with metformin [54]. Troglitazone alone and the combination of troglitazone plus clomiphene is also associated with increased rates (gamma) of ovulation and pregnancy in insulin-resistant women with PCOS [55]. One recent study found that in escalating doses letrozole was more effective than clomiphene as a fertility treatment in women with the polycystic ovary syndrome. Ovulation, conception, pregnancy, and live birth were significantly more likely after treatment with letrozole. The rate of pregnancy loss, the mean pregnancy duration and birth weight, and rates of neonatal complications (including anomalies) did not differ significantly between the letrozole and clomiphene treatment groups [56].

Though metformin is a category B medication, its use throughout pregnancy is becoming more attractive. In one retrospective study, Jakubowicz et al. found a significant reduction in the rate of early pregnancy loss for PCOS women who conceived while taking metformin and continued the agent throughout pregnancy. The rate of early pregnancy loss in the metformin group was 8.8% compared to 41.9% in controls. In the women with a prior history of miscarriage, the early loss rate was 11.1% for the metformin group compared with 58.3% in the control group [57]. The efficacy of metformin for pregnancy loss is not yet clear, and safety data for this indication are lacking. In 2007, Legro et al. published a well-designed trial that concluded that live birth rate in PCOS patients prescribed clomiphene (22.5%) was superior to metformin alone (7.2%) and not dissimilar from combination clomiphene/metformin therapy (26.8%) [58].

Metformin should not be given to those with conditions associated with elevated lactate levels, such as renal or hepatic disease, as there is a risk of lactic acidosis with an associated mortality of 50% [59]. Although most studies of metformin in PCOS used a dose of 500 mg three times daily, no studies have been performed to determine the optimal dosing regimen for improvement in insulin sensitivity, reduction of androgens, and resumption of ovulation. A dose–response study of type II diabetic patients demonstrated that the 2000-mg daily dose was optimal for improvement of glucose homeostasis [60], but the relevance of this dose to the PCOS population remains to be investigated.

Metformin should be initiated in a stepwise approach, titrating the dose slowly over several weeks in order to minimize side effects. Most patients will experience gastrointestinal symptoms such as nausea, diarrhea, indigestion, and abdominal discomfort. Side effects will resolve in several days for most patients, which allows incremental dosing increases on a weekly basis up to a maximum dose of 1000 mg bid. Baseline serum creatinine should be obtained with yearly monitoring to avoid lactic acidosis.

There are no guidelines currently on the long-term use of metformin to prevent or improve health outcomes in patients with PCOS. One of the serious reactions of TZD is hepatotoxicity. Initiation of treatment requires baseline liver function studies along with periodic monitoring.

7.6 Ovarian Surgery

7.6.1 Ovarian Wedge Resection

Ovarian wedge resection is a surgical procedure used for PCOS patients that has been found to restore both regular menses and ovulation in the majority of cases. This procedure, originally performed by laparotomy, consisted of removing a wedge of ovarian tissue and reconstructing the ovary. Laparoscopic techniques for ovarian wedge resection have described.

The primary disadvantage of this approach is the formation of significant pelvic adhesions, which occurs in at least one-third of the patients. The concern is that these adhesions might actually decrease fertility and increase the risk of pelvic pain. Another concern is that the restoration of ovulation is unlikely to be permanent, since the ovaries are not the causal agents in this complex systemic disorder. However, the actual long-term effectiveness of wedge resection has never been reported.

7.6.2 Ovarian Drilling

A laparoscopic variant with similar results to ovarian wedge resection is called ovarian drilling. This procedure involves making multiple punctures in the ovarian cortex and destroying ovarian tissue using unipolar electrosurgery or laser. The

results and complications for this approach appear to be similar or slightly less than those for ovarian wedge resection, although a prospective randomized study has never been done. There are additional concerns about long-term effects of ovarian drilling on ovarian function.

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Abnormal Uterine Bleeding

Sonia Elguero, Bansari Patel, and William W. Hurd

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8.1 Introduction

Abnormal uterine bleeding (AUB) is a common clinical problem, and is a cause of significant public health concern as it impairs quality of life by creating significant physical, emotional, sexual, social, and financial burdens [1–3].

A broad definition of AUB includes any uterine bleeding that occurs outside the parameters of normal menstruation during the reproductive years. Specifically, AUB is a term utilized to describe a spectrum of symptoms, including heavy menstrual bleeding (HMB), intermenstrual bleeding, and a combination of both heavy and prolonged menstrual bleeding. This terminology was established by the International Federation of Gynecology and Obstetrics (FIGO) Menstrual Disorders Working Group in 2011 and has since been adopted worldwide [4]. It includes bleeding originating from either the uterine fundus or cervix and does not include bleeding that originates in the lower genital tract.

It is important for gynecologists to develop a safe and cost-effective approach to the diagnosis and management of AUB. The most expedient methods for evaluation and treatment are dependent on understanding the various causes of AUB and the corresponding presenting symptoms.

Clinical Case

A 37 -year-old G0 P0 presents to her gynecologist's office with profuse vaginal bleeding that began the prior evening. Her gynecologic history is significant for a diagnosis of polycystic ovary syndrome (PCOS) and only 3–4 menses per year. She is not using a contraceptive method but has been unable to get pregnant for 3 years and is not currently pregnant. Her vital signs are stable and she has no symptoms other than bleeding. Her pelvic exam reveals clots in the vagina and blood actively coming from a normal appearing cervix. Her bimanual examination is difficult because of her obesity, but does not reveal uterine or adnexal masses or tenderness. Pelvic ultrasound shows a normal size anteverted uterus with a 16 mm endometrial stripe and what appear to be clots in the uterine cavity. Laboratory evaluation includes a negative blood hCG, white blood cell count of 9500 per mcL, hemoglobin of 8.8 g/dL, and platelet count of 250,000 per mcL. Other laboratory results are pending.

8.2 Diagnostic Criteria

AUB is broadly defined as any uterine bleeding that occurs outside the parameters of normal menstruation during the reproductive years. It includes bleeding originating from either the uterine fundus or cervix and does not include bleeding that originates in the lower genital tract (i.e., the vagina or vulva). However, these causes can be difficult to distinguish clinically. Therefore, both of these origins should be considered in all patients presenting with bleeding from the uterus. It can be further characterized in terms of volume, duration, frequency, and regularity. AUB can be classified as acute or chronic. Chronic abnormal uterine bleeding is bleeding that has occurred for at least 6 months.

The terms "menorrhagia" and "metrorrhagia" have now been replaced by more descriptive terms, including "heavy menstrual bleeding" and "intermenstrual bleeding." A new classification system is also being used to classify AUB according to the etiology [4]. The acronym PALM COEIN classifies the causes of AUB into structural abnormalities and nonstructural abnormalities (Table 8.1).

Table 8.1 PALM COEIN classification for abnormal uterine bleeding (AUB)

PALM (structural)
*P*olyp
*A*denomyosis
*L*eiomyoma
*M*alignancy and hyperplasia
COEIN (non-structural)
*C*oagulopathy
*O*vulatory dysfunction
*E*ndometrial
*I*atrogenic
*N*ot yet classified

Abnormal bleeding can occur during childhood, the reproductive years, and after menopause. Since the differential diagnoses and corresponding diagnostic approaches are markedly different during these time periods, AUB in women before and after the reproductive years is considered separately in ▶ Chaps. 4 and 10.

8.3 Prevalence

AUB accounts for approximately 30% of all gynecology visits [5]. Despite its frequency, AUB is a difficult diagnostic and therapeutic challenge and accounts for more than half of all hysterectomies performed in the USA. Approximately 20% of hysterectomy specimens removed for AUB have no visible pathology [6]. This suggests that many cases of AUB are potentially treatable using hormonal therapy or other systemic or local treatment modalities.

8.4 Normal Menstruation

A solid understanding of the normal menstrual cycle is essential to effectively evaluate and treat women with irregularities. The concept of normal menstruation is somewhat subjective and often varies between individual women and certainly between cultures. The normal menstrual cycle occurs over a span of 4.5–8 days every 24–38 days, with cycle-to-cycle variation over 12 months of ±2 to 20 days [4]. Normal menstruation should not cause severe pain or include passage of large clots.

Peri-menarchal (age <20) and peri-menopausal (age >40) stages have tremendous cycle length variation as these age ranges have the highest prevalence of anovulatory cycles [7].

The total amount of blood lost during a normal menstrual period has been estimated to average 30 mL and should be <80 mL. In most women, 90% of all blood loss per cycle occurs within the first 3 days of menstruation [8]. However, menstrual blood loss is difficult to estimate clinically, because much of the menstrual effluent is dissolved endometrium [9]. If the patient is changing pads or tampons more than once per hour, this is considered to be outside the parameters of normal menstruation.

The different bleeding patterns of AUB often give hints to the etiology and can be used to guide the appropriate diagnostic work-up. Due to the marked variation in presentation and the possible existence of multiple causes of bleeding, presentation alone cannot be used to clinically exclude common conditions.

8.5 Dysfunctional Uterine Bleeding

Dysfunctional uterine bleeding (DUB) is a term that refers to excessive uterine bleeding in cases in which no uterine pathology can be identified and is therefore a diagnosis of exclusion [10]. Due to the development of a greater understanding of AUB and the availability of more sophisticated diagnostic techniques, this term is less frequently used today.

In many cases that would have been referred to as DUB in the past, modern diagnostic techniques identify either uterine or systemic pathologies that (1) result in anovulation (e.g., hypothyroidism), (2) result from anovulation (e.g., endometrial hyperplasia or carcinoma), or (3) coexists with anovulatory bleeding but may or may not be causal (e.g., leiomyomata). Bleeding unrelated to uterine pathology can usually be determined to be a result of chronic anovulation (polycystic ovary syndrome [PCOS] and related conditions), exogenous steroid hormones (contraceptives or hormone replacement therapy), or hemostatic disorders (e.g., von Willebrand disease).

Treatment is most likely to be effective when specific causes of AUB can be identified. Since the term "DUB" is used to refer to widely divergent causes of AUB, a national consensus group recently concluded that this term is unlikely to improve diagnosis or treatment and thus no longer has any usefulness in clinical medicine [11].

8.6 Abnormal Uterine Bleeding Caused by Uterine Conditions

AUB can be grouped according to the basic pathophysiology of the various etiologies. The clinician must keep in mind that any individual patient can simultaneously have more than one

Table 8.2 Common uterine conditions associated with AUB

Pregnancy
Early normal pregnancy
Spontaneous abortion
Ectopic pregnancy
Gestational trophoblastic disease
Infection
Pelvic inflammatory disease
Endometritis
Cervicitis
Neoplasms
Benign
Leiomyoma
Endometrial polyps
Endocervical polyps
Malignant
Endometrial carcinoma
Cervical carcinoma
Adenomyosis

cause of uterine bleeding (Table 8.2). Therefore, the work-up should include an appropriate evaluation encompassing both likely and serious anatomic and systemic etiologies.

8.7 Pregnancy

It is important to exclude pregnancy in every case of AUB in a reproductive-aged woman, no matter how obvious any alternative diagnosis may be. Pregnancies are the most common cause of AUB in the reproductive age group, including normal pregnancies, spontaneous abortions, ectopic pregnancies, and gestational trophoblastic disease.

First-trimester bleeding occurs in up to 25% of all pregnancies and is associated with an increased risk of many common complications [12]. In approximately half of these cases, bleeding will be an early symptom of impending spontaneous abortion, whereas the remaining half will ultimately prove to have a viable pregnancy. Ectopic pregnancies currently make up 2% of all pregnancies and commonly present with AUB as one of the presenting symptoms [13]. Gestational trophoblastic disease is another pregnancy-related problem that presents as AUB in >80% of cases [14].

8.8 Uterine Pathology

It is a priority for gynecologists to precisely identify uterine pathology that might contribute to uterine bleeding. Most of these diagnoses can be determined to be related to infection and neoplasm. Additionally, a common uterine pathology related to AUB is adenomyosis.

8.9 Infection

Infection, in the form of endometritis, is an under-recognized cause of AUB and is often the basis of what appears to be AUB. In obvious cases of pelvic inflammatory disease, approximately 40% of patients will present with vaginal bleeding [15]. However, subclinical endometritis can also result in AUB.

Chronic endometritis was diagnosed in the past only when plasma cells were found on endometrial biopsy. More recent studies have shown an association between AUB and endometritis that manifests only as reactive changes in the surface endometrium and not in association with the presence of a particular type of inflammatory cell [16]. Other studies have verified that subclinical endometritis is a common finding in patients diagnosed with AUB and can be related to any of a number of different pathogens [17].

Cervicitis is another commonly recognized cause of AUB and is characterized by postcoital spotting. Postcoital bleeding is the most common presenting symptom in women found to have *Chlamydia* infections [18]. In addition to common sexually transmitted diseases (i.e., chlamydia and gonorrhea), other vaginal flora and pathogens can also cause cervicitis [19].

8.10 Neoplasms

AUB can be a presenting symptom for gynecologic neoplasms involving the cervix, uterine fundus, or ovaries. These neoplasms can be benign

(e.g., endometrial or endocervical polyps, leiomyoma) or malignant (e.g., endometrial or cervical carcinoma). Focal intracavitary lesions account for up to 40% of cases of AUB [20]. Neoplasms of the ovary can indirectly cause irregular bleeding by interfering with ovulation, as discussed below. Some of the most common neoplasms known to cause AUB are reviewed below.

8.11 Leiomyomas

These benign tumors of the myometrium are remarkably common and by age 50 can be found in almost 70% of white women and >80% of black women upon ultrasonographic examination [21]. Many of these leiomyomas are subclinical, and estimates of symptomatic leiomyomas range from 20–40%.

Submucosal and intracavitary leiomyomas that distort the uterine cavity are most likely to result in menorrhagia, presumably because of a direct effect on the adjacent endometrium. Large intramural leiomyomas can sometimes result in menorrhagia. However, the majority of leiomyomas that are intramural, subserosal, or pedunculated on the external uterine surface are not associated with AUB.

8.12 Adenomyosis

This benign condition involves the invasion of endometrium into the myometrium. Microscopic examination of the uterus reveals endometrial glands and stroma deep within the endometrium surrounded by hypertrophic and hyperplastic myometrium. This histopathologic diagnosis is found in over 60% of hysterectomy specimens [22]. Clinically, two-thirds of patients with adenomyosis will complain of menorrhagia and dysmenorrhea, and pelvic examination usually reveals a diffusely enlarged and tender uterus.

Diagnostic tests that help suggest the diagnosis of adenomyosis include both transvaginal ultrasonography and magnetic resonance imaging. The sensitivity for ultrasonography approaches 50%, and the sensitivity of MRI ranges from 80 to 100% [22]. Perhaps in the future, there will be more effective diagnostic testing for adenomyosis and treatments other than hysterectomy.

8.13 Endometrial Polyps

Endometrial polyps are localized overgrowths of the endometrium that project into the uterine cavity. These polyps may be broad-based (sessile) or pedunculated. Endometrial polyps are common in both pre- and postmenopausal women and are found in at least 20% of women undergoing hysteroscopy or hysterectomy [23]. The incidence of these polyps rises steadily with increasing age, peaks in the fifth decade of life, and gradually declines after menopause.

In premenopausal women complaining of AUB, studies have shown that from 5 to 33% will be found to have endometrial polyps [24]. Endometrial polyps are commonly found in patients with a long history of anovulatory bleeding, suggesting that polyps may be the result of chronic anovulation in some women. Polyps can also be found in women complaining of postmenstrual spotting or bleeding in ovulatory cycles or during cyclic hormonal therapy. Endometrial polyps in premenopausal women are almost always benign [23]. However, the risk of endometrial malignancy increases with age, and one study reported the risk of malignancy in polyps in women >65 years old was >50%.

8.14 Endometrial Hyperplasia

It is unlikely that endometrial hyperplasia causes AUB. However, this condition is most often found in premenopausal women with AUB with prolonged anovulation [25]. Although endometrial hyperplasia is not in itself a health risk, it is both a precursor to and marker for concurrent endometrial cancer, particularly in the presence of atypia.

8.15 Endometrial Cancer

The single most important disease to identify early in the evaluation of a peri- or postmenopausal woman with AUB is endometrial cancer. Approximately 20% of endometrial cancer is diagnosed in women before menopause and 5% before the age of 40 years [26]. After the menopause, approximately 10% of women with AUB will be found to have endometrial cancer, and the incidence rises with each decade of life thereafter.

8.16 Endocervical Polyps

These soft, fleshy growths originate from the mucosal surface of the endocervical canal. They usually arise from a stalk and protrude through the cervical os, although some may be broad-based. They usually range in size from 3 to 20 mm, but occasionally can be larger.

Endocervical polyps are known to be more frequent in women on oral contraceptives and with chronic cervicitis; however, the etiology remains unclear. Microscopically, endocervical polyps consist of a vascular core surrounded by a glandular mucous membrane and may be covered completely or partially with stratified squamous epithelium. In some polyps, the connective tissue core may be relatively fibrous. Endocervical polyps removed from women taking oral contraceptives often show a pattern of microglandular hyperplasia [27].

Endocervical polyps are relatively common in sexually active women, but are rare before menarche. Many endocervical polyps are asymptomatic and are discovered incidentally on visual examination of the cervix. In other instances, these polyps can manifest as intermenstrual and/or postcoital spotting.

8.17 Cervical Cancer

Cervical dysplasia can be found in up to 17% of women presenting with postcoital spotting, and 4% will have invasive cancer [28]. In the absence of a visible lesion, Papanicolaou smears and colposcopy (if indicated) are important diagnostic tools. In the presence of a visible cervical lesion, it is critical to biopsy the lesion to confirm the clinical diagnosis.

8.18 Abnormal Uterine Bleeding Unrelated to Uterine Pathology

Many women experience heavy or irregular menstrual bleeding that is not caused by an underlying condition of the uterus. Anovulatory bleeding is one of the most common underlying causes; however, a number of other unrelated causes, such as exogenous hormones and bleeding disorders, must also be considered (Table 8.3).

Table 8.3 Causes of AUB unrelated to pregnancy or uterine pathology

Exogenous hormones
Hormone contraceptives
Hormone replacement therapy
Ovulation defects
Physiologic oligo-ovulation
Perimenarchal
Perimenopasual
Polycystic ovary syndrome
Hyperandrogenic states
Congenital adrenal hyperplasia, adult-onset
Cushing's syndrome
Ovarian and adrenal tumors
Systemic diseases that interfere with ovulation
Hypothyroidism
Hyperprolactinemia
Renal failure
Liver disease
Endometrial atrophy
Menopause
Premature ovarian failure
Hypogonadotropic hypogonadism
Exogenous progestins
Hyperandrogenemia
Coagulopathy
Hereditary bleeding disorders
Von Willebrand disease
Disorders of platelet function and fibrinolysis
Acquired bleeding abnormalities
Idiopathic thrombocytopenic purpura
Leukemia
Aplastic anemia
Anticoagulation therapy

8.19 Exogenous Hormones

Hormonal therapy is one of the most common causes of AUB. Specifically, irregular bleeding is the most common symptom of women receiving contraceptive therapy and hormone replacement therapy (see ▶ Chap. 10) and the most common reason for discontinuation of these therapies.

8.20 Hormone Contraceptives

Approximately ten million women in the USA use some type of hormone contraception, including combination oral contraceptives, progestin-only pills, depot medroxyprogesterone acetate injections, progestin-containing intrauterine devices, subdermal levonorgestrel implants, transdermal combination hormone patches, and intravaginal rings. In addition to being a common reason to visit primary care physicians and gynecologists, AUB is the major reason for contraception discontinuation and subsequent unplanned pregnancy.

During the first 3 months of combination oral contraceptive use, as many as one-third of women will experience AUB. For the vast majority of women, the most effective treatment approach is patient reassurance and watchful waiting. As the uterus adapts to the new regimen of hormonal exposure, the monthly withdrawal bleeding becomes regular, lighter, and less painful than natural menstruation in most women.

If abnormal bleeding persists beyond 3 months while a patient is on hormonal contraceptives, other common causes should be excluded. In young, sexually active women, sexually transmitted diseases should be excluded, because in one study, almost one-third of women on oral contraceptives who experienced abnormal bleeding were found to have otherwise asymptomatic *Chlamydia trachomatis* infections [29]. If no cause for AUB other than hormonal therapy is found, treatment options include the use of supplemental estrogen or changing to an oral contraceptive with a different formulation that has a different progestin or higher estrogen content.

Women using progestin-only contraceptives have a greater risk of continued AUB than those using combined oral contraceptives. Prolonged exposure to progestins results in a microscopic condition sometimes called "pseudoatrophy" (see the section on Endometrial Atrophy). When reassurance is not sufficient, administration of supplemental estrogen during these bleeding episodes is sometimes useful.

8.21 Ovulation Defects

Abnormal or absent ovulation is a common cause of AUB during the reproductive years. A brief description of normal menstrual physiology (which is covered in depth in Chap. 1) is useful in understanding anovulation as an underlying cause of AUB.

8.22 Normal Menstruation

Each month, the endometrium of normally ovulating women is exposed to physiologic levels of estradiol (50–250 pg/mL), accompanied in the last 14 days of each cycle by progesterone (midluteal phase >12 nmol/L). The result is a structurally stable endometrium 5–20-mm thick as measured by transvaginal ultrasound.

Withdrawal of progesterone and estrogen results in menstruation, which involves the breakdown and uniform shedding of much of the functional layer of the endometrium, which is enzymatically dissolved by matrix metalloproteinases [30]. Normal menses occur every 28 ± 7 days, with duration of flow of 4 ± 2 days, and a blood loss of 40 ± 40 mL [31]. Hemostasis is achieved by a combination of vasoconstriction of the spiral arterioles and normal coagulation mechanisms.

8.23 Oligo- and Anovulation

Irregularity or absence of ovulation is common among reproductive-aged women not using hormonal contraception. In the perimenarchal years,

adolescents often have anovulatory cycles as part of the maturation process, but only occasionally do they complain of clinically significant AUB. In the perimenopausal years, anovulatory cycles again become more common for many women. These episodes of endometrial exposure to unopposed estrogen increase the risk of not only AUB but also endometrial hyperplasia and endometrial cancer. During the intervening years, both chronic and intermittent intervals of anovulation can occur, usually as a result of a treatable underlying condition.

8.24 Mechanism of Anovulatory Bleeding

Anovulation results in AUB as a result of chronic exposure of the endometrium to estrogen, without the effect of cyclic exposure to postovulatory progesterone. Endometrium that is exposed to unopposed estrogen becomes abnormally thickened and structurally incompetent. The result is asynchronous shedding of portions of the endometrium unaccompanied by vasoconstriction.

The bleeding associated with unopposed estrogen exposure is usually heavy. Since the blood has not been lysed by endometrial enzymes, blood clots are often passed, resulting in increased menstrual cramping in many women. Prolonged periods of bleeding also appear to predispose to subclinical endometritis, which can further exacerbate bleeding, and is often unresponsive to hormonal therapy.

8.25 Polycystic Ovary Syndrome

The most common cause of chronic anovulation is a disorder that can present with a constellation of symptoms and is referred to as PCOS (see ▸ Chap. 8). PCOS is a heterogeneous endocrine and metabolic disorder that affects 6–10% of reproductive age women [32]. This syndrome is diagnosed when a woman without an underlying condition is found to have two out of the following three criteria: (1) oligo- or anovulation, (2) clinical and/or biochemical evidence of hyperandrogenemia, and (3) polycystic ovaries [33]. These women have circulating estrogen levels in the normal range, but anovulatory progesterone levels.

PCOS is believed to result from insulin resistance in many women [32]. In today's culture, insulin resistance is often the result of obesity. However, only 70% of women with PCOS are obese [33]. Insulin resistance will be found in approximately 75% of women with PCOS who are obese, but <40% of those who are not obese [34].

The mechanism whereby insulin resistance results in PCOS is intriguing [35]. Insulin increases production of androgens by both the ovaries (primarily androstenedione and testosterone) and adrenal gland (primarily dehydroepiandrosterone). In the ovary, insulin increases androgen secretion by both theca cells, which are LH-dependent, and ovarian stroma cells. These increased androgens contribute to hirsutism and may contribute to the increased body mass often seen in PCOS patients. These androgens can be aromatized peripherally in both fat and muscle to estrogen (primarily estrone), which acts on the pituitary to increase secretion of LH, which in turn stimulates the ovaries to secrete more androgens in concert with insulin. The positive-feedback loop that results is believed to be the cause of many cases of PCOS. The accuracy of this interpretation is supported by the observation that in many overweight patients, either weight loss or the use of an insulin-sensitizing agent (e.g., metformin) will simultaneously improve insulin resistance and restore regular ovulatory cycles [35].

8.26 Systemic Diseases that can Mimic PCOS

Some patients that are oligo- or anovulatory can have an underlying systemic disease, which makes these patients clinically indistinguishable from PCOS. Although some diseases can be detected with appropriate testing, not all of these systemic conditions can be treated such that the symptoms completely resolve.

Conditions that result in signs and symptoms identical to PCOS can be divided into two groups. The first group includes conditions that cause hyperandrogenemia, which in turn can interfere with ovulation and result in a clinical picture identical to PCOS [36]. These include adult-onset congenital adrenal hyperplasia, Cushing's syndrome and disease, and androgen-secreting neoplasms of the ovary or adrenal gland. Adult-onset congenital

adrenal hyperplasia should be suspected whenever PCOS symptoms occur simultaneously with menarche. Cushing's and androgen-secreting tumors should be suspected when hyperandrogenism and ovulation dysfunction present rapidly in a woman with previously normal menstrual cycles.

The second group consists of any systemic condition that can interrupt ovulation. Both hypothyroidism and hyperprolactinemia are relatively common conditions that can have no other symptoms other than interfering with ovulation. Simple blood tests can screen for these conditions in the initial evaluation of PCOS. In addition, any serious systemic disease can interfere with ovulation—most notably, renal failure and chronic liver disease. These systemic disorders can also affect hemostasis. Patients with serious systemic diseases, however, usually manifest significant symptoms in addition to ovulatory dysfunction and AUB [37].

8.27 Endometrial Atrophy

Endometrial atrophy from any cause can result in AUB and is usually described as spotting. The significance of this type of AUB is that it is indistinguishable from the earliest symptoms of endometrial cancer and thus must be carefully evaluated in the peri- and postmenopausal woman.

Hypoestrogenemia is most commonly the result of surgical or natural menopause. Although natural menopause occurs at an average age of approximately 51 years, 2% of women undergo premature menopause before the age of 40 years. Hypoestrogenemia also occurs in women with normal ovaries who lack gonadal hormonal stimulation due to pituitary or hypothalamic pathology, descriptively grouped together as having hypogonadotropic hypogonadism. Causes of this condition include hypothalamic amenorrhea, usually secondary to conditions such as anorexia nervosa, repetitive or prolonged strenuous exercise, or starvation, and the relatively uncommon pituitary failure. Hypoestrogenemia can also occur secondary to hyperprolactinemia.

Histologically, hypoestrogenemia leads to atrophy of both endometrial glands and stroma. Scanty, small glands can be seen in dense stroma. The result is thinning of the endometrium, which can be <5-mm thick by transvaginal ultrasonography.

Prolonged exposure to exogenous progestins, with or without estrogen, can also result in endometrial atrophy. Long-term use of combined oral contraceptives results in poorly developed glands lined by a single layer of low columnar to cuboidal cells. Secretory changes are minimal, but stromal decidualization is present, resulting in discordance between small inactive glands and decidualized stroma. Numerous granular lymphocytes are often present. Progestin-only contraception results in endometrial atrophy with sparse, narrow glands lined by flattened epithelium in a spindle-cell stroma without decidual reaction. Women with hyperandrogenemia can develop a similar clinical and histological picture.

8.28 Coagulopathy

A surprisingly common cause of menorrhagia is one of the several inborn or acquired conditions that can interfere with the body's normal hemostatic mechanisms.

8.29 Hereditary Bleeding Disorders

Von Willebrand disease and less common disorders of platelet function and fibrinolysis are characterized by excessive menstrual bleeding that begins at menarche and is usually regular. As many as 20% of adolescents who present with menorrhagia significant enough to cause anemia or hospitalization have a bleeding disorder, and should therefore undergo an evaluation for coagulopathy. However, it is important to remember that most AUB in this age group is probably due to anovulation [38].

The most common bleeding disorder is von Willebrand disease, which affects 1–2% of the population [39]. This hereditary deficiency (or abnormality) of the von Willebrand factor results in decreased platelet adherence, with von Willebrand factor interacting with platelets to form a platelet plug. A fibrin clot will then form on this plug. There are three main types of von Willebrand disease. The mild form (type 1) is responsible for over 70% of cases and is characterized by an absolute decrease in the protein. The mechanism by which an abnormal factor leads to

bleeding at the level of the endometrium is unclear. The vast majority of women with this disease report AUB, specifically menorrhagia. The prevalence of this disorder in adults can range from 7 to 20%. Other inherited conditions include thrombocytopenias and rare clotting factor deficiencies (e.g., factor I, II, V, VII, X, XI, XIII).

8.30 Acquired Bleeding Disorders

New onset of extremely heavy menses not amenable to hormonal therapy can sometimes be related to acquired bleeding abnormalities. Such abnormalities include idiopathic thrombocytopenia purpura (ITP) or hematologic diseases affecting platelet production, such as leukemia. Other systemic disorders, such as sepsis and liver disorders, can also cause an acquired hemostatic disorder resulting in bleeding.

8.31 Anticoagulant Therapy

Excessive bleeding can rarely be a significant problem for women taking anticoagulant therapy, such as warfarin or heparin. Fortunately, most women taking anticoagulants do not have problems with AUB, which is considered to be an adverse reaction to anticoagulant therapy. Life-threatening genital bleeding in women taking anticoagulants is rare, but may lead to emergency hysterectomy [40].

8.32 Clinical Evaluation of AUB

The work-up for AUB should be tailored to the clinical presentation of the patient and, importantly, the age should be taken into consideration. At the same time, the clinician must be aware of common causes of AUB that might not be clinically obvious but still must be excluded.

An important point to keep in mind is that AUB can often have more than one cause. Sometimes subtle comorbid conditions, such as endometritis, can make single-factor therapy surprisingly ineffective [17]. In other women, obvious causes of chronic anovulation can be associated with endometrial hyperplasia and/or cancer. Careful evaluation of the patient for multiple simultaneous causes of AUB is important.

8.33 History

A careful history is the most important factor in determining the appropriate diagnostic approach. This should include the patient's menstrual patterns and history, the extent of recent bleeding, sexual activity, and contraception. Important questions include symptoms of pregnancy, infection, changes in body hair, excessive bleeding, and systemic disease. Current medication and information about prior Papanicolaou smears are also important. The review of systems should include symptoms of systemic disease, such as weight gain or loss, abdominal swelling, somnolence, and nipple discharge.

8.34 Pregnancy

In reproductive-aged women, the presence of signs and symptoms of pregnancy is important to ascertain. Current contraceptive methods and past pregnancy history are also important.

8.35 Characterization of Bleeding

Once pregnancy is excluded, the amount and character of the bleeding is important to ascertain. Careful, stepwise retrospective questioning will usually give a clear picture of the bleeding pattern over the previous days, months, and even years. In nonemergency cases of bleeding, the use of a prospective menstrual calendar is an excellent way to document the problem as well as the response to therapy. It is important to determine when the bleeding problems were first noticed, since menorrhagia starting at menarche should alert the clinician to the possibility of an underlying bleeding disorder.

The amount of bleeding is probably the most difficult to elicit on history, since normal or heavy menstrual bleeding can be very subjective. For research purposes, menorrhagia can be defined as a monthly blood loss of >80 mL on three consecutive menses as measured by the alkaline hematin method [41]. Unfortunately, this type of accurate evaluation is neither cost-effective nor readily available.

In adolescents with menorrhagia, it is important to determine any past history of excess bleeding during surgical, dental, or obstetric procedures

since this has been found to be predictive of von Willebrand disease [42]. Interestingly, in this same study, epistaxis and easy bruising were not clear discriminatory symptoms.

8.36 Physical Examination

The physical examination is intended to detect both gynecologic and systemic diseases. Special care should be taken to document the presence of hirsutism, acne, or other signs of excess androgens, as well as galactorrhea.

The pelvic examination begins with a speculum examination to inspect the cervix for polyps, signs of infection, or inflammation. A bimanual examination is important to determine uterine size, adnexal masses, and the presence and character of any tenderness.

8.37 Laboratory Testing

Laboratory evaluation is an important part of the initial evaluation of all patients with AUB (Table 8.4). However, rather than ordering every possible test at the first visit, laboratory tests should be obtained in a stepwise fashion based on presentation (Fig. 8.1).

The most important test for all reproductive-aged women complaining of AUB is a beta-HCG test for pregnancy. For all cases, except the most insignificant bleeding, a CBC (including platelets) is important to detect significant anemia and disorders of platelet production or survival. Unless precluded by extremely heavy bleeding, a Papanicolaou smear should be performed on any woman who has not had recent screening as per the current screening guidelines. For patients with apparent oligo- or anovulation, thyroid-stimulating hormone (TSH) and prolactin testing will detect subtle pituitary function disorders that might present with AUB as the earliest symptom. Since cervical and uterine infections are common, cervical tests for gonorrhea and chlamydia are helpful in women with intermenstrual spotting, as well as any woman at risk for these infections.

Several patient groups may require additional ancillary tests. Obese patients with apparent AUB are at increased risk for type II diabetes. Several authors recommend measurement of hemoglobin A1c (HbA1c) as a good diabetes screen that does not require fasting or a return visit for provocative testing. Patients with hirsutism or other evidence of androgen excess should be screened for ovarian and adrenal malignancies with total testosterone and DHEAS. All women >40 years old should have an endometrial biopsy after pregnancy is excluded to detect endometrial hyperplasia or cancer.

Table 8.4 Laboratory testing for AUB

All patients
Pregnancy test
Complete blood count (including platelets)
Papanicolaou smear
Cervical tests for gonorrhea and chlamydia
Anovulation
Thyroid-stimulating hormone
Prolactin
Obesity
Type II diabetes screen: HgA1c
Hirsutism
Testosterone
DHEA-S
>40 years of age
Endometrial biopsy
New-onset heavy menstrual bleeding
Prothrombin time
Activated partial thromboplastin time
Bleeding time
Heavy menstrual bleeding since menarche
Above plus
Iron profile, serum creatinine
Factor VII level
VWf antigen
Ristocetin cofactor
Platelet aggregation studies
If the above are negative, consider
Factor XI level
Euglobulin clot lysis time

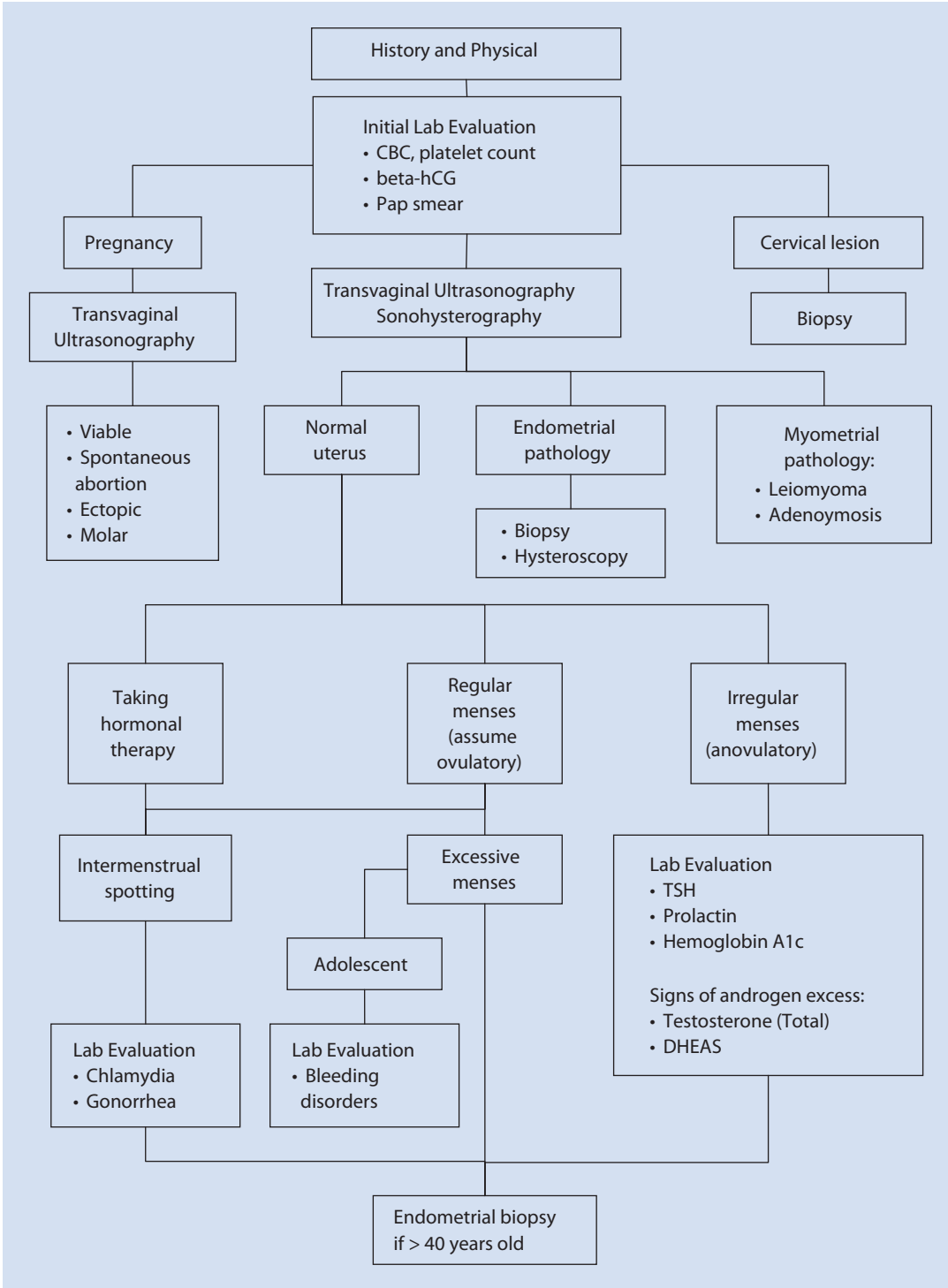

Fig. 8.1 Algorithm for evaluating women with AUB

PCOS and adult-onset congenital adrenal hyperplasia may sometimes be indistinguishable by clinical presentation, since both disorders are often characterized by hirsutism, acne, menstrual abnormalities, and infertility [43]. Unfortunately, no discriminatory screening test exists for this heterologous condition, which is most commonly caused by 21-hydroxylase or 11 beta-hydroxylase deficiency. If ovulation dysfunction and signs of androgen excess begin at the time of puberty, such women should be investigated appropriately (see ▶ Chap. 4).

8.38 Hemostatic Disorders

Patients with new onset of significant menorrhagia should be evaluated for bleeding disorders with prothrombin time, activated partial thromboplastin time, and bleeding time [44]. Any patient with a history of menorrhagia since menarche, especially with a history of surgical or dental-related bleeding or postpartum hemorrhage, should be evaluated for hereditable bleeding disorders. These tests include specific tests for von Willebrand disease, such as von Willebrand factor antigen, von Willebrand factor functional activity (ristocetin cofactor activity), and factor VIII level. These levels can fluctuate; therefore, these tests should be repeated if clinical suspicion is high. Normal ranges should be adjusted for the observation that von Willebrand factor levels are 25% lower in women with blood type O compared with other blood groups. Further studies, such as platelet aggregation studies, may also be required [44]. If these studies are negative, factor XI level and euglobulin clot lysis time can be evaluated.

8.39 Malignancies and Premalignancies

8.39.1 Endometrial Biopsy

AUB in women 40 years of age to menopause can often be attributed to anovulatory bleeding, which is a normal physiological response to declining ovarian function. However, the risk of endometrial hyperplasia and carcinoma increases with age. For this reason, once pregnancy has been excluded, an endometrial biopsy should be obtained in all women older than 45 years of age who present with AUB. Endometrial biopsy should also be performed in all women who are younger than 45 years of age who have a history of persistent AUB, unopposed estrogen exposure, or failed medical management [45].

8.39.2 Imaging and Hysteroscopy

Over the last two decades, our ability to visualize the uterine cavity and adnexa has dramatically increased. In addition to the bimanual pelvic examination, the only other available methods were hysterosalpingogram (HSG) and dilation and curettage. Although the radiation exposure and discomfort associated with HSG are both considered acceptable, this technique effectively identifies only marked abnormalities of the uterine cavity. Lesions <1 cm in size are often missed. Likewise, the previously blind procedure of dilation and curettage gave the operator only the roughest idea of the depth and contour of the uterine cavity. Intrauterine findings at the time of hysterectomy were often a surprise. In obese patients in whom bimanual examinations are difficult, unexpected ovarian masses at laparotomy were commonplace.

8.39.3 Transvaginal Ultrasonography

Today, transvaginal ultrasonography and sonohysterography have made unexpected findings at surgery a rare occurrence (see ▶ Chap. 6). Ultrasonography and sonohysterography have become important steps in the evaluation of AUB. Transvaginal ultrasonography can accurately determine uterine size and configuration, and reveal the nature of both palpable and nonpalpable adnexal masses. Knowledge about the size and location of leiomyoma and the potential that an ovarian mass might be malignant is invaluable prior to surgery. Sonohysterography can be used to accurately visualize most intrauterine abnormities once pregnancy has been excluded. Accurate evaluation of the uterine cavity is of the utmost importance for the evaluation and treatment of AUB. This procedure involves injection of sterile saline into the uterus while a transvaginal sonogram is performed. It may cause a small amount of discomfort to the patient. When the

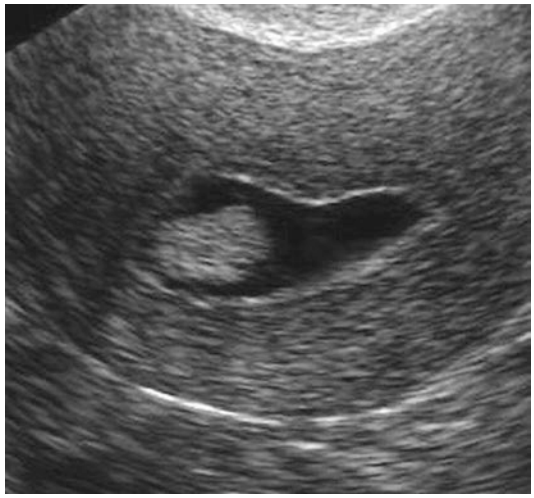

Fig. 8.2 Endometrial polyps diagnosed by sonohysterography

uterine cavity is distended with saline, intracavitary lesions (e.g., polyps, fibroids, cancer) as small as 3 mm can be clearly seen (Fig. 8.2).

8.39.4 Office Hysteroscopy

Office hysteroscopy (see ▸ Chap. 16) is another excellent outpatient method for visualizing the uterine cavity. The discomfort and risk is somewhat more than sonohysterography, and the procedure can be difficult in the presence of cervical stenosis or when the cervix is difficult to visualize. However, the color photographs depicting the lesion can be very informative for patients.

8.40 Management of AUB Unrelated to Pregnancy or Uterine Pathology

In more than half of patients presenting with AUB, the etiology will be determined to be unrelated to pregnancy or underlying uterine pathology. Treatment of these cases consists of managing underlying systemic medical conditions or comorbidities and normalizing the endometrium with exogenous hormone therapy, when necessary. In patients with hypothyroidism, for example, restoration of a euthyroid state with thyroid hormone replacement will restore normal ovulatory function in most cases. Likewise, treatment of underlying conditions, such as hyperprolactinemia, PCOS, or other endocrine dysfunction, such as an adrenal enzyme deficiency, may restore normal menstrual function.

8.41 Anovulatory Bleeding

For heavy, acute anovulatory bleeding, the primary focus of initial management is the expedient bleeding cessation by achieving rapid structural stability of the endometrium. After stabilization of the endometrium, long-term therapeutic goals for women who do not desire pregnancy include promoting synchronous endometrial shedding at regular intervals or achieving amenorrhea with exogenous hormone administration. This approach should prevent subsequent episodes of heavy bleeding that require emergent evaluation and management.

8.42 Hemodynamic Stabilization and Initial Evaluation

While hemorrhagic shock secondary to AUB is rare, many patients will present with critically low hemoglobin levels and symptomatic anemia. Healthy women of reproductive age can often compensate physiologically for severe anemia and thus have minimal symptoms. In contrast, older women, particularly those with underlying cardiovascular disease, may present with significant symptoms from heavy uterine bleeding. Initial management of hemodynamically unstable patients includes intravenous fluid resuscitation and blood product replacement, as indicated.

With stabilization, an expedient evaluation should be performed to detect pregnancy or anatomic pathology. Pertinent laboratory tests should be obtained, including CBC and quantitative beta-HCG. Transvaginal ultrasonography is an important initial diagnostic tool to evaluate for the presence of underlying anatomic pathology. However, differentiating intrauterine lesions such as leiomyomata or polyps from clots may be difficult in the setting of acute bleeding. Hormonal therapy, as discussed below, should be initiated to decrease uterine bleeding. Endometrial evaluation is an important part of the evaluation for many women, depending on age and risk factors

for endometrial hyperplasia or malignancy, but can safely be deferred until the bleeding decreases with medical management. Therefore, hormonal therapy does not have to be delayed until histologic sampling can be performed.

8.43 Dilation and Curettage

In cases in which massive, life-threatening, anovulatory uterine bleeding is present, dilation and curettage provides a rapid means to decrease bleeding and evaluate for endometrial pathology. As a surgical approach to the management of AUB, disadvantages include the risks of anesthesia, which depend on an individual patient's underlying medical conditions, and the small surgical risks of the procedure. Dilation and curettage has no long-term therapeutic effect; therefore, long-term treatment must be initiated postoperatively. In most cases of AUB, medical management can be safely used as the first-line treatment.

8.44 Intravenous Estrogen Therapy

In the absence of life-threatening bleeding requiring surgical intervention, AUB should be initially managed with hormonal therapy. Historically, first-line therapy consists of the administration of intravenous conjugated estrogens, 25 mg every 4 h, until the cessation of bleeding or for 24 h [46]. Given that this therapy is often associated with nausea, concomitant intravenous or oral antiemetics should be administered. Patients who do not respond to estrogen therapy may require dilation and curettage, as described above.

Given the pathophysiology of anovulatory bleeding, namely, prolonged exposure to unopposed estrogen, treatment with estrogen may seem paradoxical. By simulating clotting at the capillary level and promoting vasoconstriction, estrogen acutely decreases uterine bleeding related to asynchronous shedding [47].

8.45 Oral High-Dose Combined Hormonal Therapy

With heavy, non-emergent bleeding or when bleeding has decreased to a level consistent with heavy menses or less, an oral contraceptive therapy taper can be initiated (Table 8.5). This approach may be utilized for women with heavy bleeding in the outpatient setting who do not require hospitalization. As with intravenous estrogen, nausea is a common side effect, and oral antiemetics should be provided to optimize compliance.

Table 8.5 "Taper" oral contraceptive regimen for treatment of AUB using low-dose (30 μg ethinyl estradiol), monophasic oral contraceptive pills

Day	Frequency
1–2	One tablet 4 times daily
3–4	One tablet 3 times daily
5–19	One tablet daily
20–25	Expect menses
26	Start oral contraceptives at standard dosage

8.46 Women at Increased Risk for Cardiovascular Disease or Venous Thrombosis

In women at increased risk for cardiovascular disease, venous or arterial thromboembolism, estrogen-containing oral contraceptives may be relatively or absolutely contraindicated. This includes women with a history of thromboembolic disease and woman over 35 years of age who have additional risk factors for thromboembolism (e.g., cigarette smoking, hypertension, diabetes). Although no studies have been published using short-term, high-dose intravenous or oral estrogen in these patients, at least one case of fatal pulmonary thromboembolism has been reported with intravenous therapy [48]. Certainly, high-dose estrogen therapy should be used in these patients in the acute setting only if the benefits outweigh the risks. The Medical Eligibility Criteria for Contraception provides evidence-based guidelines for use of hormonal agents in the setting of various medical comorbidities and can be an important reference in the management of high-risk patients, particularly when considering options for long-term therapy [49].

8.47 Women with a Coagulopathy

Women with menorrhagia secondary to von Willebrand's disease may be successfully treated with long-term oral contraceptive therapy, as described below. Other medical treatments used by hematologists for acute episodes include desmopressin acetate, antifibrinolytic agents, and plasma-derived concentrates of von Willebrand factor.

8.48 Long-Term Treatment of AUB

The most appropriate long-term management of AUB depends on the underlying etiology of AUB as well as a patient's reproductive desires. Women who do not desire pregnancy may initiate combined oral contraception or other hormonal therapies, such as cyclic progestins or a progestin-containing intrauterine device. Additionally, non-hormonal therapy with tranexamic acid, an anti-fibrinolytic agent, may be a therapeutic option. For anovulatory women wishing to become pregnant, ovulation induction is usually the most appropriate treatment. Two important considerations, endometrial synchronization and subclinical endometritis, deserve mention, because they may serve to optimize medical management of AUB.

8.49 Endometrial Synchronization

For women with chronic irregular bleeding, synchronizing the endometrium prior to the initiation of cyclic hormones may be a helpful first step, as synchronization can reduce breakthrough bleeding encountered with subsequent therapy. Two approaches to synchronization include the use of a potent progestin or an oral contraceptive taper. Medroxyprogesterone acetate, in the dose of 10 mg per day for 14 days, usually improves the presenting bleeding episode within 2–3 days and serves to thin the endometrial lining prior to withdrawal bleeding. Patients should be counseled that they may experience moderately heavy bleeding within 1–2 days of stopping the medroxyprogesterone. Oral contraceptives should be started on the Sunday following this withdrawal bleeding. Alternately, taper therapy with oral contraceptives (Table 8.4) can be used for women presenting with heavy prolonged bleeding.

8.50 Antibiotics for Subclinical Endometritis

While few studies have evaluated the impact of subclinical endometritis on the clinical presentation of AUB, an emerging body of literature supports a relationship between these clinical diagnoses. A recent study demonstrated that chronic endometritis is the most frequent histologic finding in endometrial biopsies performed on women with AUB [50]. In another study, 81% of patients with irregular bleeding or vaginal discharge had positive endometrial cultures for *Mobiluncus*, and treatment with metronidazole resolved their irregular bleeding [51]. In a study of 100 hysterectomies performed for irregular bleeding or fibroids, 25% of the endometrial cavities were found to harbor organisms, including *Gardnerella vaginalis*, *Enterobacter* and *Streptococcus agalactiae* [52]. Finally, a study of college-age women presenting with abnormal bleeding while on oral contraceptives found that 29% were infected with *Chlamydia* [29].

These studies suggest that subclinical endometritis may impact the clinical presentation of AUB. Therefore, when apparently anovulatory AUB does not respond to standard medical management with hormonal therapy, subclinical endometritis may be a coexisting disorder to address. Cervical evaluation for common pathogens (chlamydia and gonorrhea) followed by appropriate antibiotic therapy is important. In women with negative cultures who do not respond to cyclic hormonal therapy, empiric therapy with a broad-spectrum antibiotic (e.g., metronidazole or a cephalosporin) may be considered. However, to date, prospective trials assessing the impact of treatment of clinical endometritis on AUB outcomes have not been conducted.

8.51 Ovulation Induction

Restoration of ovulation for women desiring fertility is of paramount importance. Accomplishing regular ovulatory cycles may occur with the treatment of any underlying condition responsible for anovulation. For example, in patients with hyperprolactinemia, using a dopamine agonist to normalize prolactin levels will often result in ovulation and pregnancy. In the case of PCOS, recent studies have demonstrated that insulin

sensitizing agents, such as metformin, can promote ovulation (see ▶ Chap. 8). However, a recent randomized clinical trial did not demonstrate an improvement with live birth rates when adding metformin to clomiphene citrate [53].

While waiting for systemic therapies to result in resumption of ovulation and normal menses, monthly induction of withdrawal bleeding with an oral progestin should be considered to avoid ongoing AUB. In women not using combined oral contraception, the use of micronized progesterone (200–300 mg daily for 14 days) will result in reasonable withdrawal bleeding and will be safe should pregnancy occur. For patients who do not resume ovulation with systemic therapy, induction of ovulation using clomiphene citrate or injectable medications should be considered (see ▶ Chap. 7).

8.52 Oral Contraceptives

For decades, combined oral contraceptive pills have been the first-line therapy for managing AUB, and studies have demonstrated that combined oral contraceptives decrease the duration and amount of menstrual flow as well as dysmenorrhea [54]. In addition, extending the number of consecutive days of active pills and decreasing the annual number of menses may further minimize menstrual-related symptoms [55]. Extended-cycle regimens increase the risk of spotting and breakthrough bleeding when compared with standard monthly cycle regimens, but the risk generally decreases over time [56].

8.53 Progestins

Progestins represent another option for long-term management of AUB. The administration of progestins, such as 10 mg medroxyprogesterone or 300 mg micronized progesterone, daily, from day 15 to 26 of each cycle, will regulate menses in anovulatory patients. Cyclic progestin therapy represents a safe and effective approach to managing AUB and does not have the side effects or risks associated with oral estrogen. Additionally, cyclic progestin therapy provides endometrial protection against hyperplasia and cancer. Side effects of progestin therapy include mood changes or depression, nausea, breast tenderness, and bloating. Luteal phase progestin therapy has been demonstrated to be less efficacious in ovulatory AUB (menorrhagia) when compared with nonsteroidal antiinflammatory drugs (NSAIDs), tranexamic acid, or a progesterone-releasing intrauterine system [57].

8.54 Levonorgestrel-Containing Intrauterine Device

While originally developed for contraception, the levonorgestrel intrauterine device (IUD) represents a highly effective treatment of both menorrhagia and dysmenorrhea. The local release of levonorgestrel into the uterine cavity suppresses endometrial growth and has been shown to decrease menstrual blood loss by as much as 97% [58]. While many women will experience irregular or intermenstrual bleeding in the first 6 months of use, approximately 50% will have amenorrhea by 24 months [59]. While most of the progestin acts locally within the uterus, levonorgestrel can be detected in the systemic circulation among IUD users [60]. Therefore, other side effects, such as hirsutism, acne, weight change, nausea, headache, mood changes, and breast tenderness, may occur. Although the initial costs of the levonorgestrel IUD may be higher than other medical treatment options, they provide very cost-effective long-term therapy of AUB.

8.55 GnRH Analogues

Administration of a GnRH agonist results in pituitary down-regulation, hypoestrogenemia, and complete cessation of menses. GnRH agonists initially increase ovarian stimulation, called a "flair," prior to suppression of the hypothalamic–pituitary–ovarian axis. GnRH antagonists avoid this flair, but have only recently become available, and their clinical utility remains to be determined. Symptoms of estrogen deprivation, most notably hot flashes, mood alterations, and bone loss, result from the use of all GnRH analogues. These side effects may be averted with the use of "add-back" therapy with daily administration of norethindrone 5 mg orally. While GnRH analogues play an important role in the initial management of AUB, they are rarely used for long-term therapy given the expense and side effect profile.

8.56 Tranexamic Acid

Tranexamic acid, an anti-fibrinolytic agent, is an emerging therapy for the treatment of AUB. Recent Cochrane analysis has confirmed efficacy and patient tolerance of tranexamic acid in the treatment of menorrhagia, and in Europe this medication has become the preferred treatment for women with heavy menstrual bleeding [61]. Recently, the FDA approved tranexamic acid for use in the treatment of menorrhagia. This therapy is administered orally at a dose of 1300 mg three times daily for 5 days, initiated with onset of menses. To date, studies have not demonstrated an increased risk of venous or arterial thromboembolism [62]. However, tranexamic acid should not be concomitantly administered with combined oral contraception or in women with an increased risk of thromboembolism.

8.57 Nonsteroidal Antiinflammatory Drugs

Prostaglandins significantly impact endometrial hemostasis, and by inhibiting prostaglandin synthesis, NSAIDs serve to decrease menstrual blood loss. NSAIDs may reduce menstrual blood loss by 20–40% [63]. While naproxen has been the most extensively studied NSAID, no member of the drug class offers distinct advantages for AUB [64]. Additionally, NSAIDs provide an effective treatment for dysmenorrhea, which is often present in those with AUB.

8.58 Surgical Treatment

For women with AUB refractory to medical management who do not desire childbearing, surgical treatment modalities, including endometrial ablation or hysterectomy, should be considered. Endometrial ablation is a minimally invasive surgical procedure that has been demonstrated to have less morbidity, shorter recovery, and greater cost-effectiveness than hysterectomy in the short term. Importantly, endometrial ablation does not provide reliable contraception, and patients who become pregnant after ablation have markedly increased risks of adverse pregnancy outcomes, such as PPROM or abnormal placentation. Therefore, reliable contraception is necessary, and permanent sterilization should be considered [65].

Hysterectomy remains a reasonable option for some women with AUB who fail medical management. As many as 20% of women who initially undergo endometrial ablation will require hysterectomy within 5 years, and some studies have demonstrated a higher satisfaction rate in women who initially underwent hysterectomy rather than endometrial ablation [66].

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Menopause

James H. Liu and Francisco Arredondo

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9.1 Introduction

Natural menopause occurs between the ages of 45 and 55, with a median age of 51 for women in industrialized countries [1]. Available evidence suggests that in lesser developed countries with lower socioeconomic and nutritional levels, this event takes place earlier [2, 3]. These differences in the onset of menopause support the hypothesis that menopause might not only be genetic, but rather, a biological marker echoing society's longevity.

The significant medical and technological gains of the past century in medicine, and, to some extent, the constant betterment of living standards, have yielded an improvement in life expectancy. In some countries, increases of more than 40 years of life expectancy have been observed during the last 100 years alone [4]. The life expectancy for women at birth in the USA is 80.4 years of age and is 5 years longer than men [5].

As a consequence of this demographic evolution, a large proportion of the female population will spend over one-third of their lives after the menopause. In 1950 there were 220 million women in the world over the age of 50. More than half of these women live in the so-called developed world (112 million). In 1990, there were 467 million women age 50 around the globe, and by 2030 this number is expected to approach 1.2 billion. Roughly three out of four of these menopausal women will be in the developing world (total of 912 million) [6]. At a national level, the United States Census Bureau calculated a total of 35.5 million women older than 50 during 1990, and by 1997 the menopausal population in the USA represented almost 30% of the total female population. An estimated 6000 US women reach menopause daily. By 2020, the menopausal population in the USA older than age 55 is expected to reach 46 million.

These demographic changes will reflect an "inverted pyramid" phenomenon in certain countries. In this scenario, the elderly to non-elderly population ratio will significantly change, with obvious challenging consequences in the economic balance between productivity and expenditure. For these reasons, understanding the physiological nature of the menopausal state, associated diseases, and its full impact to society is fundamental for health providers and health policy makers. The present chapter will provide a prospective of the different stages of reproductive aging, review the physiologic underpinnings of menopause and its respective signs and symptoms, highlight long term risks of the menopause, review the evidence-based medical treatment options, and discuss the lifestyle and alternative management approaches during the menopause.

Clinical Case

A 51-year-old woman consults you for symptoms that she attributes to menopause. She has not had a menstrual period for 1 year. She experiences hot flashes and night sweats, insomnia, fatigue, and overall decrease in quality of life. She has no medical problems nor any family or personal history of cancer. Her mother was diagnosed with osteoporosis at age 70 and her father died of heart attack at age 74. Her body mass index is 28 and is on a statin for high cholesterol. She takes no other medication. She consults you about hormone replacement therapy and its ability to improve symptoms and decrease her risk for heart disease.

9.2 Stages of Reproductive Aging

Until recently, there was an absence of a relevant organized nomenclature system for the different stages of female reproductive aging. With this in mind, the Stages of Reproductive Aging Workshop (STRAW) was held in Park City, Utah, on July 23 and 24, 2001, and updated 10 years later (Fig. 9.1) [7].

Conceptually, the adult life of a woman can be divided into three major periods: reproductive, menopausal transition, and postmenopause. As is noted in Fig. 9.1 the anchor for the staging system is the final menstrual period (FMP). For clinical research purposes, five stages occur before and two after this anchor point. Stages −5 to −3 cover the reproductive period; stages −2 and −1 are the menopausal transition; and stages 1 and 2 are the postmenopause.

Among the most concrete achievements of the STRAW workshops was the development of clear and specific nomenclature that was previously vague and confusing in the literature. The authors of the STRAW workshops recognize that this is an evolving field and these concepts will change as the knowledge advances

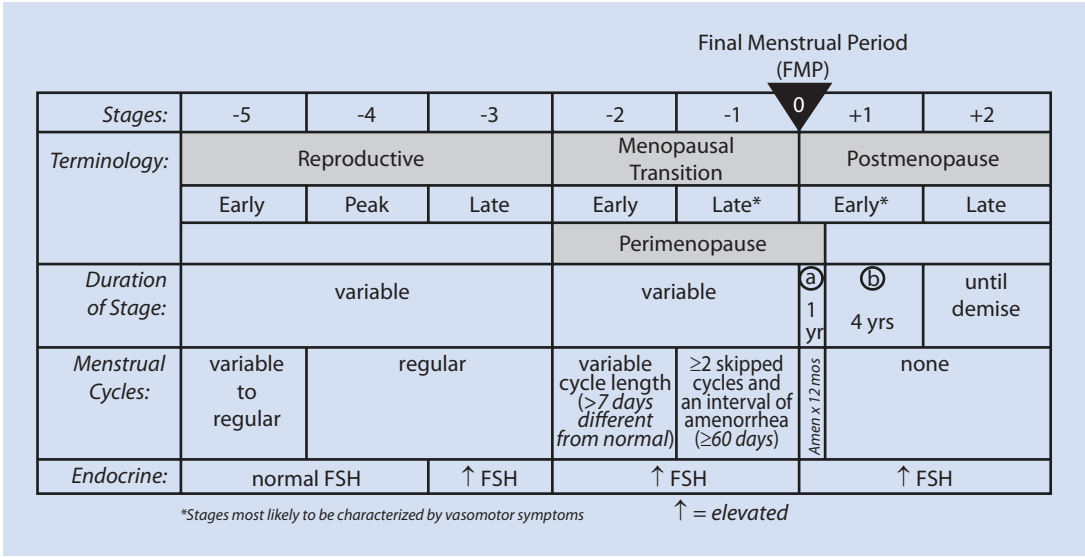


Fig. 9.1 The STRAW + 10 staging system. Reproduced with permission from Harlow SD, Gass M, Hall J, Lobo R, Maki P, Rebar RW, et al. Executive summary of stages of reproductive aging workshop + 10: addressing the unfinished agenda of staging reproductive aging. Menopause 2012;19(4):387–95

9.3 Menopausal Transition

Stage −2 (early) and −1 (late) encompass the menopausal transition and are defined by menstrual cycle and endocrine changes. The menopausal transition begins with variation in menstrual cycle length in a woman who has a monotropic FSH rise and ends with the FMP (not able to be recognized until after 12 months of amenorrhea). For most women at these stages, day 3 follicular phase FSH levels are variable and anti-Müllerian hormone levels and antral follicle counts are low.

9.4 Postmenopause

Stage +1 (early) and +2 (late) encompass the postmenopause. The early postmenopause is defined as 5–6 years since the FMP. The participants agreed this interval is relevant because it encompasses a further dampening of ovarian hormone function to a permanent level as well as accelerated bone loss. Stage +1 was further subdivided into segment "a," the first 12 months after the FMP, and segments "b and c," the next 1–5 years. Stage +2 has a definite beginning, but its duration varies, since it ends with death. Further divisions may be warranted as women live longer and more information is accumulated.

9.5 Perimenopause

Perimenopause literally means "about or around the menopause." It begins with stage −2 and ends 12 months after the FMP. "The climacteric" is a popular but vague term that we recommend be used synonymously with perimenopause. Generally speaking, the terms *perimenopause* and *climacteric* should be used only with patients and in the lay press and not in scientific papers.

9.6 Menopause Physiology and Pathophysiology

The supply of primordial follicles in the female gonad is predetermined before birth and diminishes with age until it is unable to provide enough mature follicles to sustain menstrual cyclicity [8]. The peak number of germ cell count is found at 20 weeks of gestation, with decreased numbers at birth and puberty. Based on the numbers of follicles at three successive stages of development which were obtained by counting follicles in histological sections of ovaries from 52 normal women, a mathematical model was developed to describe the rates of growth and death of ovarian follicles (atresia) in human ovaries between ages 19 and 50 [9]. While the number of

oocytes dwindles throughout a woman's life [10], there seems to be a transition at age 38 when the rate of follicle disappearance is augmented considerably with age. As a consequence, an estimated total of 1500 follicles remained at 50 years of age from the 300,000 present at age 19 years. This rate of decline in small follicles appears to be responsible for ovarian failure, and therefore menopause, and the transition to midlife in our species.

Recently, these concepts have been modified by Tilly and coworkers, who observed in the mouse model that there is a pool of germ cells that can proliferate and sustain oocyte and primordial follicle development in the *postnatal* mammalian ovary [11]. Moreover, in a chemotherapy-induced ovarian failure mouse model, his group was able to demonstrate that bone marrow transplantation is associated with a new population of immature oocytes [12]. Taken together, these new observations suggest that the control mechanism(s) of oocyte atresia can be potentially modified.

9.7 Age of Menopause

The mean age of menopause in normal women in the USA ranges between 50 and 52 years [1]. This number was based on a cross-sectional study, which is associated with recall bias. However, these findings have been confirmed in a large prospective cohort study of mid-aged women: The Study of Women's Health Across the Nation (SWAN) cohort consisting of African-American (n = 916), Caucasians (n = 1533), Chinese (n = 248), Hispanic (n = 277), and Japanese (n = 279) women from premenopause to their final menstrual period. The median age of menopause was 52.54 years and was similar for all five ethnic groups [13].

In other parts of the world, population-based surveys have shown an earlier onset of menopause. For example, the median age of onset of menopause was 48 in a survey of 742 United Arab Emirates women [14]. In certain regions of India, such as the state of Himachal Pradesh, the mean age onset of menopause can be as low as 43.5 years, according to data from 500 postmenopausal women [15].

9.8 Factors Affecting the Onset of Menopause

Table 9.1 includes several factors that have been linked with an earlier onset of menopause. Among these factors, smoking has been the most often linked environmental agent to early age onset of menopause. However, these studies have been inconclusive with regard to duration and intensity of smoking. Midgette et al. concluded after a review of 14 studies that the risk of being menopausal was approximately doubled for current smokers compared with nonsmokers among women 44–55 years of age [16]. However, in 2004 van Asselt et al., using data from a Dutch population-based cohort of 5544 women, assessed the effect of smoking duration and intensity on age at menopause correcting for the chronologic age-dependency of the variables concerned. After their modeling, they concluded, like previous researchers, that smoking lowers the menopausal age. However, the reduction in the menopausal age appears not to be dependent on smoking duration and that smoking cigarettes could have

Table 9.1 Factors associated with earlier onset of menopause

Smoking
Genetic factors
Family history of early menopause
Pelvic surgery
Total abdominal hysterectomy
Unilateral oophorectomy Ovarian cystectomy
Metabolic factors
Type 1 diabetes mellitus
Galactose consumption
Galactose-1-phosphate uridyl transferase deficiency
Ovulation patterns
Nulliparity
Shorter menstrual cycles
Non-use of birth control pills

an effect only around the time of menopause itself [17]. In other words, the number of cigarettes smoked during perimenopause is apparently more significant than smoking history as the culprit of earlier age of onset of menopause among smoking women.

Women treated with total abdominal hysterectomy appear to be at risk of early menopause. Concurrent unilateral oophorectomy or ovarian cystectomy was associated with an even earlier onset. However, previous tubal ligation does not influence the age of menopause.

9.9 Time Course of Oocyte Pool Depletion

It is estimated that the entire process from initiation of the primordial follicle growth until complete maturation and finally ovulation is over 150 days [18]. The majority of follicles will experience atresia by apoptosis at some point in their developmental process [19]. Fifty percent of apoptosis occurs at the small antral follicle stage of 2.1–5 mm. On the other hand, the atresia of the resting follicles in the human fetus seems to be set off by a process of necrosis rather than apoptosis [20]. It seems clear now that the age-related decline in ovarian function in women is the result of the decline in both quantity and quality of the resting ovarian follicle pool. Recently, a total of 182 resting follicles from a young cadre of women (25–32 years) was compared with 81 resting follicles from the less young group (38–45 years) for signs of age-related changes by transmission-electron microscopy. De Bruin et al. concluded that, in resting follicles, the morphological changes with age are different from the changes seen in quality decline by atresia. The morphological changes with age specifically comprise the mitochondria, the dilated smooth endoplasmic reticulum, and the Golgi complex.

9.10 Genetic Contribution

The age of natural menopause is determined by the interplay of genetic and environmental factors [21]. There are some cross-sectional [22] and case–control [23] population studies suggesting the existence of genetic variability in the age of menopause to be as high as 70%. However, a study conducted in the Netherlands in 164 mother–daughter pairs with a natural menopausal age estimated a hereditability of 44% (95% CI 36%, 50%). The authors conclude that these estimates are more accurate because previous studies that were done in twins and siblings are overestimates because siblings shared many environmental factors [24].

9.11 Clinical Signs and Symptoms of Menopause

The diagnosis of menopause can be established when the absolute level of serum FSH is elevated. The threshold for the diagnosis of menopause will vary according to the assay employed. In any event, the level will be two standard deviations above from the normal value of a reproductive-age woman on cycle day 3. Anti-Müllerian hormone levels will typically be very low and may be undetectable. LH is of little value in the evaluation or diagnosis of menopause.

The clinical diagnosis of menopause is established retrospectively once a patient has had more than 12 months of amenorrhea in conjunction with vasomotor symptoms such as hot flashes and headaches. At this point, the patient has made the transition in the STRAW classification from −1 to +1. The range of symptoms due to immediate estrogen deficiency in women during STRAW stages −1 to +1 includes hot flashes and urogenital changes.

9.12 Hot Flashes

Vasomotor symptoms (hot flashes) are the most characteristic trait of estrogen deficiency. It is experienced at least once in 75% of women during menopause. It is also one of the most puzzling symptoms of menopause, because the etiology and physiology remain incompletely understood [25]. It is thought to be the result of a hypothalamic dysregulation from estrogen withdrawal that culminates in peripheral vasodilatation and increase in blood flow. This results in heat loss and a decrease in core body temperature. The hypothalamic dysfunction is also manifested by

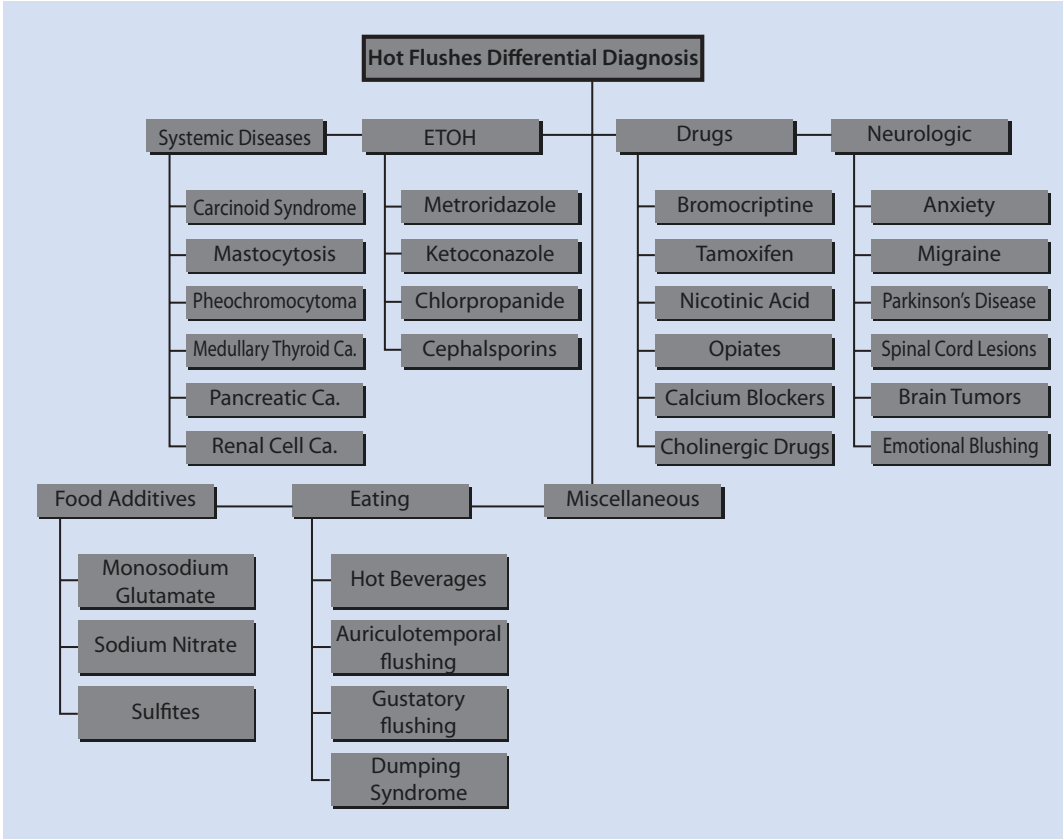


Fig. 9.2 Differential diagnosis of flushing. Adapted from [27]

simultaneous pulse of LH and presumably GnRH that is coincident with the hot flash. "Hot flash," "hot flush," "night sweats," and vasomotor symptoms are words frequently used to describe the same experience. Hot flashes are defined subjectively as the recurrent transient sensation of heat, and could be accompanied by palpitations, perspiration, chills, shivering, and feeling of anxiety. It is then followed by a heat dissipation response that habitually begins in the face, neck, chest, and often becomes generalized [26].

While menopause is the most common cause of hot flashes, there are other causes that should be considered. Fever by far is the most common cause of hot flashes, especially when coupled with a night sweat; thus, if during an episode of hot flashes the oral temperature is elevated, then the cause of the fever should be sought.

In general we can divide the potential causes of hot flashes into seven categories: systemic diseases, neurological, alcohol–medication interaction, drugs, food additives, eating, and miscellaneous [27] (Fig. 9.2). However, it is important to emphasize that these other causes are much less common than those associated with low estrogen levels.

9.13 Systemic Diseases

The most common systemic disorders associated with hot flashes are carcinoid syndrome, mastocytosis, pheochromocytoma, medullary thyroid carcinoma, pancreatic carcinoma, and renal cell carcinoma.

9.14 Carcinoid Syndrome

These patients present with neuroendocrine tumors of the bowel. Carcinoid tumors can be localized to the bronchus, pancreatic islets, retroperitoneum, liver [28], and even in the ovary

[29]. It is thought these tumors probably arise from gastrointestinal or bronchopulmonary pluripotential stem cells [30]. The carcinoid syndrome clinically has a classic triad of diarrhea, flushing, and valvular heart lesions. Skin flushing is the most common sign and is present in over 90% of patients. The mechanism of flushing is at least partially due to serotonin release, but other substances such as kinins, substance P, neurotensin, and prostaglandin may play a role [37].

9.15 Mastocytosis

Mast cell proliferations can be limited to the skin (cutaneous) or can spread to extracutaneous tissues (systematic). Vasomotor-like symptoms may be present in these patients because the mast cell granules contain a number of acid hydrolases, leukotrienes, histamine, heparin, and slow-reacting substance [31].

9.16 Pheochromocytoma

These tumors often arise from the adrenal medulla. Most of their clinical characteristics are due to the production, storage, and secretion of catecholamines. A key finding in these patients is hypertension, which is present in over 60% of patients. A significant number of them suffer from hot flashes. Documenting increased urinary catecholamines makes the diagnosis [32].

9.17 Medullary Carcinoma of the Thyroid

This is a malignant tumor that originates from the parafollicular or thyroid C cells. These tumor cells typically produce an early biochemical signal (hypersecretion of calcitonin) [33]. This cancer can occur sporadically, but many times is inherited in an autosomal-dominant pattern as a part of a syndrome: multiple endocrine neoplasia type 2 [34]. Other bioactive substances that can be secreted by medullary carcinomas and may be responsible for the vasomotor symptoms include ACTH, corticotropin-releasing hormone, and prostaglandins [34].

9.18 Neurological Flushing

Anxiety or emotional blushing, migraines, Parkinson's disease, spinal cord lesions (autonomic hyporeflexia), and brain tumors can also be associated with hot flashes.

9.19 Alcohol–Medication Interaction

Alcohol and numerous drugs are associated with vasomotor symptoms. In some cases, the drug is not the real vasoactive agent, but rather, a metabolite or another mediator triggered by the drug ingested (i.e., histamine release). Some drugs will only create vasomotor symptoms when combined with alcohol [35]. Others, such as calcium channel blockers, have a direct impact in the vessels [36]. On the other hand, tamoxifen or bromocriptine produces hot flashes by triggering different mediators.

Vasodilators (nitroglycerin, prostaglandins), calcium channel blockers, nicotinic acid, opiates (i.e., morphine), amyl nitrite, cholinergic drugs, bromocriptine, thyrotropin-releasing hormone, tamoxifen, clomiphene, triamcinolone, and cyclosporine are the most commonly cited medications.

9.20 Food Additives and Eating Habits

Monosodium glutamate, sodium nitrite, and sulfites are the most common food additives associated with hot flashes. Hot beverages, auriculotemporal flushing (cheese, chocolate, lemon, highly spicy foods), gustatory flushing (chewing chili pepper), dumping syndrome (seen in patients after gastric surgery and triggered by a meal, hot fluid, or hypertonic glucose) are examples of symptoms associated with ingesting food or beverage.

The main limitation in our ability to assess the value of a treatment for hot flashes is the lack of an objective measure. This complex task is due to the current inability to reliably identify when a hot flash has taken place. The main objective method used today is sternal skin conductance monitoring, which has some limitations, but the main weakness is the failure of sternal skin conductance

to provide any information on duration, intensity, and interference with patient activities. Therefore, all data are derived from imperfect methods [32].

9.21 Morbidity Associated with Hot Flashes

9.21.1 Sleep Disturbances

The relationship between hot flashes and interference with sleep is controversial. Multiple epidemiological studies have shown an association between awakening and arousal from sleep and hot flashes in menopausal women [37–39]. This has led to the commonly held conception that hot flashes and night sweats cause awakening, which subsequently create fatigue, and possibly decrease performance and quality of life [40]. The flaw in these studies is that these hypotheses have not been properly tested in controlled laboratory investigations. Also, neither of these investigations screened out patients with apnea and other sleep disturbances that are also prevalent in menopause and may represent a confounder factor.

Recently, Freedman et al. studied 31 patients between the ages of 46 and 51 who were classified into three groups: premenopausal asymptomatic (cycling), postmenopausal asymptomatic (asymptomatic), and postmenopausal symptomatic (symptomatic). They then assessed several outcome measures: sleep electroencephalogram recordings, sternal skin conductance to record hot flashes, multiple sleep latency test to assess sleepiness, simple and divided attention performance tests, sleep and fatigue questionnaires. There were no significant differences among the three groups on any sleep variable. Of the awakenings taking place within 2 min of a hot flash, 55.2% happened before the hot flash, 40.0% after the hot flash, and 5% simultaneously. Of arousals taking place within 2 min of a hot flash, 46.7% occurred before, 46.7% after, and 5.6% simultaneously. There were no significant group differences on any self-report measure or on any performance measure. They concluded that there is no evidence that hot flashes produce sleep disturbance in symptomatic postmenopausal women [41]. Although the use of estrogen will effectively relieve postmenopausal nocturnal vasomotor symptoms, sleep quality was not changed [42].

9.21.2 Migraines

There is sufficient observational data to suggest a link between hormones and migraines [43, 44].

However the relationships between menopause and migraine are still debated. Observational studies suggest that migraine worsens just before menopause and improves after cessation of menses in approximately two-thirds of cases.

Neri studied 556 postmenopausal women for the prevalence and characteristics of headaches and found that many had migraine with aura. Interestingly, women with prior migraine generally improved with the onset of spontaneous menopause. In contrast, women with bilateral oophorectomy usually experienced worsening of their migraines [45]. More recently, a cross-sectional community-based study of 1436 women using the 1988 International Headache Society Criteria showed the highest prevalence of migraines in the perimenopausal group (31%) and the lowest (7%) in the postmenopausal group [46].

9.21.3 Urogenital Changes

The lack of estrogen has been associated with the onset of vulvo-vaginal atrophy with thinning of the cells lining the vulva, urethra, and vagina. The terminology describing these constellations of changes has changed from "Vulvo-Vaginal Atrophy" to Genito- urinary Syndrome of Menopause (GSM) [47]. Epithelial secretions decline and with time lead to dryness of the vaginal tissues. The vaginal pH will increase and the bacterial flora will change. The percentage of superficial vaginal cells will decline and basal-appearing cells will predominate. The persistent dryness of the vaginal mucosal surfaces may lead to symptoms of vaginitis, pruritus, dyspareunia, and even stenosis. Other symptoms that may be related to estrogen deprivation in the urogenital tissues are dysuria, urgency incontinence, and urinary frequency. It is unclear whether all these symptoms are related to the lack of estrogen or whether they are part of a degenerative process of aging. It is postulated that changes in estrogen levels changes the composition of the collagen content and the connective tissues in the urogenital area [48].

Data suggest that lack of estrogen increases the likelihood of menopausal women to experience recurrent urinary tract infections (UTIs).

A randomized, placebo-controlled trial of vaginal estrogen in 93 postmenopausal women demonstrated that patients being treated could reduce their number of UTIs per year. This study observed 0.5 episodes of UTI in the treatment groups vs. 5.9 episodes in the placebo group [49]. On the other hand, data from the Heart and Estrogen/Progestin Replacement Study (HERS) showed that UTI frequency was higher in the group randomized to hormone treatment, although the difference was not statistically significant (odds ratio 1.16, 95% confidence interval 0.99, 1.37) [50].

However conflicting these results are, from the clinician's point of view it seems prudent to attempt a trial of vaginal estrogen therapy to address those postmenopausal with urogenital symptoms. It should be considered in the presence of a vaginal pH greater than 4.5. Like FSH, elevated vaginal pH appears to be a good prediction of estrogen status [51].

9.22 Long-Term Morbidity Associated with Postmenopausal Status

The two major long-term risks associated with menopause are osteoporosis and cardiovascular disease.

9.22.1 Osteoporosis

In this section, we will discuss its relationship as a long-term risk factor in menopausal women. The demographic changes and longevity increase described at the beginning of this chapter, coupled with the fact that osteoporosis rises dramatically with age, make [52] osteoporosis a serious economical burden for health care systems in our society. The estimated total direct expenditure (hospitals and nursing homes) for osteoporotic and associated fractures was $17 billion in 2001 ($47 million each day) [53]. The National Osteoporosis Foundation estimates that 50% of white women will suffer at least one osteoporosis-related fracture in their remaining lifetime. At least 90% of hip and spine fractures among elderly women can be attributed to osteoporosis [54]. These statistics are the result of an accelerated decline in bone-mass after menopause. However, this decline begins approximately at age 35, when bone resorption is greater than bone formation. After menopause there are two periods of net bone loss: An accelerated stage that begins with the onset of menopause (1–3 years) and continues for 5–8 years [55] (STRAW 0, 1, and 2) and a prolonged, slower stage of bone loss that remains throughout STRAW 2. The initial accelerated phase may account for bone loss of up to 30% [56].

The three most common osteoporotic-related fractures are hip, vertebral, and wrist. The most common are vertebral fractures, accounting for 700,000 cases a year in the USA. These should be suspected in postmenopausal women with back pain, loss of height, and kyphosis. In one observational study of 7223 postmenopausal women over 65 years of age, patients with radiographically detected vertebral fractures were found to have significantly more limited-activity days, whether they were symptomatic or not [57]. These data should raise awareness for the clinician of the decreased quality of life that menopausal patients may experience even with asymptomatic fractures.

The second most common fracture is hip fracture, accounting for 300,000 cases a year in the USA. These are without doubt the more serious consequence of osteoporosis in postmenopausal women. One in five women will die within 1 year postfracture and one in two will have permanent loss of function [58]. Lastly, distal forearm fractures occur in 250,000 patients a year in the USA. Only half of the patients that suffer these fractures recover full function of the arm in 6 months [59].

9.22.2 Cardiovascular Disease

The American Heart Association has designated cardiovascular disease a "silent epidemic." Despite the overall decline in the mortality rate due to cardiovascular disease in the USA, the absolute number of deaths due to cardiovascular disease is actually increasing [60], in part due to the demographic changes in our society described in the introduction of this chapter. Cardiovascular disease that encompasses heart attacks and strokes combined are responsible for more deaths than all other causes combined in postmenopausal women [61, 67]. The burden and threat of this disease during menopause is in part due to the lack of perception of its magnitude by both physicians and patients. Nothing exemplifies this better than a 1995 Gallup survey, which revealed that four out

of five women aged 45–75 were unaware that cardiovascular disease was the first cause of death for their age group. Instead, most of the women quoted cancer, specifically breast cancer, as their most probable cause of death. In reality this represents only 4% of the causes of death in this age group. The primary care physicians questioned did not do much better. Thirty-two percent were unaware that heart disease was the main cause of death in this age group of women [62].

The incidence of cardiovascular disease and particularly myocardial infarction dramatically increases after menopause and approximates the mortality of this entity in men [63,64]. Furthermore, bilateral oophorectomy or premature ovarian failure increases the risk of cardiovascular disease beyond that of natural menopause [65]. Despite this seemingly logical association between estrogen cardioprotection and other coherent data from observational studies, the Vasomotor symptoms and the HERS have found no role for estrogen as a primary or secondary prevention for cardiovascular disease in postmenopausal women.

Substantive evidence from epidemiological studies and clinical research indicate that the best tools are preventive measures and lifestyle habit modifications: smoking cessation, blood pressure control, lowering cholesterol, and promoting exercise.

9.23 Medical Treatment of Menopause

The results of the Women's Health Initiative study (WHI) have altered the principles of medical practice in menopausal women. We have changed from the concept of prevention of chronic diseases encompassed in the term "hormone replacement therapy" to the concept of "hormone therapy." Thus, the US Food and Drug Administration (FDA) and professional organizations such as the American Congress of Obstetricians and Gynecologists recommend that the use of estrogenic-containing medications be restricted to the treatment of vasomotor and vaginal symptoms. They also affirm that the lowest effective dose be prescribed for the shortest duration of time [66–68].

More detailed analysis of the WHI results has suggested a more individualized approach to hormone therapy. For this reason, the North American Menopause Society and the Endocrine Society have modified their guidelines for hormone therapy in 2012 [69]. Current evidence suggests that the absolute risks of estrogen therapy between the ages of 50 and 59 are low and the individual benefit may favor a longer duration of therapy. In contrast, the duration of use of estrogen with progestin should be limited because of the increased risk of breast cancer associated with 3–5 years of use.

9.24 Principles of Hormone Therapy

During the last 3 decades, the clinical opinion on the use of estrogen during menopause has changed drastically. Initially, estrogen was recommended as a short-term treatment for menopausal symptoms. Later, on the basis of observational studies, estrogen was given for long-term prevention of heart disease and an improved quality of life. The Women's Health Initiative Hormone Therapy trial, however, demonstrated that estrogen was not effective for the prevention of cardiovascular disease.

9.25 Key Findings from the Women's Health Initiative

The WHI was a group of clinical trials designed to examine the impact of hormone therapy on cardiovascular disease and breast cancer, the effect of low-fat diet on breast and colon cancer, and the impact of vitamin D in calcium supplementation on fractures and colon cancer [70].

These trials included:

- A randomized controlled trial of 16,608 asymptomatic postmenopausal women ages 50–79 years with a uterus comparing conjugated estrogens (0.625 g) and a progestin, medroxyprogesterone (MPA) (2.5), daily vs. placebo. The primary outcome measure of this trial was coronary heart disease (CHD) and breast cancer. The secondary outcome measures were stroke,

congestive heart failure, angina, peripheral vascular disease, coronary revascularization, pulmonary embolism, deep venous thrombosis, ovarian cancer, endometrial cancer, hip fractures, diabetes mellitus requiring therapy, death from any cause, and quality-of-life measures
- A randomized controlled trial on 10,739 asymptomatic postmenopausal women 50–79 years without a uterus (hysterectomized) comparing conjugated estrogens (0.625 mg/day) vs. placebo
- A dietary modification randomized controlled trial of 48,837 postmenopausal women 50–79 years to either sustained low fat (20%) or self-determined diet. The primary outcome measures were breast and colorectal cancer. The secondary outcome measures included stroke, congestive heart failure, angina, peripheral vascular disease, coronary revascularization, ovarian cancer, endometrial cancer, hip fractures, diabetes mellitus requiring therapy, and death from any cause
- A calcium/vitamin D supplementation diet trial of 38,282 postmenopausal women in which the primary outcome measure was hip fractures and the secondary outcome measures were death from any cause, breast and colon cancer
- A cohort observation group of 93,676 postmenopausal patients

In May of 2002 the clinical trial that aimed to assess the cardiovascular effects of estrogen and progestin therapy in postmenopausal women with intact uterus was halted. The Data and Safety Monitoring Board reported that the estrogen/progestin treatment group had an increased risk in cardiovascular disease, thromboembolism, and breast cancer after 5.2 years of follow-up [71]. In 2004, after 6.8 years of follow-up, the estrogen-alone trial was halted [72]. In this clinical trial, estrogen-only treatment demonstrated an increase risk in strokes similar to the one found in the estrogen–progestin clinical trial previously halted. There was also reported a lack of benefit on cardiovascular disease incidents and a probable increase in dementia. Surprisingly, the breast cancer risks in the estrogen treatment group were lower than the placebo. The risks and benefits findings of the WHI are summarized in Table 9.2 [73].

It should be emphasized that the WHI trial did not intend to evaluate the effects of estrogen or estrogen–progesterone on vasomotor symptoms because hot flashes were not the major complaint among the majority of subjects. Therefore, these results must be translated into the specific needs of our patients when they request relief for hot flashes or other postmenopausal symptoms. It is also critical to point out that the serious adverse events in patients treated with estrogen therapy is low and calculated to be 2 out of 1000 women treated per year [74]. Health care providers and patients must balance the benefits of estrogen treatment vs. the absolute risk for adverse events. Today, more than ever, the concept of individualized menopausal care should be applied in the clinical setting.

The WHI investigators have performed a secondary, stratified analysis of the cardiovascular risk by age. These data suggest that estrogen or estrogen with progestin have potential cardiovascular benefits if started early in the menopause (aged 50–55 or less than 10 years from menopause), whereas those started on hormone therapy after 60 or more than 10 years from menopause are at increased risk (Fig. 9.3). These newer findings are in keeping with observational studies and other cardiovascular preventive trials. Taken together, these data suggest that there is a "window or time" of opportunity for use of estrogen therapy and it has been termed the "timing hypothesis" [75, 76]. Studies to test this hypothesis are ongoing.

The WHI hormone trial is the first randomized controlled trial that demonstrates that estrogen actually decreases the risk of fractures in a low-risk population. However, when all the risks and benefits are weighted, it can be concluded in this study that estrogens are not indicated as an overall preventive measure in postmenopausal women and the potential harm outweighs the potential long-term benefit. Thus, at the present time, use of estrogen should be limited to the treatment of symptomatic menopausal women for the shortest possible time at the lowest effective dose. Table 9.3 shows a comparison between the WHI and HERS trials.

Table 9.2 Women's Health Initiative Findings: outcomes associated with use of combined estrogen and progestin and estrogen alone in healthy postmenopausal women, aged 50–79 years

Outcome	Estrogen and progestin	Average absolute risk difference[b]	Estrogen[a]	Average absolute risk difference[b]
	RR (95% CI)		RR (95% CI)	
Cardiovascular				
Deep venous thrombosis	2.07 (1.49–2.87)	13	1.47 (1.04–2.08)	6
Pulmonary embolism	2.13 (1.39–3.25)	8	1.34 (0.87–2.06)	11
Coronary heart disease	1.24 (1.00–1.54)	7	0.91 (0.75–1.12)	−5
Ischemic stroke	1.44 (1.09–1.90)	8	1.39 (1.10–1.77)	12
Cancer				
Breast	1.24 (1.02–1.50)	8	0.77 (0.59–1.01)	−7
Colorectal	0.63 (0.43–0.92)	−6	1.08 (0.75–1.55)	1
Ovarian	1.58 (0.77–3.24)	8	NYR	NYR
Endometrial	0.81 (0.48–1.36)	−4	N/A	N/A
Other				
Probable dementia[c]	2.05 (1.21–3.48)	23	NYR	12
All fractures	0.76 (0.69–0.83)	−44	0.70 (0.63–0.79)	−56
Hip fractures	0.67 (0.47–0.96)	−5	0.61 (0.41–0.91)	−6
Mortality	0.98 (0.82–1.18)	−1	1.04 (0.88–1.22)	+3

RR relative risk compared to placebo, *CI* confidence interval, *N/A* not applicable (hysterectomized women), *NYR* not yet reported
[a]Hysterectomized
[b]Annual per 10,000 women
[c]Women aged 65–79 years

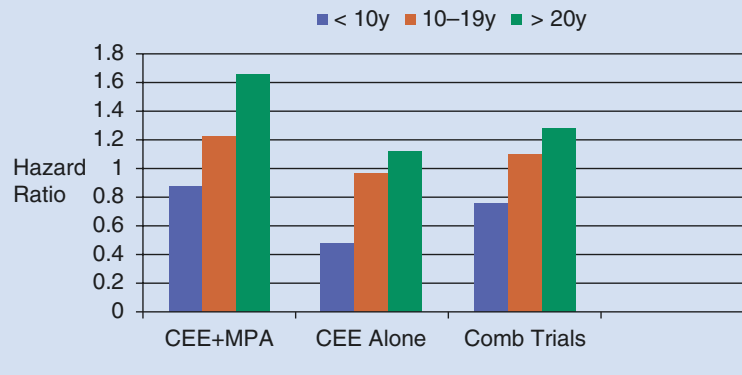


Fig. 9.3 Hazard ratio of cardiovascular risk for estrogen alone and estrogen with progestin relative to placebo controls in the WHI. Created with data from [75]

Table 9.3 Impact of estrogen–progestin treatment on cardiac events in a healthy menopausal population (WHI) vs. a population with a prior history of cardiac events (HERS)

WHI-CHD by year of follow-up				HERS-risk of cardiac events		
Year	Estrogen–progestin	Placebo	Hazard ratio and confidence interval	Estrogen–progestin	Placebo	Relative hazard (risk) and Confidence Interval
	n = 8000+			*n* = 1383	*n* = 1380	
1	42 cases	23 cases	1.81 (1.09–3.01)	57 cases	38 cases	1.52 (1.01–2.29)
2	38	28	1.34 (0.82–2.18)	47	48	1.00 (0.67–1.49)
3	19	15	1.27 (0.64–2.50)	35	41	0.87 (0.55–1.37)
4	32	25	1.25 (0.74–2.12)	33	49	0.67 (0.43–1.04)
5	29	19	1.45 (0.81–2.59)			1.06 (0.69–1.62)
>6	28	37	0.70 (0.42–1.14)			0.98 (0.72–1.34)

9.26 Key Findings of the Heart and Estrogen/Progestin Replacement Study

While the WHI aimed to test the hypothesis that hormone therapy prevented cardiovascular disease in healthy postmenopausal women (primary prevention), the HERS intended to evaluate whether hormone therapy decreased the risk of CHD in postmenopausal women with established coronary disease. In this randomized trial, all the 2763 postmenopausal women had uteruses, and were allocated to either placebo (n = 1383) or 0.625 mg of conjugated equine estrogens plus 2.5 mg of MPA daily (n = 1380) [77, 78]. The primary outcome measures were (1) nonfatal myocardial infarction and (2) CHD death.

The results of HERS trial have been reported in two publications, HERS and HERS II. The HERS report is the result of randomized, blinded, placebo-controlled trial for 4.1 years and the HERS II reflects the unblinded follow-up for 2.7 more years [84, 85]. Both of the studies demonstrated that in patients with established heart disease, the use of estrogen and progestin does not prevent additional cardiovascular events. There were no differences in the primary or secondary outcomes of patients in the placebo or the treatment group.

The conclusion of HERS and HERS II studies is that postmenopausal hormone therapy should not be recommended for the purpose of reducing the risk of cardiovascular events.

9.27 Key Findings from the ELITE and KEEPS Randomized Trials

To examine the possibility that HRT was beneficial in early menopausal women and to test the "timing hypothesis" suggested by the WHI data, two small focused randomized trials were conducted.

The first randomized trial to study the "timing hypothesis" was the Early versus Late Intervention Trial with Estradiol (ELITE) [79]. The trial enrolled 643 postmenopausal women in two strata: those that were <6 years or >10 years postmenopause. Each group received either placebo or oral estradiol 1 mg/day with progesterone gel (45 mg/day) given for 10 days every 30 days for endometrial protection. The primary outcome was the rate of change in carotid-artery intima-media thickness (CIMT). This is a sensitive, reproducible biomarker for atherosclerosis progression. The key finding from this trial demonstrated that early menopausal women had a slower progression of CIMT increase 0.0044 mm/year versus placebo 0.0078 mm/year, p = 0.008. Whereas the late menopause women had similar rates of progression to placebo 0.0088 and 0.0100 mm/year, p = 0.29).

The Kronos Early Estrogen Prevention Study (KEEPS) was a four-year randomized trial involving more than 700 early menopausal women given either a low-dose oral conjugated equine estrogen (CEE) 0.45 mg/day, transdermal estradiol 50 μg/day, or placebo with 200 mg of

micronized progesterone days 1–12 each month. Assessment of progression of atherosclerosis by carotid artery intima-media thickening demonstrated similar rates of progression of arterial wall thickness among the three groups [80]. KEEPS also evaluated the impact of estrogen on cognitive function. Treatment with CEE or transdermal estradiol did not alter cognitive performance. However, improvements in depression and anxiety symptoms were noted in women receiving CEE but not transdermal estradiol or placebo groups [81].

Collectively, these studies support the "timing hypothesis" that HRT given to early menopausal women may be beneficial for reducing cardiovascular risks whereas late postmenopausal use of HRT has no additional cardiovascular benefits.

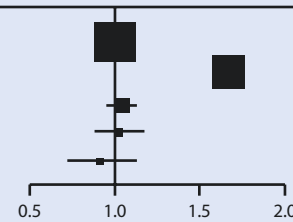
9.28 Key Findings the Million Women Study

The Million Women Study is a prospective observational study that included 1,084,110 British women aged 50–64 recruited between 1996 and 2001. This study was undertaken by the UK National Health Service Breast Screening Programme targeting women ages 50–64 undergoing routine screening once every 3 years. Because approximately only half of these patients had ever taken estrogen after menopause, the aim of the study was to investigate the relation between various combinations of hormone therapy and two main outcomes: breast cancer and mortality [82]. All patients filled out a questionnaire and were monitored in this manner. This questionnaire is available at ▶ http://www.millionwomenstudy.org. The major strength of this study was the unparalleled database size, which was sufficiently powered to quantify absolute and relative risks and enabled researchers to discern the effects among different preparations of hormones in use among postmenopausal women. One weakness of the study was that hormone use vs. non-use was determined on admission to the study and was not modified during follow-up, even though there were potential multiple crossover treatments in some of the subjects. The authors reached these conclusions:

- Current use of hormone therapy is associated with an increased risk of incident and lethal breast cancer
- The risk is substantially greater for estrogen–progestin combinations of postmenopausal hormone therapy
- The mortality rate due to breast cancer was 27% less in hormone users than in non-hormone users. This could be explained in part by more frequent medical care and early detection

The relative risks for invasive breast cancer in relation to current use of hormone therapy and type of hormone preparations are illustrated in ◘ Figs. 9.4 and 9.5.

HRT use at baseline	Cases/population	Relative risk (95% FCI)*
Never users	2894/392 757	1.00 (0.97–1.04)
Current users	3202/285 987	1.66 (1.60–1.72)
Last use <5 years previously	579/81 875	1.04 (0.95–1.12)
Last use 5–9 years previously	207/29 395	1.01 (0.88–1.16)
Last use >10 years previously	79/12 568	0.90 (0.72–1.12)



X^2 for heterogeneity between ever users=161.5. p<0.0001

◘ **Fig. 9.4** Relative risk of incident invasive breast cancer in relation to recency of use of HRT. *FCI* floated CI. *Relative to never users, stratified by age at first birth, family history, of breast cancer, body-mass index, region, and deprivation index. Reprinted from The Lancet, 362, Breast cancer and hormone-replacement therapy in the Million Women Study, 419–27, Copyright 2003, with permission from Elsevier

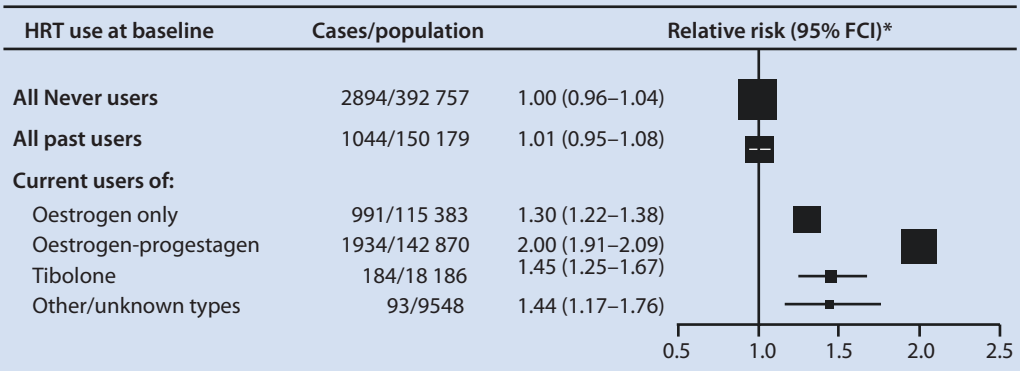


Fig. 9.5 Relative risk of incident invasive breast cancer in relation to recency and type of HRT used. *FCI* floated CI. *Relative to never users, stratified by age at first birth, family history, of breast cancer, body-mass index, region, and deprivation index. Reprinted from The Lancet, 362, Breast cancer and hormone-replacement therapy in the Million Women Study, 419–27, Copyright 2003, with permission from Elsevier

9.29 Candidates for Hormone Therapy during Menopause

It is clear that the treatment paradigm has shifted when it comes to whom should we recommend the use of estrogens during menopause. After reviewing the evidence in the randomized controlled trials, it seems prudent to state that estrogen should not be used to prevent long-term chronic diseases such as cardiovascular disease and dementia.

Nevertheless, patients with moderate to severe vasomotor symptoms should weigh the risk and benefits and might consider using estrogen. It seems that the group of patients with vasomotor symptoms and osteoporosis or at risk for osteoporosis are good candidates to consider prescribing estrogen. Estrogen is the most effective treatment to alleviate vasomotor symptoms and has been shown to reduce the fracture risk in postmenopausal women.

For these reasons, medical management of menopausal symptoms has evolved to a personalized approach. Personal choice is important and some will never take hormone therapy under any circumstances. The physician must consider many variables before offering hormone therapy in light of individual cardiovascular, osteoporosis, and breast cancer risk and the severity of estrogen-deficient symptoms.

9.30 General Principles of Drug Therapy

For patients on hormone replacement therapy, there are two important concepts that must be discussed. First, is the projected duration of therapy. Professional societies such as the North American Menopause Society and the Endocrine Society suggest that the lowest effective dose be utilized consistent with the treatment goals. If it is for the treatment of vasomotor symptoms, it could be for a limited period of time. The duration of therapy may vary depending on the individual. Those begun on hormone therapy earlier in the menopause (prior to age 55) appear to have a cardiovascular benefit. Secondly, the effectiveness of therapy should be quantified. Typically, this consists of assessing symptoms and signs of hypoestrogenemia and monitoring bone mineral density.

The choice of drug regimen should be based on the specific needs of the patient. For example, a breast cancer patient with severe genitourinary atrophy who has failed nonhormonal regimens may benefit from a locally applied estrogen medication that has minimal systemic absorption, provided this is with the acquiescence of her oncologists.

Hot flashes can be controlled with continuous estrogens rather than cyclic. If the hot flashes are not controlled, a progestin can be added if this has

not already been used. Increased metabolism of estrogen by concomitant use of anti-seizure drugs should be considered and substitution with a transdermal delivery with reduced hepatic metabolism should be considered. Patients that do not or cannot use estrogens can be offered a selective serotonin reuptake inhibitor.

All patients with a uterus should be given a progestin. Table 9.4 shows the most common estrogen–progestin regimens. The progestin can be given cyclically, such as the first 12–14 days of each month or continuously (daily). The majority of patients on cyclic therapy will have a monthly withdrawal bleed. The continuous regimen will induce irregular bleeding initially but will eventually result in amenorrhea. Patients with a uterus require endometrial monitoring to detect possible hyperplasia or cancer. With cyclic regimens, bleeding occurs after 10–12 days of progestin therapy. Unscheduled bleeding or a change in the pattern of bleeding warrants investigation. A transvaginal ultrasound should be performed. If the endometrial thickness is less than 5 mm, endometrial cancer is ruled out. If the endometrial thickness is 5 mm or greater, an endometrial sampling should be performed. If the thickness is less than 5 mm but bleeding persists or recurs, then endometrial sampling should also be performed.

Absolute contraindications to estrogens include acute venous thrombosis, pulmonary embolism, cardiovascular or liver disease. Untreated endocrine-sensitive tumors and undiagnosed vaginal bleeding are also contraindications. Relative contraindications include the chronic forms of the previous mentioned conditions as well as uncontrolled hypertension and hypertriglyceridemia. Some traditional relative contraindications, such as seizure disorders, migraines without scotomata, gallbladder disease, or myomas, are a matter of debate. Patients with a personal or family history of venous thromboembolism should be screened for a possible thrombophilia before starting hormone replacement therapy.

9.31 Hormone Preparations for Menopausal Therapy

The use of hormone therapy has some documented increased risks including stroke, breast cancer, CHD, and venous thromboembolism. Nevertheless, this does not mean that postmenopausal hormone therapy must never be used. Postmenopausal women with severe vasomotor symptoms, vaginal dryness, and/or other symptoms that decrease the quality of life continue to be appropriate indications in the absence of contraindications such as history of coronary artery disease, thrombophilia, and history of thromboembolism. Here we will discuss the different estrogen preparations to address menopausal symptoms.

The concept of "bioidentical" has been promoted by the public and some practitioners. This refers to the use of steroids that are naturally found in humans and sometimes to the concept of individualizing the doses by specifically compounding a product. There are no data to support the superiority or the perceived safety of this approach.

9.32 Systemic Estrogen Therapy

Systemic absorption can occur with oral, transdermal, or vaginal preparations. All estrogens are metabolized in the liver but the oral forms will

Table 9.4 Common estrogen and progestin treatment regimens

Regimen	Bleeding pattern	Possible side effects
E days 1–30, cyclic	Bleeding ~ day	Breast tenderness, mood disturbances, headaches
P days 1–12	14 or 15	
E days 1–25, cyclic	Bleeding ~ day	Same as above, may have hot flashes days 27–30
P days 14–25	27 or 28	
Long cycle (low-dose E daily, P every 3 months for 14 days	Occasional spotting, with quarterly withdrawal bleeding	Same as above, but on a quarterly basis

have a dominant "first pass effect" that is manifested by elevated binding globulins, triglycerides, and clotting factors. Estrogen therapy may increase thyroxine requirements due to the induced hepatic production of thyroid-binding globulin.

9.33 Oral Estrogen

There are several preparations in the oral form of estrogen. The most commonly used preparation is Premarin (Table 9.5). This compound of conjugated equine estrogens derived from pregnant mare's urine consists mostly of estrone sulfate, equilin sulfate, dihydroequilin sulfate, and many other minor estrogenic compounds. There are also synthetic conjugated synthetics such as Cenestin that are derived from plant sources. Esterified estrogens, such as Estratab or Menest, are derived from plant sources as well. Ethinyl estradiol is found in oral contraceptives but also in one product for hormone therapy called Femhrt. Estrace is a micronized form of estradiol. As described in Table 9.5, there are many options available with different dosages and potencies. However, there is no major difference in their efficacy provided that equivalent doses are used [83].

Lower-dose regimens have been approved by the FDA for the treatment of vasomotor symptoms and osteoporosis and include Prempro 0.45 mg conjugated estrogen/1.5 mg MPA, and Prempro 0.3 mg/1.5 mg. Trials with these combination estrogen–progestin regimens show no evidence of endometrial hyperplasia in women treated regardless of the dose [84]. These clinical trials have also demonstrated that low doses of estrogen are adequate for the prevention of bone loss. It is important to emphasize a basic tenet of hormone therapy that the lowest dose should be used to achieve the desired clinical response.

Table 9.5 Estrogen products, routes of administration, and available doses

Product	Available doses
Oral	
Synthetic conjugated estrogens (Cenestin)	0.3 mg, 0.625 mg, 1.25 mg
Equine conjugated estrogens (Premarin)	0.3 mg, 0.45 mg, 0.625 mg
	0.9 mg, 1.25 mg, 2.5 mg
Micronized estradiol (Estrace, Gynodiol)	0.5 mg, 1.0 mg, 2 mg
Esterified estrogens (Menest)	0.3 mg, 0.625 mg, 1.25 mg
	2.5 mg
Estropipate (Ogen, Ortho-Est)	0.625 mg, 1.25 mg, 2.5 mg
Transdermal systems	
17-beta estradiol (Estraderm, Vivelle, Alora Climara, Esclim, Menostar)	0.025 mg, 0.0375 mg, 0.05 mg
	0.075 mg–0.1 mg/day
	14 μg/day
17-beta estradiol plus	0.045–0.05 mg/day plus
norethindrone acetate or	0.14, 0.15, 0.25 mg/day
Levonorgestrel (Combipatch, Climara Pro)	
Topical	
17-beta estradiol (Estrasorb, EstroGel)	3.48 g/day delivers 0.5 mg/estradiol/day
	1.25 g/day delivers 0.75 mg/estradiol/day

(continued)

Table 9.5 (continued)

Product	Available doses
Injectable	
Estradiol valerate in oil (Delestrogen)	10–40 mg/mL
Estradiol cypionate in oil (Depo-Estradiol)	5 mg/mL
Vaginal	
Tablets (Vagifem)	10, 25 μg estradiol/tablet
Creams	
Estrace	0.1 mg estradiol/g
Premarin	0.625 conjugated equine estrogen mg/g
Ogen	1.5 mg estropipate/g
Rings	
Estring	Estradiol 2 mg/3 months/ring (7.5 μg/day)
Femring	Estradiol 0.05 mg to 1 mg/day for 90 days (systemic absorption)

9.34 Transdermal Estrogen

All transdermal systems currently available contain the same estrogen: 17β-estradiol at different dosages. A dose of 50 μg/day in a transdermal delivery system is bioequivalent to an oral dose of 0.625 of conjugated equine estrogens [85]. The transdermal system delivery with the lowest dose that has been approved for prevention of osteoporosis only has 0.014 mg/day of estradiol (Menostar) [86].

9.35 Topical Estrogen

There are multiple products: Estrogel, Divigel, Elestrin, Evamist, and Estrasorb. A randomized controlled trial of 225 menopausal women demonstrated that a topical gel containing 1.25 or 2.5 g of gel containing 17ß (beta)-estradiol was much more effective than placebo in alleviating the frequency and intensity of moderate and severe hot flashes in symptomatic women [87].

9.36 Vaginal Estrogen

When the target organ of the hormonal preparation is the urogenital tissue, vaginal route of administration seems to be the most appropriate way to provide relief to symptomatic postmenopausal women. The safety and efficacy of the vaginal delivery have been widely studied, and there are cream, tablets, and ring preparations available in the market (see Table 9.4). The Estring vaginal ring delivers local estradiol without significant estrogen absorption. It is effective for 3 months. Other intravaginal products such as Femring release estradiol at a rate of 50 μg/day, which will have systemic absorption that necessitates consideration of an endometrial effect.

There are some general rules of equivalence between these products. Ethinyl estradiol 5 μg, 0.625 mg of conjugated or esterified estrogens, 2 mg of micronized estradiol, and 0.05 mg of a transdermal estradiol appear to be equivalent.

9.37 Selective Estrogen Receptor Modulators

New pharmaceutical agents to treat or prevent chronic diseases such as osteoporosis are being developed. The mixed estrogen agonist–antagonist action such as selective estrogen receptor modulators (SERMs) has been developed to specifically target tissues such as bone, vaginal tissues, and their selective binding with estrogen receptors makes them a good option for some

patients [88]. There are many reasons why SERMs produce the tissue-selective effects such as differences in receptor binding affinities and interaction with different coactivators and corepressors that are present in target tissues.

9.38 Tamoxifen

Tamoxifen is a SERM that may act as an estrogen receptor agonist (i.e., uterus) or as an antagonist (i.e., breast) [89]. As a result, tamoxifen can have beneficial effects for osteoporosis, reduction in breast cell proliferation, and cardiovascular disease. Unfortunately, tamoxifen also stimulates the endometrial proliferation and increases vasomotor symptoms.

In the largest prevention-oriented randomized clinical trial aimed to test the hypothesis that tamoxifen reduces the incidence of breast cancer in patients at high risk for the disease, fracture risk was determined as a secondary endpoint. In the National Surgical Adjuvant Breast and Bowel Project (NSABP) P-1 Trial, 13,338 women were randomized to placebo or 20 mg of tamoxifen a day for 5 years. The trial demonstrated a significant decrease in the incidence of invasive and noninvasive breast cancer, and a decrease in the incidence of vertebral, wrist (Colle's), and hip fractures in the treatment group when compared with the placebo. In this same study, patients in the tamoxifen group did not experience a higher rate in ischemic heart disease. However, the risk for endometrial cancer, especially in women over 50 years of age, was increased (risk ratio = 2.53; 95% confidence interval = 1.35–4.97) with all of the endometrial cancers detected in early stage (I) [90].

9.39 Raloxifene

Raloxifene was discovered over 20 years ago in an effort to develop an antiestrogenic drug to treat and/or prevent breast cancer and was previously known as keoxifene [91]. Raloxifene reduced the risk of osteoporosis in postmenopausal women without increasing their risk for uterine cancer or endometrial hyperplasia [92]. Raloxifene at 60 mg/day is currently approved for the prevention and treatment of osteoporosis.

Some clinicians prefer to use bisphosphonates as a first option because of their more potent antiresorptive activity than raloxifene. A comparative trial compared the two classes of drugs. In the "EFFECT" trial (*EF*ficacy of *F*OSAMAX vs. *E*VISTA *C*omparison *T*rial), patients in the alendronate group were found to have substantially greater increases in bone mineral density (BMD) than raloxifene at both lumbar spine and hip sites at 12 months. Lumbar spine BMD increased 4.8% with alendronate vs. 2.2% with raloxifene ($P < 0.001$). The increase in total hip BMD was 2.3% with alendronate vs. 0.8% with raloxifene ($P < 0.001$). Decreased bone turnover was more significant with alendronate than raloxifene. Overall tolerability was comparable. However, the number of patients reporting vasomotor symptoms was considerably higher with raloxifene (9.5%) than with alendronate (3.7%, $P = 0.010$), while an equal number of patients reported adverse gastrointestinal side effects [93].

Besides the positive impact in intermediate outcome measures such as BMD and bone markers, raloxifene has been proven to reduce the risk of osteoporotic vertebral fractures. In the MORE trial (Multiple Outcomes of Raloxifene Evaluation), a multicenter, international, double-blind placebo controlled trial, 7705 women aged 31–80 years in 25 countries who had been postmenopausal for at least 2 years were randomized to either of three groups: 60 mg/day, 120 mg/day of raloxifene, or to placebo. After 36 months of follow-up, the risk of vertebral fracture was reduced in both study groups receiving raloxifene (for 60 mg/day group: relative risk [RR], 0.7; 95% confidence interval [CI], 0.5–0.8; for 120-mg/day group: RR, 0.5; 95% CI, 0.4–0.7) [94]. This study also demonstrated that the rate of breast cancer was less common in the treatment arms than in the placebo. Thirteen cases of breast cancer were confirmed among the women assigned to raloxifene vs. 27 among the women assigned to placebo (relative risk [RR], 0.24; 95% confidence interval [CI], 0.13–0.44; $P < 0.001$) [95]. Like the tamoxifen P-1 Trial, the Study of Tamoxifen and Raloxifene (STAR) P-2 Trial demonstrated that raloxifene was as effective as tamoxifen with a reduction in breast cancer risk of 50%. Women on raloxifene had 36% fewer uterine cancers and 29% fewer blood clots [96].

Raloxifene studies have also shown improvement in some intermediate cardiovascular biomarkers such as serum LDL, lipoprotein (a), homocysteine, and plasma fibrinogen [97, 98]. To test the hypothesis that raloxifene reduces risk of

coronary events (coronary death, nonfatal myocardial infarction [MI], or hospitalized acute coronary syndromes other than MI) the Raloxifene Use for the Heart (RUTH) trial was begun in 1998. A total of 10,101 menopausal women were randomized to raloxifene 60 mg/day or placebo and followed for a median for 5.6 years. Raloxifene did not alter the risk of CHD [99].

9.40 Ospemifene

Ospemifene is a SERM that was approved by the FDA in 2013 for the treatment of moderate-to-severe dyspareunia at a dose of 60 mg/day. Clinical studies demonstrated an improvement in vaginal superficial cells, reduction in parabasal cells, reduction in vaginal pH, vaginal dryness, and dyspareunia [100]. Patients did report an increase in hot flashes relative to placebo during the 12-week study and the 1 year extension. There was an increase in endometrial thickness and uterine polyps observed during the 1 year extension trial, however no cases of hyperplasia or endometrial cancers were observed. Ospemifene labeling contains a black box warning regarding risks for endometrial stimulation, VTE, and stroke although these are lower than those taking estrogen alone.

9.41 SERM-Estrogen Combination

A new formulation, the tissue selective estrogen complex (TSEC), pairs a specific SERM with a specific estrogen and appears to be a favorable alternative to traditional hormone therapy, taking advantage of the estrogen-antagonistic properties of the SERM while offering the benefits of estrogen therapy. In 2013, the FDA approved bazedoxifene (BZA) combined with conjugated equine estrogen (CE) (at a dose of 20 mg BZA/0.45 mg CE) to relieve hot flashes and prevent osteoporosis [101]. This combination is the first TSEC marketed in the USA. BZA was selected as the SERM because it possesses sufficient antagonist effect on uterine tissue to be paired with a conjugated estrogen.

The BZA/CE combination would be an ideal option for women taking estrogen therapy for the treatment of vasomotor symptoms who do not tolerate progesterone therapy or who have a history of irregular bleeding or a thickened endometrial stripe. BZA/CE is also a good first option for symptomatic menopausal women with a uterus who have hot flashes, night sweats, or VVA and desire bone loss prevention without monthly vaginal bleeding.

9.41.1 Tibolone

Tibolone is a synthetic steroid that has shown to be effective in alleviating menopausal symptoms and preventing bone loss. It has been widely used in Europe and the rest of the world since 1988 but is not available in the USA. Tibolone has estrogenic actions in the brain, vagina, and bone tissues, but lacks estrogenic activity in the endometrium and in the breast tissue. Its multifaceted hormone properties appear to be due to its rapid conversion into three metabolites: 3α(alpha)- and 3β(beta)-hydroxy-tibolone, each with estrogenic effects, and the Δ(delta)^4-isomer, with progestogenic and androgenic effects. The tissue-selective actions of tibolone are the product of metabolism, enzyme control, and receptor activation that vary in responsive target tissues. This biotransformation takes place principally at the liver and intestine. These pharmacological characteristics make tibolone unique and different from the typical SERMs [102].

More recently, an even lower dose of tibolone was evaluated in an RCT. This study included 90 postmenopausal women who were followed for 2 years and were allocated to tibolone 2.5 mg (n = 30), tibolone 1.25 mg (n = 30), and a control group (n = 30). All subjects received 1000 mg of calcium per day. Gambacciani et al. demonstrated tibolone to be effective at lower doses not only at preventing bone loss as measured by BMD but also was successful at alleviating vasomotor symptoms [103].

Another interesting potential benefit of tibolone on postmenopausal women is the positive effect shown on libido in some clinical trials. In a small randomized trial, patients with postmenopausal changes in sexual desire were allocated to 2.5 mg/day of tibolone (n = 14) or to 500 mg/day of calcium [104]. This trial showed that the patients treated with tibolone experience an improvement in sexual desire after the third month of treatment, which was maintained until the end of treatment (12 months) [103]. Similar

beneficial findings in sexual desire were shown in a small study of 50 postmenopausal patients that were treated with either tibolone or conjugated estrogens and medroxyprogesterone [103].

In the large cohort Million Women Study, there was increased relative risk of breast cancer in current users of tibolone (RR 1.45, 95% confidence intervals 1.25–1.68). These results were not anticipated, since tibolone does not increase breast density and has been shown to have much less estrogenic activity in breast tissues than other agents in postmenopausal clinical trials [105, 106].

In summary, clinical data support the use of tibolone as a viable option for hormone therapy during menopause. Its versatile actions in different target tissues have demonstrated to have beneficial effects for the relief of vasomotor symptoms and treatment of osteoporosis. There is suggestive evidence that tibolone may positively impact libido in postmenopausal women. Unfortunately, there are few randomized trials, and there is lack of the long-term clinical evidence of tibolone benefits on cardiovascular and neurological systems.

9.41.2 Androgens

The use of androgens for postmenopausal women is the subject of much debate. Reduced androgen levels in the menopausal women are due in part to a gradual decline of adrenal precursors such as DHEA and DHEAS with a further decline in ovarian production of testosterone. Combinations of esterified estrogens and methyltestosterone have been used for decades for the treatment of severe hot flashes especially in younger, surgical menopause women [107], whereas the use of methyltestosterone alone at low doses did not relieve hot flashes [108]. Currently, no combination estrogen–androgen products are available in the USA.

Testosterone is the key sex steroid implicated in the mediation of sexual desire and coital frequency in both men and women. In contrast to women after surgical menopause, women undergoing natural menopause do not experience an abrupt decline in testosterone levels [109]. The production of testosterone from the menopausal ovary will gradually decline with aging. The concept of sexuality in women is complex and not directly linked to levels of serum androgens. Nonetheless, there have been many clinical trials that have assessed the use of testosterone. In a recent cross-sectional cohort study of 2311 perimenopausal women, the Study of Women Across the Nation (SWAN), longitudinal data indicate that total testosterone levels declined from baseline by −12.8, −21.4, −22.4, and −26.4% during the initial 4 years of follow-up. Other adrenal-derived, less potent androgens such as DHEA sulfate did not appreciably change [110]. Taken together, these observations suggest that an androgen deficiency state may not be unique to women with surgical menopause but rather the decline in androgens may be clinically significant in women who are undergoing the natural transition into the menopause. What remains unclear is whether these inevitable physiological changes in circulating testosterone concentrations are associated with an increased incidence of hypoactive sexual desire disorder (HSDD) as defined by a loss of libido that is associated with psychological distress.

Validated instruments to evaluate and quantify sexual desire in surgically menopausal women [111] have been established. In randomized, double-blinded, placebo-controlled clinical trials, transdermal testosterone administration at a dose of 300 μg/day to surgically menopausal women significantly enhanced sexual functioning and increased the frequency of total satisfying sexual activity [112–114]. These testosterone patches are currently not available in the USA.

9.41.3 Progestins

Oral progestins that are typically used are MPA, micronized progesterone (Prometrium), and norethindrone. These hormones can be used cyclically or continuously. MPA 5–10 mg or micronized 200 mg progesterone can be given for the first 12 days of each month. Lower doses are used for continuous use. Typical doses for this are 2.5 mg MPA (Prempro), 1 mg norethindrone (Femhrt), or 100 mg micronized progesterone.

Some progestins are formulated with an estrogen such as Prempro, which contains MPA, or Femhrt, which contains norethindrone 0.5–1 mg. A low-dose levonorgestrel intrauterine device is under investigation as a method of endometrial protection.

9.42 Nonhormonal Therapies

It is clear that estrogen is the most effective and studied medication to treat vasomotor symptoms. However, given the results of the WHI, more patients and physicians have considered non-estrogen treatment methods for the treatment of vasomotor symptoms.

9.42.1 Clonidine

Clonidine is an antihypertensive medication that acts centrally as an α2-adrenergic agonist and can be administered orally or via a transdermal delivery system. Clonidine has been used to treat hot flashes not only in postmenopausal women but also in post-orchiectomized men [115]. The evidence to support its use is limited. One randomized controlled trial 15 patients to placebo and 14 to transdermal clonidine. Subjects were followed for 8 weeks, and 86% of the patients in the treatment group had a significant decrease in the frequency of vasomotor episodes, 73% decrease in the severity of flushes, and 67% decrease in the duration. In the placebo groups, these benefits were present in 36%, 29%, and 21%, respectively [116]. One characteristic consistent throughout the retrospective and prospective clinical trials of clonidine was the high percentage of patients with side effect: dry mouth in up to 40% of patients, drowsiness and dizziness in up to 35%, skin rash and irritation in 15% of oral and up to 50% in the transdermal system.

If no other treatment fits the need of the symptomatic postmenopausal patient, clonidine can be tried in a dose of 2.5 mg of a transdermal system and changed every week. Clonidine also may be prescribed and divided orally in doses of 0.1–0.4 mg/day.

9.43 Selective Serotonin and Norepinephrine Reuptake Inhibitors

This group of drugs (Table 9.6) seems to effectively decrease vasomotor instability in menopausal women by increasing the availability of serotonin and/or norepinephrine in the central nervous system. These compounds should be considered a possible option to control hot flashes in women not willing to take estrogen or they are contraindicated. There are several open label trials and enough randomized controlled trials to currently recommend them as a reasonable option. In 2013, the FDA approved paroxetine 7.5 mg/day (Brisdelle) to treat

Table 9.6 Randomized, controlled trials of selective serotonin reuptake inhibitors (SSRIs) for the treatment of vasomotor symptoms in postmenopausal women

SSRI/groups	Subjects	Endpoint	Length of the study	Comments
Venlafaxine (2000)	191	Reduction average daily hot flashes	4 weeks	Side effects: nausea, dry mouth, constipation, decreased appetite
Placebo	50	27%		
37.5 mg/day	49	37%		Other benefits: decreased depression scores, improved quality-of-life scores
75 mg/day	43	61%		
150 mg/day	49	61%		
Venlafaxine (2005)	61	Reduction patient perceived hot flash score	12 weeks	Side effects: dry mouth, sleeplessness, decreased appetite
Placebo	32	15%		
37.5 mg/ day × 1 week then				
75 mg/ day × 11 weeks	29	51%		

Table 9.6 (continued)

SSRI/groups	Subjects	Endpoint	Length of the study	Comments
Paroxetine (2003)	165	Mean change daily hot flash composite score	6 weeks	Clinical global impression also improved significantly in treatment groups
Placebo	56	37.8%		
12.5 mg/day	51	62.2%		
25 mg/day	58	64.6%		
Fluoxetine (2002)	81	Hot flash frequency and hot flash score	1-week documentation	Randomized cross-over trial
Placebo/fluoxetine 20 mg	41	36%	4 weeks each period	Well tolerability of fluoxetine
Fluoxetine 20 mg/ placebo	40	50%	Total of 9 weeks	

moderate to severe hot flashes associated with menopause [117]. There are studies that evaluate citalopram, paroxetine, and sertraline. One of the first compound to be studied and used is venlafaxine at doses of 37.5–75 mg/day. Venlafaxine and its active metabolite desvenlafaxine 100 mg/day (Pristiq) have both serotonin and norepinephrine reuptake inhibition. In general, the efficacy of these compounds is approximately a 50–60% reduction in severe hot flash frequency relative to baseline. The placebo response rates in these same study populations range from 30 to 40% [118].

9.44 Gabapentin

Gabapentin is a (gamma)-aminobutyric acid analogue approved in 1994 for the treatment of seizures and has been used by neurologists to treat neuropathic pain, essential tremor, and migraines [119]. In a randomized, double-blind controlled trial that included 59 menopausal women with seven or more severe hot flashes a day, a dose of 900 mg of gabapentin a day was given for 12 weeks. Gabapentin was associated with a 45% reduction in hot flash frequency and a 54% reduction in hot flash composite score (frequency and severity combined into one score) from baseline, compared with 29% ($P = 0.02$) and 31% ($P = 0.01$) reductions, respectively, for placebo [120]. One out of five patients taking gabapentin will experience dizziness and somnolence.

9.45 Paced Respirations

This behavioral modification technique uses a slow, deep breathing exercise to control hot flashes. Freedman and Woodward first described this technique and validated that the deep breathing component alone was effective in reducing hot flashes, whereas progressive muscle relaxation techniques were ineffective [121]. The protocol utilized by Freedman consists of slow, deliberate, deep breathing at a target rate of six to eight breaths per minute to be done for 15 min twice a day. Additionally, deep breathing is applied during a hot flash. This regimen is risk-free and could be considered a first-line treatment for vasomotor symptoms.

9.46 Dietary and Lifestyle Recommendations during Menopause

There is little doubt that prevention is the best strategy to live a healthy life. As health care providers, we should regard the onset of menopause as a signal for future living rather than as the decline of a woman's life. This phase is a great window of opportunity for health care providers to help patients to establish dietary and lifestyle habits to maximize her physical, social, mental, and sexual opportunities.

9.46.1 Calcium Recommendations

One of the key elements to prevent osteoporosis is to maintain a positive calcium balance. This task becomes challenging during menopause because calcium absorption declines with age. Furthermore, the lower levels of estrogen during menopause decrease the levels of 1,25-dihydroxyvitamin D, with the subsequent consequence of even less calcium absorption [122]. Nordin et al. measured radiocalcium absorption in 262 postmenopausal women ages 40–87 and demonstrated a late age-related decrease (only after >75 years of age) in calcium absorption in postmenopausal women in addition to the decline that occurs at menopause. They concluded that this decrease could be due to a decline in either the active calcium transport or diffusion component of the calcium absorption system [123].

The following are the recommendations for calcium intake in postmenopausal women [124]:

- 1000 mg/day for women between 25 and 50 years
- 1000 mg/day for postmenopausal women on estrogen replacement therapy
- 1200 mg/day for postmenopausal women not on estrogen therapy
- For all women over 50 years, daily intake is recommended to be 1200 mg/day, although further research is needed in this age group
- Adequate vitamin D is essential for optimal calcium absorption. Dietary supplements, hormones, drugs, age, sun exposure, and genetic factors influence the amount of calcium required for optimal skeletal health
- Calcium intake, up to a total intake of 2000 mg/day, appears to be safe in most individuals
- The preferred source of calcium is through calcium-rich foods such as dairy products. Calcium-fortified foods and calcium supplements are other means by which optimal calcium intake can be reached in those who cannot meet this need by ingesting conventional foods

These guidelines are based upon calcium from the diet plus any calcium taken in supplemental form and all are based on elemental calcium.

The preferred method of reaching optimal calcium intake is through dietary sources. Among the foods with higher content of calcium are dairy products, green vegetables (e.g., broccoli, kale, turnip greens, Chinese cabbage), calcium-set tofu, some legumes, canned fish, seeds, nuts, and certain fortified food products, like bread and cereal. A good rule of thumb to calculate the daily intake of calcium is to multiply the number of milk servings (8 oz. = 240 mL = 1 cup) by 300 mg [125].

Due to the challenges of achieving adequate calcium intake via food sources, many physicians advocate the use of calcium supplements. There are two main types of calcium compounds available in the market: calcium carbonate and calcium citrate. Some authors advocate the use of calcium citrate (Citracal) over calcium carbonate (Os-Cal), arguing that calcium citrate is better absorbed than calcium carbonate, especially with an empty stomach but even when taken with a meal [126, 127]. On the other hand, the calcium carbonate is usually less expensive, and according to at least one study in absorbability, bioavailability, and cost-effectiveness, the carbonate formulation is a better supplement choice in a population at risk for both low BMD and hip fracture [128].

9.46.2 Vitamin D

Vitamin D is not usually found in food. It is produced by the skin in response to light exposure. There is controversy regarding the right amount of oral intake of vitamin D that is required. A serum 25-OH vitamin D level of >than 20 ng/mL is considered adequate for bone and overall health. The dose recommended by the Recommended Dietary Allowance (RDA) in women 50–70 years old is 600 IU/day. For those >70 years, a dose of 800 IU/day is recommended. Those living in Northern climates should consider a higher daily dose not to exceed 4000 IU/day [129].

9.46.3 Exercise

Many exercise recommendations to maintain a health-related fitness is based on studies done in men. However, a recent systematic review of all the randomized controlled exercise trials in postmenopausal women delineates some benefits in intermediate outcome measures. In this systematic review, Asikainen et al. assessed 28 randomized controlled trials with 2646 subjects. Based on this review of the studies on postmenopausal

women, they describe specific guidelines for health professionals to counsel their patient to adequately develop an exercise program [130]:

- Early postmenopausal women could benefit from 30 min of daily moderate walking in one to three episodes combined with a resistance-training program twice a week
- For a sedentary person, walking is a feasible way to start exercise by incorporating it into everyday life
- A feasible way to start resistance training is to perform 8–10 repetitions of 8–10 exercises for major muscle groups starting with 40% of 1 repetition maximum
- Resistance training initially requires professional instruction, but can thereafter be performed at home with little or no equipment as an alternative for a gym with weight machines
- Warm-up and cool-down with stretching should be a part of every exercise session

The training described above probably will preserve normal bodyweight. When combined with a weight-reducing diet, exercise will likely reduce bone loss and increase muscle strength. Based on limited evidence, such exercise might also improve flexibility, balance, and coordination, and decrease hypertension and improve dyslipidemia [130].

9.46.4 Tobacco and Alcohol

Cigarette smoking and excessive alcohol intakes are related to increase risk of bone loss and cardiovascular disease. In women, more than two drinks per day is associated with an elevated risk of developing hypertension. On the other hand, mild consumption of alcohol (≤7 units/week) in women may be associated with a cardioprotective effect. In a 12-year prospective study that included 85,709 healthy women aged 35–59, Fuchs et al. concluded that mild to moderate consumption of alcohol could be associated with a decreased mortality rate for women, but mainly for those at high risk for CHD [131]. Nevertheless, we ought to be cautious when counseling for mild alcohol intake, since it is also associated with increased incidence of breast cancer [132]. These confounders make it difficult to provide a solid recommendation for alcohol consumption for women.

9.47 Alternative Medicine during Menopause

9.47.1 Botanical Dietary Supplements

In recent years more natural products have gained popularity as alternatives for treatments of vasomotor symptoms. Among these products phytoestrogens are perhaps the ones with more widespread use. Phytoestrogens are plant-derived substances structurally related to estrogens that have weak affinity to estrogen receptors [133]. Phytoestrogen-containing dietary supplements commonly employed to relieve vasomotor symptoms include flaxseed, red clover extract, evening primrose oil, and soy compounds among others. The difficulty of consuming these botanical dietary supplements is that the purity, potency, and effectiveness are not well established; however, they are popularly believed to be safe and effective for the treatment of menopausal symptoms [134]. These products are used by as many as 46–79% of menopausal patients in some surveys [135, 136].

A review of randomized controlled trials of complementary and alternative medicine for the treatment of menopausal symptoms by Esenberg et al. evaluated a total of 29 randomized controlled trials of complementary and alternative therapies for hot flashes and other menopausal symptoms; of these, 12 dealt with soy or soy extracts, 10 with herbs, and 7 with other therapies. They concluded that while soy products seem to have modest benefit for hot flashes, studies are not definitive. Isoflavone preparations seem to be even less effective than soy products. Black cohosh may be effective for menopausal symptoms, especially hot flashes, but the lack of adequate long-term safety data (mainly on estrogenic stimulation of the breast or endometrium) precludes recommending long-term use [137].

Also, a more recent systematic review of 25 randomized controlled trials from the Cochrane Library and MEDLINE from 1966 to March 2004 involving a total of 2348 patients concluded that the available evidence suggests that phytoestrogens available as soy foods, soy extracts, and red clover extracts do not improve hot flushes or other menopausal symptoms [138].

In summary, the current clinical data on the effectiveness of botanical dietary supplement is

mainly from open label trials, which are burdened with methodological flaws because of the large placebo effect. For this reason, it is useful to refer to the North American Menopause Society position statement on the treatment of vasomotor symptoms:

» For mild hot flashes, lifestyle-related strategies such as keeping the core body temperature cool, participating in regular exercise, and using paced respiration have shown some efficacy without adverse effects. Among nonprescription remedies, clinical trial results are insufficient to either support or refute efficacy for soy foods and isoflavone supplements (from either soy or red clover), black cohosh, or vitamin E; however, no serious side effects have been associated with short-term use of these therapies [139].

9.48 Progesterone Cream from Yam Root

Progesterone can be synthesized commercially from the wild yam. Its use for alleviating menopausal symptoms is growing in the USA. The scientific evidence is controversial at best. A 12-month randomized controlled trial of 102 healthy postmenopausal women given either a quarter teaspoon of cream (containing 20 mg progesterone) or placebo to the skin daily showed that while there was a significant improvement in the relief of vasomotor symptoms (83% treatment group vs. 19% in the placebo group), there was no difference in the BMD [140].

In contrast, a randomized, double-blind, placebo-controlled, crossover trial performed in 23 postmenopausal women with vasomotor symptoms, using a topical cream containing wild yam extract (*Dioscorea villosa*), vitamin E, and other oils, found no detrimental side effects, nor any improvement in menopausal symptoms as documented in weekly diaries for 3 months [141]. Similar results were found in a parallel, double-blind, randomized, placebo-controlled trial in 80 symptomatic postmenopausal women comparing the effect of a transdermal cream containing a progesterone (32 mg/daily) with a placebo cream. This study showed no changes in mood characteristics or sexual feelings nor was there any change in blood lipid levels or in bone metabolic markers, despite a slight elevation of blood progesterone levels [142].

9.49 Acupuncture

A limited number of studies have evaluated Chinese acupuncture in alleviating mainly vasomotor symptoms. The lack of systematic controls in some of these studies makes it difficult to interpret their effectiveness.

In a small prospective open trial, 11 menopausal symptomatic patients underwent acupuncture for 5 weeks and experienced a significant improvement in menopausal vasomotor symptoms without any changes in reproductive hormones or psychosocial or sexual symptoms as measured by the Menopause Specific Quality of Life Questionnaire [143].

A pilot study evaluated the effectiveness of acupuncture for the treatment of menopausal symptoms in 15 patients with breast cancer treated with tamoxifen 20 mg/day. With the exception of libido, all the other dimensions of the Green Menopause Index showed significant improvement ($P < 0.001$) with acupuncture [144].

Wyon et al. randomized 45 postmenopausal women with complaints of vasomotor symptoms to three study groups: electro-acupuncture, superficial needle insertion, or oral estradiol treatments for 12 weeks with a 6-month follow-up. The patients in the electro-acupuncture group had a decrease in the mean number of hot flashes from 7.3 to 3.5, while superficial needle insertion (i.e., placebo) patients decreased the mean number of hot flashes from 8.1 to 3.8. In the estrogen group, the number of flushes decreased from 8.4 to 0.8. The Kupperman index and the general climacteric symptoms score decreased, and remained unchanged for 24 weeks after treatment in all groups. Superficial needle insertion and electro-needle stimulation were similar in efficacy for acupuncture treatment of vasomotor symptoms, although not as effective as estrogen [145].

In Sweden, a randomized single-blind controlled design was used to evaluate the effects of electro-acupuncture on general psychological distress and relate to experience of climacteric symptoms in 30 postmenopausal women. This study had a group of patients with electro-acupuncture and another group with extremely superficial needle insertion, this one functioning as a near-placebo control. Patients were treated for 12 weeks, and general psychological wellbeing, mood, and experience of climacteric symptoms were used as

outcome measures. This study showed enhancement in the mood scale in the acupuncture group, but climacteric symptoms and psychological wellbeing improved in both placebo and treatment groups, suggesting that electro-acupuncture is not any better than superficial needle insertion for amelioration of climacteric symptoms or improvement of wellbeing [146].

Acupuncture may create tissue damage occasionally, but usually will not cause serious complications (i.e., pneumothorax, cardiac tamponade). The most common serious risk is the transmission of hepatitis viruses or other infection agents through inadequately sterilized needles. Disposable needles, the standard of care in the USA, have eliminated this threat. In summary, alternative treatment for vasomotor symptoms lack carefully conducted placebo-controlled trials. Until these are evaluated, all recommendations should be guarded.

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Osteoporosis for the Female Patient

Heather D. Hirsch, Andrea Sikon, and Holly L. Thacker

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10.1 Introduction

The women's health physician and/or gynecologist needs to be acutely aware of the risks of undiagnosed osteoporosis (OP), as more women will suffer from an osteoporotic fracture than from myocardial infarction, strokes and cancers combined [1–3]. The resultant risks of disability, institutionalization, and mortality from complications are the real threats after an osteoporotic fracture. As OP carries no symptoms until it clinically manifests as a fracture, it often goes undiagnosed and undertreated.

Clinical Case

A 43-year-old married female presents to the office with hot flashes, trouble sleeping, and some episodes of dyspareunia. She underwent hysterectomy secondary to fibroids with bilateral removal of the ovaries 6 months ago. She is slender with a BMI of 21 and she walks for exercise 3 days per week. As her fibroids caused significant pain during her young adulthood, she took Lupron and then Depo-Provera for about a decade to help control her painful periods. Her fibroids caused infertility and she was unable to conceive. She tells you that her mother was just diagnosed with osteoporosis after a hip fracture and that she is worried about her bone health. She reports no falls, fractures, or history of fracture herself. She takes vitamin D in her daily multivitamin. She is a vegetarian. How would you proceed with her concerns about her bone health?

10.2 Prevalence

Data from the National Health and Nutrition Examination Survey III (NHANES III) and the National Osteoporosis Foundation (NOF) have estimated that 10 million Americans have OP with nearly 52% of women over the age of 80 [4]. This number is only expected to steadily increase as the baby boomer generation ages [5]. Data from 2013 estimates that upwards of $16.9 billion is spent annually on osteoporotic fractures, with the highest costs related to the non-vertebral fracture burden [6]. As medical practice continues to emphasize cost effective medicine, preventing osteoporotic fractures can have a significant impact on value based care.

10.2.1 Diagnostic Criteria

The diagnostic criteria for OP rests mostly on identification of risk factors, bone mineral density (BMD) results, and a history of fragility fracture. It is first important to understand some basics of underlying bone physiology. Osteoblasts govern the synthesis and calcification of new bone, while osteoclasts function to maintain bone structure by removal of old bone matrix through a lifelong process called bone turnover [7]. Under normal physiologic conditions, this process should be balanced, referred to as "coupling" [8]. Any pathology of the osteoblast can lead to malformation of the bone while overstimulation of osteoclasts can also lead to loss of bone integrity and strength. OP results when such disruptions lead to diminished bone strength that predisposes the patient to an increased risk of a fracture. Bone strength is comprised of both bone mineral density (BMD) and bone quality. See Fig. 10.1.

10.3 Diagnostic Tests

Diagnosing OP starts with a thorough history and physical, and should include documentation of risk factors as well as accurate height (or loss of height) and weight to calculate BMI. (Table 10.1)

BMD is measured standardly by dual energy absorptiometry (DXA); however assessing bone quality is more complex. Bone microarchitecture can be evaluated with high resolution quantitative computed tomography (QCT) [9], high resolution magnetic resonance imaging (MRI) [10], or double tetracycline-labeled transiliac bone biopsy with histomorphometry, but these are not commonly used in clinical practice [11].

A DXA study calculates both a *T*- and *Z*-score and provides these to the clinician for interpretation. The *T*-score is a range based on standard deviations (SD) below that of a young adult at peak bone density, which is used as the reference

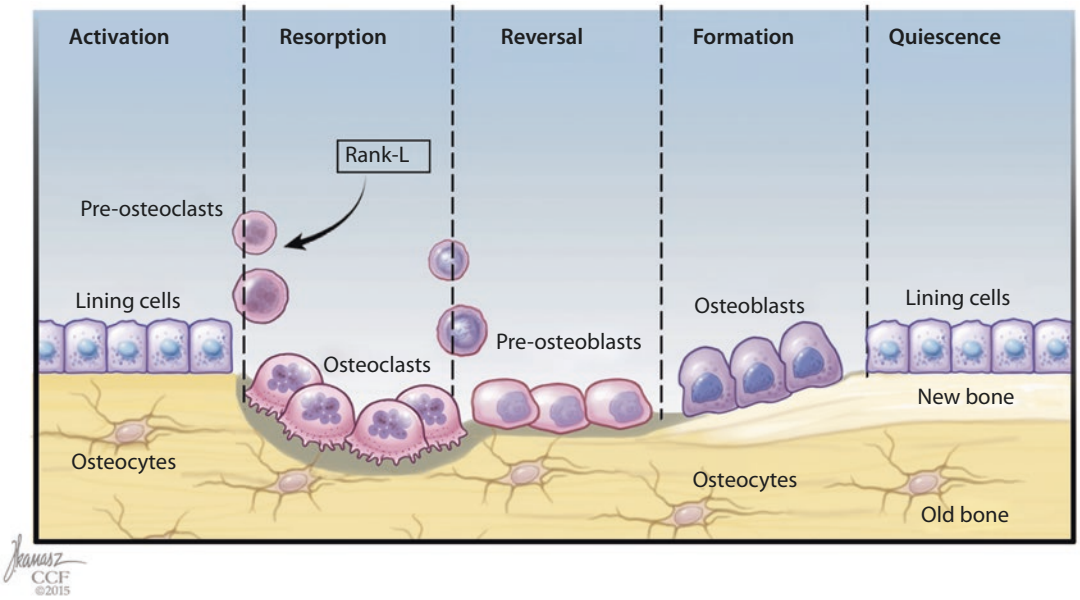

Fig. 10.1 Illustration of bone resorption and bone formation

Table 10.1 Medical risk factors for primary osteoporosis

Advancing age
Past history of fragility fracture
Frequent falls
Low body weight or BMI
Family history of osteoporotic fractures
Early menopause before the age of 45 should be listed individually as should Premature Ovarian Insufficiency* Surgical menopause (bilateral salpingo-oophorectomy)
Sedentary lifestyle
Excessive alcohol (>1 drink per day for women or >14 drinks per week) Excessive caffeine intake (>3 large cups per day) Active tobacco exposure
Low vitamin D (under 20 ng/dL)
Low calcium intake

*They are risk factors for osteoporosis

population, which is unique to the actual DXA scanner. In contrast, the *Z*-score refers to a BMD that uses an age matched reference population. Specific BMD based cutoffs as defined by the WHO for a diagnosis of OP are outlined in Table 10.2 [12].

As BMD is a surrogate marker of bone strength and there are many other factors that contribute, a diagnosis of clinical osteoporosis is made regardless of the BMD result in the setting of a fragility fracture. Osteoporotic fragility fractures are those occurring from a fall or less than standing height,

Table 10.2 WHO diagnosis of OP by DXA score

Definition	BMD measurement	*T*-score
Normal	BMD within 1.0 SD of the mean bone density of the young adult population	+2.5 to −1.0
Low bone mass (osteopenia)	BMD 1.0–2.5 SD below the mean for young adult reference population	−1.0 to −2.4
Osteoporosis	BMD > or equal to 2.5 SD below the normal mean for young adult reference population	Less than or equal to −2.5
Severe Osteoporosis	BMD > or equal to 2.5 SD below the normal mean for young adult reference population *plus* history of fracture	Less than or equal to −2.5 plus fragility fracture(s)

Table 10.3 Important co-morbidities leading to secondary osteoporosis

Diagnosis	Pathophysiology
Cushing syndrome	Excess endogenous cortisol
Gastrointestinal disorders	Decreased gut absorption of Ca/Vit D
Hypogonadism	Decreased endogenous estrogen
Hyperthyroidism (Graves or multinodular goiter)	Increased osteoclast activity
Multiple myeloma	Up-regulation of osteoclasts
Primary hyperparathyroidism	Increased bone reabsorption
Transplantation	Multi-factorial
Vitamin D deficiency	Malabsorption, kidney failure, liver failure
Hypercalciuria	Increased calcium loss in the urine

occurring separately from any trauma such as a motor vehicle accident or other major trauma (excluding toes, nose, and fingers), regardless of *T*- and *Z*-score. This is especially important as many women and providers often mistake fractures obtained from a slip and fall as expected rather than recognizing the pathological nature which requires further investigation and treatment.

Screening all women with a DXA starts at age 65, unless a woman meets criteria for having known risk factors and/or a low bone density result might influence a woman's decision to initiate hormonal therapy for other menopausal symptoms [13]. In premenopausal women and women under the age of 50 who are not yet menopausal, race-adjusted *Z*-scores should be used when interpreting the DXA. For postmenopausal women, the *T*-score is used.

Secondary osteoporosis is OP caused by another condition or medication and may go undetected as there are numerous potential causes, several of which are somewhat obscure (Table 10.3).

Approximately half of premenopausal women with OP and one-fifth of postmenopausal women have a secondary cause [14]. A cursory evaluation should thus be completed for all patients with OP before proceeding to treatment, tailored further by the patients' history and physical. Secondary causes should also be revisited in patients who lose bone density and/or fracture despite therapy [15]. Although there is not 100% consensus amongst the many

Table 10.4 laboratory testing to work up secondary causes of osteoporosis

Cushing syndrome	24 h urinary free cortisol Dexamethasone suppression test
Diabetes mellitus	HgA1c Fasting glucose Glucose tolerance test
Gastrointestinal disorders	Celiac disease – endomysial IgA antibiotics – anti-tissue transglutaminase – IgA and IgG antibodies to synthetic deamidated gliadin peptide and/or duodenal biopsy Hemochromatosis – serum total iron binding capacity – transferrin – plasma ferritin
Hypogonadism	Estradiol level FSH Prolactin Anti-Müllerianhormone
Hyperthyroidism	TSH, Free T4 Total T3
Hyperparathyroidism	Parathyroid hormone

Table 10.5 Important co-morbidities leading to secondary osteoporosis

Diagnosis	Pathophysiology
Cushing syndrome	Excess endogenous cortisol
Gastrointestinal disorders	Decreased gut absorption of Ca/Vit D
Hypogonadism	Decreased endogenous estrogen
Hyperthyroidism (Graves or multinodular goiter)	Increased Osteoclast activity
Multiple myeloma	Up-regulation of osteoclasts
Primary hyperparathyroidism	Increased bone reabsorption
Transplantation	Multi-factorial
Vitamin D Deficiency	Malabsorption, kidney failure, liver failure
Hypercalciuria	Increased calcium loss in the urine

organizations that have guidelines on OP as to precisely what an initial evaluation should entail, most include those in Table 10.4. When *Z*-scores are low, one should especially consider evaluating for secondary causes of OP (Table 10.5).

10.4 Treatment

Additional pharmacologic therapy is indicated in all patients with OP, diagnosed either by BMD or clinically by presence of a fragility fracture [12]. For those with low bone density, the Fracture Risk

Assessment Tool (FRAX®) is utilized to calculate absolute 10 year fracture risk. Based off of cost benefit analysis, if the calculated 10 year risk of a hip fracture is greater than or equal to 3%, or if any major osteoporotic-related fracture probability is equal to or greater than 20%, then the patient should be started on treatment after excluding and/or treating secondary causes of OP. The FRAX® calculation considers the patient's femoral neck bone mineral density and personalized risk factors. FRAX calculation takes into account the patient's femoral neck bone mineral density, (not vertebral measurement) and personalized risk factors, which include the patient's age, sex, BMI, personal history of prior fracture, family history of an osteoporotic fracture, smoking status, personal history of glucocorticoid use (>5 mg/day or longer than 3 months in a lifetime), rheumatoid arthritis, or other diagnosis of secondary osteoporosis, and alcohol intake. FRAX® scores can quickly be calculated using an online tool: [► http://www.shef.ac.uk/FRAX]

FRAX® is to be used as a guideline, and clinical consideration should still be utilized, especially as several limitations exist [16]. First, the FRAX® calculation only uses the femoral neck BMD, which can underestimate risk in patients who have low lumbar spine but normal femoral neck measurement, a common scenario in the early years following menopause. Also, only dichotomous yes/no responses are allowed for smoking, alcohol and steroid use, despite it being well known that higher doses and longer durations of these can disproportionately impact bone strength. Also, no consideration is given to fall risk, which is a significant fracture risk. Fall history is included in the Asian Garvan fracture risk calculator, an alternative to FRAX® [17].

Universal recommendations for all patients with OP and low bone density include weight bearing exercise to increase bone density and muscle strength. Mechanical loading through exercise increases bone mass [18], while immobility is strongly correlated with declining bone density [19, 20]. Assessing for fall assessment and prevention (vision, home safety, balance training and physical therapy) becomes more critical as the woman advances into the geriatric years [20]. Smoking should be eliminated [21] and alcohol intake limited to <14 drinks/week in women (1 drink = 1.5 fl oz. spirits or 5 fl oz. of wine or 12 fl oz. beer) [22]. All women should maintain adequate intake and serum levels of vitamin D, which is difficult to obtain adequately from the diet so supplementation is usually needed (vit d3 800–1000 IU/day in most and higher doses required in some women) [23, 24, 25].

All women should be advised to ingest adequate daily calcium in their diet and likewise to obtain adequate levels of vitamin D. The recommended daily intake of calcium is 1000 mg for premenopausal women and 1200 mg for postmenopausal women daily [26] and is optimally obtained from dietary sources such as dairy (low-fat milk, cheese, and yogurt), salmon, tofu, broccoli, almond milk, and some fortified cereals. Calcium supplements should be added only when an intake of 1200 mg daily in the diet is not obtainable. Some studies have suggested that calcium supplementation may increase cardiovascular risk [27]. These have been variable and the data remains unclear. As calcium is readily available from a variety of dietary sources, it is encouraged to reach daily recommended intake levels through diet when possible.

Estrogen decreases osteoclast activity and stimulates osteoclast apoptosis. It also functions to block RANK-L by inhibiting TNF-alpha. In the 5–7 years following the onset of menopause, with reductions of estrogen, women can lose up to 20% of their bone mass, primarily of cancellous bone (i.e., spine and calcaneus) [28]. In the Women's Health Initiative (WHI), hormone therapy (HT) reduced the risk of hip fracture by 50%. In the estrogen only arm, fracture reduction was seen at all sites [28]. In both the estrogen (ET) and the estrogen + medroxyprogesterone acetate (MPA) treatment arms (EPT), hip fractures were reduced compared to placebo [29]. Post hoc analysis of the WHI has showed additional benefits, particularly for women within 10 years of menopause, now referred to as the *timing hypothesis*. An apparent reduction in the risk of cardiovascular events was seen in this group of women who used HT within the first 10 years of menopause onset [30]. HT in both arms decreased the risk of colon cancer and diabetes. Furthermore, women in the estrogen only arm had no increased risk of breast cancer compared to placebo. The increased risk of breast cancer in the EPT arm seemed to increase significantly after 5 years of therapy [31].

HT increased stroke risk after 3 years of therapy in both arms; however, the increased absolute

risk with EPT was only 3 additional strokes per 10,000 women per year of therapy in those within 5 years of menopause and 6 within 10 years of menopause [32]. The ET arm showed an increased risk only in women age 60 and over. All of these absolute risks are actually defined as rare events by the WHO criteria [33].

Estrogen is also the most efficacious treatment option for postmenopausal women suffering from severe vasomotor symptoms (VMS), which are experienced by upwards of 60–90% of women, of which nearly a quarter of women report these symptoms as unbearable [34]. Therefore, estrogen may be ideal in women in the first 5 years postmenopausally without significant risks for stroke or breast cancer that have low BMD and are also seeking relief of concomitant VMS and genitourinary syndrome of menopause (GSM). Estrogen has a dose dependent effect on bone and its effects are lost with cessation of therapy.

In postmenopausal women ages 60–80 who have osteopenia but are without other symptoms of menopause that require systemic hormonal therapy, ultra-low dose transdermal estradiol (Menostar®) can be used for the prevention of OP. Due to the relatively low dose of estrogen, only twice annual progestin is needed for women with a uterus.

Bisphosphonates (BPS) are anti-resorptive medications that activate osteoclasts. BPS have many advantages being overall very safe, effective, affordable, and are available in a variety of formulations. Many formulations exist, but no head-to-head studies are available. Multiple large meta-analyses have concluded that the four available BPS—alendronate, risedronate, ibandronate, and zoledronic acid— reduce fractures in postmenopausal women compared to placebo, but ibandronate does not have data showing reductions in hip fracture or non-vertebral fractures [35]. Before starting treatment, vitamin D deficiency and hypocalcemia (both of which are common in patients post gastric bypass) [36] should be corrected. Serum creatinine should be measured as BPS are contraindicated with a creatinine clearance of less than or equal to 35 mL/min. [37] (Table 10.6).

BPS's main negative side effects when administered orally can be esophageal irritation/reflux and dyspepsia, and so should be used with caution in those with gastroesophageal reflux, swallowing disorders, and a history of gastric surgeries. As a result, patients should be instructed to remain upright for 30 min following administration. Additionally, BPS are very poorly absorbed when taken orally at less than 1% and consequently significant instruction to take it on an empty stomach with 8 oz. of plain water, without the addition of other foods, liquids, or medicines becomes essential [38]. Risedronate sodium (Atelvia®) is a newer formulation that can be taken on a full stomach after a meal at 35 mg/week. GI side effects overall can also be averted if given intravenously.

Osteonecrosis of the Jaw (ONJ) is a rare potential side effect that has created unwarranted fear in many patients. The absolute risk of ONJ is approximately 1 case per 100,000 person-years [39], and must be accurately balanced with the comparative significantly increased risk of fracture for postmenopausal women, which can be as high as 1 in 2 postmenopausal women. Most cases are reported in patients actively undergoing cancer treatment with intravenous dosing. The risk of ONJ may be increased when treatment duration is over 3 years [40].

Atypical femur fracture (AFF) from BPS usage is also rare, with the absolute risk between 2 and 78 cases per 100,000 person years [39]. AFFs are thought to be due to microfractures in the bone from prolonged BPS use and should be suspected when patients complain of upper thigh pain that is often bilateral for months preceding a fracture. A meta-analysis of three large randomized trials showed that BPS treatment for greater than 10 years carries a low and not statistically significant risk of atypical fractures [41], and the benefits of treatment overall far outweigh these risks. However, due to these small risks, treatment "holidays" (drug free intervals) for 1–2 years may be considered in patients after they have received treatment for 10 years on alendronate and 3 years for zoledronic acid.

Intravenous administration has also been associated with an acute phase response, in which flu like symptoms are reported within 72 h after infusion, often associated more frequently with the initial dose. This incidence and severity of symptoms tends to lessen with subsequent infusions and can be pre-treated with acetaminophen or ibuprofen [42].

Receptor activation of NK-kB (RANK) ligand plays an essential role in mediating resorption through osteoclast formation, function, and

Table 10.6 Summary of OP treatments

Agent	Dosing	Cost	Hip fracture risk reduction	Vertebral and non-vert. fracture risk reduction	Additional benefits	Risks
Antiresorptives						
Bisphosphonates						
Alendronate (Fosamax®)	Prevention: 5 mg daily/35 mg weekly Treatment: 10 daily or 70 mg weekly	generic $8/month	Yes	Yes	Numerous formulations Generic is inexpensive Bone specific, limited system side effects Long lasting effect	Abnormalities of the esophagus that delay emptying if oral formulations Avoid if have Hypocalcemia Avoid in renal insufficiency (<30 to 35 mL/min creatinine clearance)
Risedronate (Actonel®; Atelvia®)	Prevention/treatment: 5 mg daily/35 mg weekly		Yes	Yes		
Ibandronate (Boniva®)	Treatment: 150 mg orally monthly; intravenous 3 mg every 3 months		Yes	No		
Zoledronic acid (Reclast®)	Prevention: Intravenous 5 mg every 2 years. Treatment: Intravenous 5 mg yearly	$90/month				
Estrogens						
	Prevention: dose dependent effect on bone: 0.3, 0.45, 0.625 mg, 1 mg estradiol ± progestin (needed if uterus)	$17-190/month	Yes	Yes	Vasomotor symptom control GMS control Short half life	Timing hypothesis—increased risks if initiated ≥10 years PM Increased breast ca after 5 yrs. in EPT Increased VTE Increased CVA after 3 yrs. (although absolute risk is "rare" event)
Estradiol Menostar®	Prevention: Estradiol 0.014 mcg/day weekly transdermal					Requires cycled progestin for 2 weeks every 6-12 months
Estrogen Receptor Agonists and Antagonists (ERAAS)						
Raloxifene(Evista®)	1 Barrett-Connor E; et al. RUTH trial NEJM 2006 Jul 13;355(2):125-37. 2. Vogel VG; et al. Star trial. JAMA. 2006 Jun 21;295(23):2727-41. Epub 2006 Jun 5	$100/month			Reduces risk of invasive breast cancer Absolute RR 1.2 cases per 1000 women/year	VTE: Absolute risk increase 1.3 per 1000/year. [1] Death from stroke Absolute risk 7/1000/year. [2]

Estrogen-ERAAS combination						
Bazedoxifene + conjugated estrogen (Duavee®)						
Rank ligand inhibitor						
Need to list D enosumaub (Prolia)		$150/month	Yes	Yes	No adjustment needed for decreased renal or hepatic dysfunction	Small risk for osteonecrosis of the jaw (ONJ) Small risk for atypical femur fxs. (AFF) May want to avoid w/ immunosuppressants Avoid in hemodialysis and hypocalcemia
Miscellaneous						
Calcitonin (Miacalcin®)		$60/month	No	Vertebral only (no non-vert)	Short-term reduction of acute pain in patients sustaining a vertebral fracture	No longer recommended due to increased risk of malignancy
Anabolics						
Teriparatide (Forteo®)		$900/month	Unclear	Yes		Avoid if increased risk of osteosarcoma: - Paget's bone disease Prior radiation therapy of the skeleton Bone mets or bone malignancies Open epiphyses Renal or hepatic impairment Unexplained elevation in alkaline phosphatase Prior bone disease other than osteoporosis Preexisting hypercalcemia Hyperpara-thyroidism

survival [43]. Denosumab (Prolia®) is a human monoclonal antibody that directly blocks the RANK ligand pathway. In the FREEDOM trial, a randomized double-blinded placebo controlled 3 year multicenter study of women aged 60 to 80 with OP showed that denosumab increased BMD of the lumbar spine and total hip compared with placebo and significantly reduced the risk of new vertebral fractures by 68%, hip fractures by 40%, and non-vertebral fractures by 20% [44]. Denosumab was FDA approved in 2010 for the treatment of osteoporosis and is conveniently administered by subcutaneous route every 6 months. Denosumab is cleared hepatically, and thus can be used in patients with creatinine clearance of less than or equal to 35 mL/min.; however, caution should be taken to ensure that low BMD is not due to renal osteodystrophy rather than primary OP, as treatments differ. In the FREEDOM Extension trial, which extended another 5 years after the original FREEDOM trial, for a total treatment duration of 10 years, participants showed an even further increase in BMD at the lumbar spine and total hip at 7.7% and 4.0%, respectively. There were no adverse events reported with the longer duration of therapy. In the cross over group, there were two reported cases of ONJ, yet the drug was deemed to have a favorable risk/benefit profile and overall, 5 years of treatment in women with postmenopausal osteoporosis showed marked BMD improvement [43].

The group of *estrogen receptor agonists and antagonists (ERAAs, previously called SERMs)* are referred to as "designer" estrogens for their specific target tissue effect. ERAAs act directly on estrogen receptors exerting different outcomes in various tissues, allowing for the possibility to selectively stimulate or inhibit estrogen-like downstream effects in those target sites [45]. Although tamoxifen works as an estrogen agonist at the bone, fracture reductions did not reach statistical significance in the large Breast Cancer Prevention Trial [46], it is *not* FDA approved to treat OP.

Raloxifene, another second generation ERAAs (Evista®), has FDA approval for both the prevention and treatment of OP and for the reduction of invasive estrogen receptor (ER) positive breast cancer in women at high risk. In the Multiple Outcomes of Raloxifene (MORE) trial, a 30% relative risk reduction of new vertebral fracture was seen; however, no significant effect was seen on the reduction of hip or non-vertebral fractures [47, 48]. While there is an increased risk of mortality post stroke and VTE, pooled analysis of mortality data from the MORE, CORE, and RUTH trials found all-cause mortality was 10% lower in older postmenopausal women taking 60 mg of raloxifene daily compared to placebo [49].

A third generation ERAAs, bazedoxifene combined with conjugated estrogen (CE/BZE, Duavee®) gained FDA approval in 2013 for the treatment of moderate to severe VMS associated with menopause and also for the prevention of postmenopausal osteoporosis in women with an intact uterus. The SMART-1 trial showed the CE/BZE 0.45/20 mg and 0.625/20 mg showed significant increases from baseline in mean lumbar spine BMD and total hip BMD at 12 and 24 months compared to placebo [50]. At 24 months, the increase in BMD was even greater when compared to women treated with raloxifene [51]. A 7 year study of the use of bazedoxifene without CE found a significantly lower percentage of new vertebral fractures compared to placebo, however this effect was not seen for non-vertebral fractures [52].

For midlife women who are seeking treatment for VMS, GSM or a reduction in the risk of breast cancer, these morbidities can be weighed with the selection of choosing an ERAA that may also act in favor of the bones.

Calcitonin-salmon nasal spray (Miacalcin) has a short-term reduction of acute pain in patients sustaining a vertebral fracture. Results from the PROOF study, a large randomized trial, showed no significant decrease in non-vertebral fractures, only a statistically significant reduction in vertebral fractures [53]. However, a 2014 meta-analysis of 21 randomized controlled trials suggested a potential increased risk of overall cases of new malignancies in calcitonin-salmon treated patients compared to placebo (1.54 times greater with 95% CI: 1.06–2.23) [54]. With this new data the FDA has issued a new warning on its use, resulting in an abandonment of its regular use.

Parathyroid hormone (PTH) and terminal fragments of its amino acid structure have been shown to increase bone mass, increase bone strength, and reduce bone loss when given in a pulsatile manner [55] Teriparatide, (Forteo®) is the only anabolic agent currently available in the USA. It is a recombinant form of the 34 amino-terminal residues of human PTH as a daily 20 μg

subcutaneous injection [56] FDA approved for 2 years. Concerns over rat studies showing an increased risk of osteosarcoma, and two human cases detected by 2010, led to this 2 year recommendation. Teriparatide is thus not to be administered to postmenopausal women with hypercalcemia, bone metastases, disorders that predispose them to bone tumors such as Paget's disease, or those who received prior skeletal irradiation. Bone formation with teriparatide is rapid acting, and peaks at 6–12 months of therapy; however, its high price and route of administration can be prohibitive to some. While expense may be an issue, it has been shown that is likely cost effective for women with severe postmenopausal osteoporosis [57]. Current investigation of its use as oral, nasal, or transdermal preparations is underway.

Combining treatment options that may result in small additional increments in bone density is not usually recommended due to cost. In postmenopausal women with low bone mass, BMD improvements in the spine and hip with combined alendronate and HT were significantly greater than results for either treatment option alone at 8.3% vs 6.0% [58]. Combined risedronate and HT also have shown favorable, although modest, BMD effects compared with either agent alone [59]. It is unclear whether these increases in BMD result in better fracture protection. Combining antiresorptive agents with an anabolic agent such as teriparatide has been considered, but is typically done in a sequential manner.

As OP is a lifelong disease and fracture risk generally increases with age, the notion of treatment duration can be complex. The concept of initiating a drug "holiday" or interval cessation of an OP pharmacologic resulted due to the widespread use of BPS and the fears around potential side effects of atypical femur fractures (AFF). The exact mechanism behind AFF is not currently understood; however, theories propose that as BPS incorporate into bone for a longer duration of time (zoledronic acid and alendronate having the longest half lives in bone), that bone turnover may become over suppressed with a subsequent increased risk of stress fracture. From this proposed explanation, consideration has been explored around treatment intervals with breaks in therapy to allow for a partial "wash out" of the medication effects. This applies mostly to the BPS due to their prolonged half lives in bone. As the other non-BPS bone therapeutics have short half-lives, drug holidays are not necessary considerations in their use.

Several medications are in development as future therapies for OP. Cathepsin K inhibitors (catK) are a class of anti-catabolic agents that act to decrease bone resorption. Cathepsin K *(Odanacatib)* is a protease expressed in osteoclasts, and breaks down type 1 collagen in bone. Inhibitors such as odanacatib block this destruction and have been shown to increase BMD in postmenopausal women in a 2 year study [60]. While it has a strong safety profile, it has yet to show a significant reduction in fractures.

Lasofoxifene, another third generation ERAAS, has been studied in the PEARL study, at two doses of 0.25 and 0.5 mg orally in women with T scores of −2.5 or less at the femoral neck or spine. In this 3 year study both doses showed an increase in lumbar spine BMD by 3.0% and 3.1%, respectively, and increased femoral neck BMD by 2.9 and 3.0%, respectively, to placebo [61]. Importantly, both doses also reduced the risk of vertebral fractures by 31% and 42%, respectively, while non-vertebral fractures were significantly reduced by 22% with the higher dose. Both doses also reduced the risk of ER positive breast cancer by 81% and 49%, respectively.

10.5 Case Discussion

This patient underwent surgical menopause and is symptomatic. She should be started on HT for her VMS, and this therapy will also be beneficial for her bone strength. A DXA should be done now based on her personal and family history. She needs daily Vitamin D through supplements, calcium in her diet, and to add weight bearing activity. Another bone agent could be added based on her *T*-score.

10.6 Follow-up

Women who are treated for OP or low bone density need appropriate follow-up to monitor adherence and effectiveness. Fracture while on therapy can simply reflect a patient's underlying high fracture risk. Missed secondary causes and medication non-adherence, though, should be

Table 10.7 Recommended follow-up on pharmacologic therapy

Test	Frequency	What to measure
BMD* *Re-measure on-SAME MACHINE	Every 6 months- 1 year if on glucocorticosteroids Every 1 year if Hyperparathyroid Every 2 years (+) for patients with primary OP	Hip and spine Hip, spine *and* distal 1/3 radius Hip and spine
Bone resorption markers Bone formation markers	Baseline Within first 3–6 months after initiating tx. for OP: should decrease 30-50% Every 12 months when a patient is taken off of BPS therapy Bi-annually if tx. with Teriparatide	Serum: C-terminal telopeptide(CTX) Urine: N-terminal telopeptide (NTX) (Fasting 2nd morning void) Ser: Osteocalcin & P1NP should rise
Clearance	Yearly	Serum Cr/GFR estimate
25 OH vit D	Baseline Consider repeat based on initial value and ongoing adherence	Vit D 25 OH

*All serial repeat BMD should be done on the same machine so that there can be a valid comparison of increase in BMD, decrease in BMD or stable/no change. Once can NOT compare BMD values between machines

considered when new fractures and/or a loss of BMD occur despite therapy. BMD measurement with DXA, bone turnover markers (BTM) in certain circumstances, creatinine clearance and vit D 25 OH levels are all considered in the recommended follow-up on pharmacologic therapy (Table 10.7).

In women with unusual features and/or failure of therapy may also warrant referral to a specialist in metabolic bone disease.

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Male Infertility

Mark Gibson and Ahmad Hammoud

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11.1 Introduction

The male accounts for nearly half of known instances of infertility. Determining the prevalence of male infertility is hampered by lack of thresholds for normal and infertile measures in the semen analysis and other tests of sperm quality and function. Thorough evaluation of the male factor should be a part of the infertility and is essential for defining the course and content of a couple's care. This chapter identifies diagnoses as found in the history, by examination, and in the laboratory; each contributes valuable information. Pathophysiology and management for principal diagnoses are also presented. For many infertile men, attribution of cause for semen abnormalities is not possible. For them, and for men whose conditions are not amenable to specific therapy, intrauterine insemination (IUI) and assisted reproductive technology (ART) with intracytoplasmic spermatozoa injection (ICSI) offer pathways to fertility.

Clinical Case

A couple was referred for management of male infertility. They have been trying to conceive for the last 18th month unsuccessfully. The female partner is 28 years old with normal ovulation and patent fallopian tubes. The male partner is 32 years old without erectile dysfunction. Two semen analyses showed low sperm count and morphology. He had a negative medical history, normal physical exam and hormonal evaluation. Artificial insemination and/or artificial reproductive technologies (ART) were recommended.

11.2 History

11.2.1 Coital Function

Adequate frequency of intercourse and erectile function with ejaculation are essential. Semen quality may decline with daily ejaculation, leading to prescription for alternate day intercourse. However, many studies show better semen quality with daily or more frequent ejaculation [1–5]. Prescribed timing for intercourse can create dysfunction and marital stress related to on-demand performance [6]. Because of this, and because ovulation prediction may have up to a day of error, advice to have intercourse "every day or two" during the fertile portion of the cycle without reference to ovulation prediction may be helpful. It allows some spontaneity without compromise of the chance for optimal timing. Sexual dysfunction frequently accompanies infertility [7]. Men unable to achieve appropriate coital frequency and function should be evaluated for hypogonadism (▶ see Sect. 11.5.4) [8]. Findings will usually be normal and provision for marital/sexual counseling is then appropriate [9].

Ejaculatory dysfunction may be psychogenic, occurs after retroperitoneal node dissection, results from use of some medications, is common in men with diabetes, and is not possible for most men with spinal injury [10, 11]. Induction of ejaculation is often successful using high-amplitude vibratory stimulation, which is less stressful than electroejaculation, and allows for home use and home insemination for some men [12–16]. Induced ejaculation can be complicated by autonomic dysreflexia, so initial attempts should include monitoring for this complication, which can be blunted with the use of nifedipine [17]. Semen quality is often poor in men with spinal cord injury, so the principal benefit of induced ejaculation may be avoidance of testicular spermatozoa extraction (TESE) for ICSI. When azoospermia is found on an initial induced ejaculation, second attempts or use of other methods may yield semen with sperm sufficient for ART with ICSI [18–20].

11.2.2 Surgery, Injury, and Infection

Childhood surgery involving the reproductive tract and/or inguinal region can imply abnormalities resulting from defective androgen synthesis or action that may explain later impaired spermatogenesis. Alternatively, surgery may injure the ductal system. The assemblage of abnormal androgen-dependent development, genital malignancy, and impaired spermatogenesis is suggested to comprise "testicular dysgenesis syndrome" [21–24]. A history of injury, torsion, or vasectomy reversal may explain subsequent infertility. Genital tract infection may compromise semen quality, but does not cause infertility except in cases of post-infective

obstruction [11, 25–27]. Retroperitoneal node dissection compromises ejaculatory function, adding to the damage to germinal epithelium from chemotherapy [12].

11.2.3 Cytotoxic Medications

Chemotherapy for malignant or rheumatologic disease frequently causes azoospermia. This effect depends on the agents used, their doses, whether there was also radiation used [28–31]. Effects may be transient [29]. There is no evidence that these exposures affect health of offspring [30]. Resources for fertility preservation should be provided for men anticipating cytotoxic therapy [32, 33].

11.2.4 Lifestyle

Evidence for adverse effects of tobacco and alcohol on fertility is mixed [34–40]. Studies have not linked recreational drugs to infertility [39]. Obesity is associated with poor semen and reduced fertility in some, but not all studies [39, 41–45]. Genetic variation in hormone metabolism may explain a portion of the variability in obesity's effect on semen quality [46]. Effects of obesity may be mediated by coexisting disturbances in insulin resistance, leptin, systemic inflammation, sleep apnea, and testicular thermoregulation and expressed through altered epigenetic controls [43, 47, 48].

Reversibility of obesity's effect has not been shown, and rapid weight loss may harm semen quality [48]. High intensity endurance training alters hormone levels and some semen measures but has not been shown to cause infertility [49–51]. Most medications have not been investigated for effects on human male fertility. Agents of concern for semen quality or ejaculation include serotonin reuptake inhibitors, anti alpha-adrenergic antihypertensives, and sulfasalazine [11, 44, 52]. Anabolic steroid abuse and testosterone replacement reversibly cause fertility impairment [53, 54]. Nutrition is important for spermatogenesis; dietary elements of interest include selenium, dietary antioxidants, zinc, folate, and folate indirectly via its metabolism [55–61]. Clear linkage between adequacy of these in the diet and infertility has not been shown and limited studies of supplementation have not shown benefit [60, 62].

11.2.5 Environmental Exposures

Air pollutants and heavy metal exposure may impair semen quality but evidence for causation of infertility is weak [63–65]. Recent interest has focused on organochlorines, dioxins, phthalates, phytoestrogens, and chemical mixtures as found in pesticides and tobacco smoke because of mechanistic hypotheses for endocrine disruption and some demonstrated effects on semen quality [66, 67]. Effects are found for PCBs inconsistently, and DDT exposure appears to have minimal effect on semen parameters [68–70]. Pesticide exposure may affect semen quality and fecundability [71]. Overall, however, the literature is not consistent regarding effects of man-made xenobiotics [72–74].

11.3 Physical Examination

11.3.1 Clinical Signs of Hypogonadism

Clinical hypogonadism may be suspected with findings of decreased muscle mass, decreased sexual hair, and increased subcutaneous tissue but there is wide normal variation in expression of sexually dimorphic characteristics.

11.3.2 Examination of Scrotal Contents

Testis size reflects aggregate seminiferous apparatus, not endocrine tissue, and is normal at 30 cc, or a length of 4 cm. Testes smaller than this may reflect decreased sperm production, especially when they exhibit less-than-normal turgor. The vas deferens should be palpable. Absence is usually bilateral, with normal testis findings and azoospermia, and is usually a result of mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) complex. Before undertaking ART, partners should be screened to exclude abnormal CFTR genes that would place offspring at risk for cystic fibrosis. Unilateral absence of the vas deferens may indicate CFTR mutations or renal abnormalities and calls for genetic evaluation and urinary tract imaging [75].

11.3.3 Varicocele

Varicocele is posturally expressed and more often left sided. Clinically inevident varicocele is of questionable significance, though can be identified with ultrasound, which may be useful when findings are equivocal. Implications of varicocele for fertility (and response to corrective treatment) are proportional to size of the lesion, but a standard classification is lacking [76]. It is common—the prevalence generally given is 15%—and though more prevalent among infertile men is also commonly innocent. Men with varicocele may have normal semen and fertility or may have infertility, reduced testicular volume, and severely abnormal semen [77, 78]. Proposed mechanisms for infertility include altered testicular thermoregulation, increased seminal plasma reactive oxygen species, and compromised testosterone production [79]. Varicocele effects on semen may be progressive, which argues for surgical treatment for men with normal semen except that this effect is neither consistent nor predictable [80, 81]. Treatment of varicocele is discussed below, under treatment of oligoasthenoteratospermia (OAT).

The Role of Ultrasound in Male Infertility

A role of ultrasound in the diagnosis of male infertility is slowly emerging. Both gray-scale and color Doppler ultrasonography are becoming useful tools in the assessment of male genital tract disorders. Ultrasound can extend the physical exam and explore in more detail the genital area through both the scrotal and trans-rectal approaches.

Scrotal ultrasound is typically performed with patient lying in dorsal supine position, using a high frequency transducer (7–12 MHz) of adequate length to encompass the longitudinal axis of the testicle. Normal ultrasonographic testicular volume is thought to be 12–15 mL. [82] The ultrasound of the scrotal region can also look at parameters such as testicular texture, lesions, and vascularization, the presence of varicocele, epididymal diameter, texture, and vascularization, and the presence of vas deferens. [83] Trans-rectal ultrasound can help in evaluating prostate volume, and texture, and presence of median prostate cysts, ejaculatory ducts cysts, and seminal vesicle volumes. [83]

Clinically, scrotal ultrasound can assist in the diagnosis of absence of vas deferens. It also has a role in confirming the diagnosis of a clinically palpable varicocele. Only patients with palpable varicoceles are thought to benefit from surgical intervention (see below). The role of surgical correction of varicocele diagnosed on ultrasound but not clinically palpable is more controversial. Trans-rectal ultrasound can establish the diagnosis of ejaculatory duct obstruction or CBAVD. Male genital tract color Doppler ultrasound either scrotally and trans-rectally can play an important role in diagnosis of obstructive azoospermia. However, both approaches have more specificity than sensitivity for this diagnosis, indicating that ultrasound is more suitable for exclusion rather diagnosis of obstructive azoospermia. Its current role for other diagnoses of male infertility is limited. [84]

11.4 The Laboratory

11.4.1 Semen Analysis

Pioneering work by Macleod established norms for semen measures based on time required to achieve pregnancy in subjects with currently pregnant partners [82, 83]. Standards for semen analysis were promulgated by the World Health Organization (WHO) in 1987, 1992, 1999, and most recently in 2010 [84]. Yet, excepting the most severely abnormal specimens, semen analysis does not clearly distinguish men with normal fertility from men with infertility [85, 86]. Moreover, because infertility often is multifactorial, and semen exhibits large intra-individual variation, assigning a precise contribution of semen findings to a couple's infertility is usually not feasible [87, 88]. The WHO guidelines published in 2010 are aggregated from findings among fertile men in five studies in eight countries (Table 11.1) [84, 85]. Cutoffs stipulated that 95% of the values were normal, with a one-sided distribution that assumed upper end values do not represent disease. Values below the fifth percentile for these fertile subjects were designated abnormal. The new guidelines simplify quantification of motility from designation of grades to "progressive" or "nonprogressive." Morphology is described by "strict" (or "Tygerberg") criteria and poses some difficulties, as this important measure is subjectively

Table 11.1 Lower reference limits (fifth percentile) and their 95% confidence intervals for semen parameters and 50th percentile values from fertile men whose partners had a time-to-pregnancy of 12 months or less

	N	5th percentile (95% CI)	50th percentile
Semen volume (mL)	1941	1.5 (1.4–1.7)	3.7
Sperm concentration (106/mL)	1859	15 (12–16)	73
Total number (106/ejaculate)	1859	39 (33–46)	255
Total motility (PR, NP, %)[a]	1781	40 (38–42)	61
Progressive motility (PR, %)[a]	1780	32 (31–34)	55
Normal forms (%)	1851	4 (3.0–4.0)	15
Vitality (%)	428	58 (55–63)	79

Adapted from [85]
[a]PR, progressive motility (WHO, 1999 grades a, b); NP, nonprogressive motility (WHO, 1999 grade c)

determined and difficult to standardize across laboratories [82, 89–92]. The new, often lower, cutoffs for normal do not solve the problem of finding values denoting infertility, and have raised concerns regarding clinical application [93–96]. Nonetheless, semen analysis can be used effectively when its limitations are understood [97]. Fertility is a continuously varying characteristic such that reference values cannot segregate absolute fertility from absolute infertility and instead lie within a zone of ill-defined subfertility [86, 92]. Very importantly, decisions to treat with the assumption of infertility on the part of the male (e.g., varicocelectomy, decision for ART/ICSI) should not be made on the basis of results of a single semen analysis, owing to large variations in an individual's semen measures over time [87, 88].

11.4.2 Morphology: A Key Measure of Fertility

Efforts to correlate semen findings with fertility consistently highlight the importance of sperm morphology [83, 86]. Refined standards for morphology, in part based on mucus penetrating capability, led to elaboration of the strict criteria [98]. Morphology by strict criteria varies independently of sperm density and motility and independently predicts success with IUI and ART [90, 99–103]. Strict morphology assessment correlates only roughly with tests of function (e.g., hamster egg penetration test) and has replaced these as predictors of fertilization success in vitro or requirement for ICSI in most ART programs.

11.4.3 Other Measures for Sperm

The advent of ART and ICSI gave impetus to the quest for measures predicting ability to fertilize oocytes and to produce successful embryos. Efforts to this end centered on two principal arenas: first, functional tests of the binding of sperm to the oocyte with execution of the acrosome reaction and second, the integrity of sperm DNA. Examples of functional tests include the hamster egg penetration test, hemizona binding assay, and the zona-induced acrosome reaction (ZIAR) [90, 101, 104–106]. Results of such tests show correlation with morphology, particularly of the acrosome, but are not inconsistently aligned with measures of strict morphology [98, 107, 108]. Sperm function tests remain poorly validated and standardized, their clinical relevance is uncertain, and they are not part of the routine fertility assessment [97, 109].

Fragmentation of DNA is present to varying degrees in sperm, and its extent can be assessed by techniques such as the flow cytometry-based sperm chromatin structure assays (SCSA), terminal deoxynucleotidyl transferase-mediated fluorescein-dUTP nick-end labeling (TUNEL), the single-cell gel electrophoresis assay (also

known as COMET), among several [110–112]. The correlation of findings from these tests with semen measures is often poor, and their ability to predict natural fertility, IUI success, and ART outcomes is variable [110, 112–115]. DNA damage is increased in the presence of varicocele and improves after repair [116]. Measures for DNA damage may be markers for toxicant exposure and oxidative injury to sperm, inflammation, or exposure to certain medications [67, 117–120]. Because of differing results among different tests, lack of standardization, and conflicted data as to utility routine use of DNA fragmentation testing is not justified by current evidence [94, 112, 121, 122]. Emerging work suggests that epigenetic alterations and defects in DNA packaging (protamines, histones) may reflect abnormal spermatogenesis or constitute primary disorders of fertility [123–127].

Special Findings in the Evaluation of Semen

Agglutination of sperm is detected and graded on wet-mount examination. If extensive and associated with a history testicular trauma or vasectomy reversal, it suggests the presence of antisperm antibodies. Evidence suggesting antibodies should be substantiated with specific testing, most commonly with the immunobead test [127–129]. Antibodies may be directed at a variety of antigens on different regions of spermatozoa, with differing consequences for fertility [130, 131]. Pregnancy can occur spontaneously in the presence of antisperm antibodies, but IUI has been used successfully when it does not [132]. Rapid dilution of semen upon collection for IUI may be beneficial [133]. ART has also been used as treatment for antisperm antibodies [132]. The addition of ICSI addresses concern for interference of antibodies with fertilization, but is currently without evidence for clinical benefit [134, 135].

Absent motility occurring with normal measures of vitality indicates one of several ultrastructural defects affecting ciliary function in primary ciliary dyskinesia syndrome. With chronic respiratory infection and situs inversus, the diagnosis of Kartagener's syndrome can be made [136–138]. These disorders are autosomal recessive gene defects affecting the several proteins critical for normal ciliary ultrastructure and movement. Chronic/recurrent respiratory function in these men is due to impaired mucociliary function. Evaluation of sperm tail ultrastructure by electron microscopy can confirm diagnosis, but the classic findings on semen analysis with a typical history of respiratory disease and clinical findings for situs inversus are sufficient for clinical diagnosis. Pregnancy is achieved with ART and ICSI [139, 140].

Absent or minimal ejaculate after orgasmic masturbation suggests retrograde ejaculation or ejaculatory duct obstruction. Distinction between the two depends upon post-ejaculation urine analysis, which will show abnormally elevated sperm numbers after retrograde ejaculation. Causes include anatomic disruptions from prostate surgery, and neurologic dysfunction related to diabetes, demyelinating disorders, or sequelae of retroperitoneal node dissection. Pharmacologic disruption of the ejaculatory signaling pathway may occur with alpha-adrenergic blockers used for urine flow with prostatic hyperplasia. Medical treatments using alpha sympathomimetic agents (ephedrine, phenylephrine) or tricyclic antidepressants may help in some instances [141, 142]. More often, harvesting of sperm from post-ejaculatory urine that has been alkalinized by bicarbonate ingestion is done so that IUI or ART may be undertaken [143–145].

11.5 Clinical Categories of Severe Semen Abnormalities

11.5.1 Oligoasthenoteratospermia

Abnormalities of semen among infertile males are rarely limited to a single parameter and commonly present as subnormal values for sperm density, motility, and normal morphology. This constellation is often termed OAT, or OAT syndrome. OAT, when severe, should be evaluated for genetic, chromosomal, and endocrine origins as described below for the evaluation of azoospermia. OAT is often idiopathic [146]. Two groups of OAT have been described, one affected primarily in density and motility and the other in sperm morphology, with the latter showing higher correlation with the presence of sperm aneuploidy [147, 148]. OAT is associated with sperm aneuploidy in many studies, with the rate of aneuploidy found in normal and abnormally formed sperm from men with OAT being similar to that

in abnormally formed sperm from normal semen specimens [149–152]. Elevated frequencies of aneuploidy in sperm from men with OAT likely explain the increased aneuploidy in embryos created from their sperm using ICSI [153]. Other sperm abnormalities in OAT include increased DNA fragmentation, mitochondrial abnormalities, epigenetic alterations, and disordered chromatin organization [154–156]. Management of infertility due to OAT includes consideration of donor insemination, ART with ICSI, and attempts at medical therapy (discussed below). Surgical or embolic treatment for varicocele for men with OAT may be appropriate.

11.5.2 Treatment of Varicocele

Treatment of varicocele with surgery or embolization is performed for discomfort and for infertility. Additionally, when low serum testosterone levels are present, they may be corrected with surgical treatment [78, 157]. Improvement in semen varies widely after varicocelectomy, and may be more likely in younger individuals. [158] Even azoospermic individuals may show return of sperm to the ejaculate after surgery [158]. Surgical techniques have advanced in recent decades to reduce unintended vascular or lymphatic injury [159, 160].

Despite beneficial effects of varicocelectomy on semen quality, endocrine function, and pain, its role as a fertility treatment is controversial; analyses of literature using live birth as the outcome of interest yields conflicting conclusions [159, 161–163]. The advent of ART and ICSI for male factor infertility adds complexity to knowing varicocelectomy's role [163, 164]. Pregnancy as a result of varicocelectomy may occur over an extended window of time (as is true for pregnancy among normally fertile couples) and, therefore, youth of a couple, and lack of urgency would favor an attempt at correcting infertility with varicocelectomy, prior to advancement of care to ART. The presence of pain or hypoandrogenism would favor surgery, as would religious or other barriers to ART. Alternatively, progression to ART, without varicocele correction, may be preferred for couples not comfortable accepting its uncertain benefit and for couples for whom time to pregnancy is a concern, especially where female age is a factor.

11.5.3 Azoospermia

Azoospermia requires examination of centrifuged semen for confirmation. It may be due to obstruction, inadequate gonadotropins, or defective germinal epithelium. The latter two comprise the two causes for nonobstructive azoospermia (NOA), and the laboratory plays an important role in differentiating between them. The nature of the semen is helpful in distinguishing the causes of azoospermia [165]. Smaller semen volumes and greater acidity without fructose suggest absent seminal vesicles (CBAVD), which can be confirmed on scrotal examination. Smaller semen volumes will also be seen in severe hypogonadism. Normal semen volumes with normal pH suggest primary testicular (germinal) defects or ductal obstruction at the level of the vas or epididymis. Surgery for cryptorchidism in childhood is a risk factor for NOA and these occurring together may implicate the putative "testicular dysgenesis syndrome," warranting careful testicular examination for mass [21, 24, 166]. Surgery for cryptorchidism or for torsion that harms the contralateral ducts or history of prior epididymitis may account for proximal ductal obstruction. Exogenous androgen use may profoundly suppress spermatogenesis [53, 54]. Examination of the scrotal contents helps differentiation of ductal disorders (the presence of normal testicular volume and turgor, palpable ductal abnormalities) from primary testicular and endocrine control disorders (small testes of reduced turgor). Congenital bilateral absence of the vas is evident from palpation and has implications for CFTR mutation screening in the event of ART.

11.5.4 Endocrine Evaluation

The principal value of endocrine testing in evaluation of azoospermia lies in distinguishing testicular from central causes of NOA. It is rarely helpful in the evaluation of OAT or sexual dysfunction. Some degree of elevation of follicle-stimulating hormone (FSH) levels is expected with primary testicular disorders, and though cutoff values for this are elusive, such findings usefully contrast with the very low FSH, luteinizing hormone (LH), and testosterone levels seen in hypogonadotropic disorders [167].

Principal hypogonadotropic disorders are either congenital (frequently as classic Kallman's syndrome, which includes anosmia), in which impaired pubertal development may have led to androgen replacement, or acquired disorders, in which the principal concern is pituitary or juxtapituitary neoplasm [168–170]. Therefore, when adult-onset hypogonadotropic hypogonadism is diagnosed, the serum prolactin levels should be determined; CNS and pituitary imaging should be performed if it is elevated, or if there is evidence for global pituitary insufficiency (central hypothyroidism, hypoadrenalism, or diabetes insipidus) or symptoms of intracranial mass. Treatment of endocrine disorders for fertility restoration is discussed below.

11.5.5 Chromosomal and Genetic Evaluation

NOA of testicular origin and severe OAT warrant genetic and chromosomal evaluation, especially prior to ART. Identifiable chromosomal and genetic abnormalities are common among men requiring ICSI [171–174]. Five percent of ICSI patients exhibit chromosome errors, and these involve the sex chromosomes in approximately two-thirds of instances. Frequency of chromosomal errors increases with the severity of semen impairment, reaching 10% or greater among men with the most profound deficits in sperm density [171, 175]. Y-chromosomal microdeletion is roughly as common as chromosomal error in this population and also most prevalent among men with the greatest depression of spermatogenesis [171, 172].

Sex chromosome aberrations are the most frequent of the few chromosome abnormalities compatible with adult life in men. Klinefelter syndrome (XXY and mosaics) and XYY syndrome both include infertility and each occurs in one to two per 1000 births [173, 174, 176]. Nonmosaic Klinefelter's syndrome is common among azoospermic individuals, and men mosaic for the disorder frequently present with abnormal semen [175]. Klinefelter's patients have harvestable sperm with TESE, more often than not. Testicular volume and hormone levels are of limited utility in predicting TESE success [167, 177, 178]. Because spermatogenesis may be focal, microdissection may provide the best TESE success rate [177, 179]. Fluorescence in situ hybridization (FISH) to exclude embryonic aneuploidy should be considered if ART/ICSI is undertaken for Klinefelter's syndrome [180]. Autosomal-balanced translocations (and the Robertsonian translocation form of these) are associated with infertility, recurrent abortion, and rarely, offspring with deficits owed to unbalanced chromosomes [173–175]. Effects of autosomal translocations and inversions arise through disruption of normal meiotic bivalents such that azoospermia, due to meiotic arrest, or oligospermia occurs. Interchromosomal effects, whereby other, normal chromosomes are collaterally damaged during meiotic errors, add to the reproductive morbidity of autosomal chromosomal rearrangements [181]. Frequencies of sperm aneuploidy, likelihood of embryonic aneuploidy, and successful reproduction vary widely according to the defect present [173, 182].

High percentages of embryos from men with structural rearrangements have aneuploidy, and preimplantation genetic diagnosis (PGD) can increase the likelihood of transferring normal embryos [183]. It is most clearly of use in cases of recurrent abortion, in which ART/PGD may shorten the time to implantation of a normal embryo, or in the uncommon cases in which abnormal offspring with unbalanced chromosomal complement have been born [184]. PGD technologies will be transformed by the advent of emerging array technologies [173].

It is likely that genetic lesions often explain severe male infertility, but only a few are described [185, 186]. Microdeletions in the AZF region of the Y-chromosome long arm have been extensively studied. A prevalence of 7.4% among infertile men is estimated, and their likelihood is proportional to the severity of spermatogenic abnormality. Deletions in the various AZF subregions ("a," "b," or "c") or combinations of them occur with differing frequencies and with differing implications for the degree of impairment of spermatogenesis, and the likelihood of retrievable sperm if there is NOA. Large areas of deletion and deletions involving the a and b subregions are associated with failure to retrieve sperm on TESE [180, 185, 187]. Because of this, testing for AZF deletion is advisable before attempting TESE for NOA. Mutations with severe functional consequence for the androgen receptor lead to infertility and intersex conditions and lesser lesions to infertility alone [188–190]. Such mutations were

found in 1% of a large series of men with severe oligospermia undergoing ICSI [172].

Identification of chromosomal or genetic causes for male infertility thus has implications for health of embryos and offspring, and can predict TESE success for NOA. Using results of genetic and chromosomal evaluation of the male to provide counseling about pregnancy likelihood and outcomes is an important element of care for couples treated for severe male factor infertility [171–173, 187, 191].

11.5.6 Treatment of Azoospermia and OAT

Treatments available for azoospermic disorders include donor insemination in all cases, surgical repair for some ductal obstructions, and ART with TESE and ICSI in selected instances [192–194]. Genetic and chromosome evaluation should be encouraged prior to ART for non-ductal azoospermia (see above). CBAVD signals a high likelihood of CFTR mutation, and ART care should always include screening for these in the female partner prior to ART. When azoospermia is associated with varicocele, treatment may restore sperm to the ejaculate of some patients [158, 195, 196]. Administration of gonadotropins can be effective as sole therapy for hypogonadotropic disorders or to provide ejaculated sperm for ICSI. Fertility can be restored with dopamine agonists for most men with pituitary tumors; when treated surgically, gonadotropins are usually required (see below).

11.5.7 Medical Regimens

Medical therapy for male infertility falls into three categories: replacement of deficient gonadotropins for men with hypogonadism of central origin, empiric direct or indirect augmentation of gonadotropins for men with unexplained infertility, and use of nutritionals and supplements.

11.5.8 Hypogonadal Males with Central Deficiencies of Gonadotropins

Induction of spermatogenesis in constitutional hypogonadotropic hypogonadism, including patients with anosmia (Kallman's syndrome), can be accomplished with administration of pulsatile gonadotropin-releasing hormone (GnRH), which precisely targets the pathophysiology, but is cumbersome [197]. Fertility in Kallman's syndrome and idiopathic or postsurgical hypogonadotropic states is often achievable with administration of human chorionic gonadotropin (hCG) alone, typically in doses of 1500–2000 IU twice weekly, but many patients will require co-administration of FSH [198, 199]. When required, FSH doses as low as 150–225 IU weekly may be sufficient [200]. Pregnancies often occur once there are sperm densities that are usually considered oligospermic [199].

Surgical management of prolactinomas in men is complicated by a high rate of persistent hypogonadotropic hypogonadism and recurrent hyperprolactinemia such that replacement gonadotropins are still necessary for fertility [201]. Medical therapy has emerged as a preferable course for most cases. Treatment with the dopamine agonist Cabergoline allows for regression of lesion size, normalization of the hypothalamic–pituitary–testicular axis, normalization of androgens, and restoration of spermatogenesis in a majority of instances, including cases of large prolactinomas [169, 170, 202].

11.5.9 Empiric Therapies for Idiopathic OAT

There is a limited literature supporting a variety of empiric therapies for unexplained male infertility [203]. These treatments presume etiologies such as minimally defective gonadotropin secretion, oxidative insult, or nutritional deficiency. Administration of gonadotropins for men with apparently intact hypothalamic–pituitary–gonadal axes and poor semen quality is supported by limited trial data [204, 205]. Indirect enhancement of gonadotropin secretion with antiestrogens (tamoxifen citrate and clomiphene citrate) is less cumbersome and costlier than gonadotropin administration and is also supported by limited evidence [204, 206–209]. Use of aromatase inhibitors has also shown some promise, especially for men with low ratios of circulating testosterone to estradiol [210, 211]. Among supplements, zinc and folate may improve semen parameters [212]. Studies of carnitines and of antioxidants suggest possible benefits [209, 213–215]. Evidence for these several putative therapies for unexplained

male infertility is hampered by high intra-individual variation in semen values, limited expression of results in terms of pregnancies, and the likely heterogeneous nature of underlying causes. None of these is adequately supported by high-quality evidence [216]. Large and carefully conducted clinical trials for the treatment of idiopathic OAT that utilize pregnancy as the outcome are needed [146].

11.5.10 Intrauterine Insemination

IUI is widely employed for infertility due to mild or moderate male factor or unknown cause. Often the latter, may have undiagnosed mild male factor. The rationale for IUI is based on several conjectures, including the bypass a hostile vaginal and or cervical environment, reducing the distance for sperm transport, selection of the most fertile sperm, concentrating fertile sperm at the site competition for fertilization, reducing the concentration of spermatotoxic molecules in the seminal fluid (capacitation inhibitors, free radical, etc.), and improved timing of the ovum–sperm exposure. IUI require that the female has spontaneous or inducible ovulation, and has normal anatomy, including normal uterine cavity and patent fallopian tubes. Timing IUI is determined by home kits for detection of the LH surge, or by a triggering injection of hCG given when follicle diameters reach at least 18–20 mm. If hCG is used, artificial insemination is timed 32–36 h after the injection. Success rates are not affected by whether the endogenous LH surge or hCG administration is used for timing [217]. Frequent intercourse through midcycle appears preferable to prescribed abstinence prior to collection of the specimen for IUI [1, 2, 4]. Semen is prepared for insemination using one of several aimed at selecting the most fertile pool of sperm and, importantly, to separate sperm from seminal prostaglandins which can cause painful contractions. Density gradient preparation is commonly used, although there is no evidence of the superiority of any of the sperm preparation techniques [218].

The prepared sperm is typically concentrated to a volume of ½ to 1 cc and injected into the uterine cavity gently, using a sterile catheter passed through the cervical canal after wiping the cervix free of secretions or excess mucus. Triggering of upper reproductive tract infection with IUI is a rare complication. IUI shows a higher pregnancy rate when compared to intracervical insemination in couples with unexplained infertility but may not be superior to timed intercourse when done without superovulation [219, 220]. Although superovulation adds efficacy to IUI, and pregnancy rates show some proportionality to numbers of maturing follicles, this must be balanced against significant risks for high-order multiple pregnancy [219, 221–223]. Double inseminations have been proposed to increase success. However, most studies show little evidence of the benefit of this maneuver, which increases the cost and complexity of IUI considerably [224–227]. IUI success depends on female age and quality of semen. Older women fare poorly, and pregnancies are uncommon if they are older than 40 [228]. Pregnancy success is a function of total motile sperm in the insemination; a preparation that contains 5 million motile sperm appears to be the threshold for benefit of IUI, although preparations with fewer sperm may rarely yield pregnancy [223, 229, 230]. Artificial insemination is typically attempted for 3–4 cycles; series do not show a significant increase in cumulative pregnancies beyond that [223]. A recent critical review found a limited number of adequately conducted trials, few subjects overall for evaluation, and thus limited evidence for a benefit for IUI vs. timed intercourse [220]. It is likely that couples vary in the degree to which IUI might benefit them, and that substantial benefit for some couples, and little benefit for others, underlies the generally low statistics for IUI outcomes. A trial of IUI is often selected in hopes that success will obviate the need to progress to the more invasive and costly undertaking of ART.

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Female Infertility

Hope Y. Yu, Roxanne Vrees, and Gary N. Frishman

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12.1 Introduction

Infertility may be narrowly defined as 1 year of unprotected intercourse without successful conception. Increasing recognition is being given to the impact of a women's age, and it is recommended to begin an evaluation after only 6 months in women over 35 years [1]. It also makes sense to begin treatment immediately when there is a known significant issue such as amenorrhea associated with polycystic ovarian syndrome (PCOS), recognized azoospermia following cancer treatment, etc. Based on observational data, infertility affects up to 15% of all couples [2, 3]. Infertility can be further classified as primary and secondary. Primary infertility is described in a patient who has never been pregnant. In contrast, secondary infertility is seen in a patient with a previous history of a pregnancy regardless of outcome, i.e., spontaneous abortion, ectopic pregnancy, still birth, or live birth. From the perspective of evaluation, taking into account insurance coverage issues, consideration should be given to both defining infertility and initiating an evaluation based on the lack of delivery rather than lack of conception. As such, a 36-year-old woman who conceived twice in the past year but miscarried without a delivery should be evaluated. The Centers for Disease Control and Prevention estimates that 6.7 million women were either unable to conceive or carry a baby to term and that 7.4 million women had, at some point, used fertility services [4].

12

Clinical Case

A 35-year-old woman with a 32-year-old male partner presents with inability to conceive for 18 months. She has regular cycles, conceived once 3 years ago but miscarried and now has been using luteinizing hormone (LH) ovulation predictor kits which demonstrate clear signs of ovulation. She was evaluated by her primary care provider 6 months ago and was told that she will probably get pregnant and should try to take a more relaxing attitude. In talking to her, she confides that her infertility and the associated stress have been affecting both her work and marriage.

12.2 Diagnostic Criteria

As noted above, the inability to carry a pregnancy to term after 1 year in a younger woman or for 6 months in women over 35 years of age is a working definition of infertility. There are many causes, with actual percentages ranging widely across different studies. The Centers for Disease Control and Prevention reported the following statistics on primary etiologies in 2013: male factor (33%), unexplained infertility (13%), diminished ovarian reserve (32%), tubal factor (13%), ovulatory dysfunction (14%), endometriosis (9%), uterine (5%), multiple factors (12%), and other (15%) [5]. Of note, the percentage of couples without a diagnosis (unexplained infertility) has not significantly changed over time.

12.3 Initial Evaluation of the Infertile Couple

A primary focus on the initial evaluation of the infertile couple is identifying potential etiologies and, ideally, modifiable factors that can improve the chances of a couple establishing a successful pregnancy. As with all new patients, a comprehensive history and physical examination should be performed. However, the initial office visit is also an opportunity to establish a sense of trust and collaboration with the couple in what is usually already a stressful situation. In addition to a discussion regarding the medical approach and plan, a review and provision of resources for the emotional aspects associated with infertility should be routinely included.

12.3.1 History

The information gathered from a thorough medical history will enable the clinician to narrow down the wide range of potential etiologies and may permit a more targeted initial evaluation and treatment plan.

Demographics

The age of a female patient can have a significant influence on her fertility. Data have suggested that fertility in women peaks between ages 20 and 24 [6, 7], remains relatively stable until approximately

ages 30–32 at which time it begins to decline progressively [8, 9]. This decline accelerates significantly after age 40. Women at the age of 20 have a fecundity rate of approximately 20% per cycle, considered to be the peak fecundity rate. Subsequently, the chance of conceiving any given month decreases to 18.4–19.2% in women ages 25–29; 16.2–17% by ages 30–34; 10.8–14.8% by ages 35–39; and 1% by ages 40–45 [10, 11].

Gynecological History

The menstrual history is a crucial aspect of the gynecological history as it can not only clarify a patient's ovulatory pattern, which might indicate a thyroid, prolactin or other hormonal etiology, but can also provide useful information about risk factors for other conditions such as endometriosis. Furthermore, characteristics such as shortened cycles might be a potential indicator for diminished ovarian reserve. Details about the following should be obtained:

- Age of onset of menses
- Development of secondary sexual characteristics including breast (thelarche), pubic hair (pubarche), and axillary hair and the prepubertal growth spurt (adrenarche).
- Menstrual cycle characteristics including duration, flow, mid-cycle spotting, pre-menstrual symptoms, and changes from previous norm (including shortening of the normal cycle length),
- Symptoms of endometriosis such as dysmenorrhea and dyspareunia

Other aspects of the gynecological history that are critical include:

- History of sexually transmitted diseases
- Previous methods of contraception
- Previous abnormal Pap smears and any associated interventions
- History of endometriosis, fibroids, ovarian cysts, and any associated interventions

Obstetrical History

The following should be reviewed:

- Gravity, parity, pregnancy outcomes, and any associated complications
- Interval to conception with any previous pregnancy including whether fertility drugs or other interventions were used and whether the pregnancy was with a prior partner
- How each delivery was accomplished (vaginal or cesarean) along with indications and any complications (e.g. retained placenta, post-partum endometritis, etc.) that might contribute to infertility such as Asherman's syndrome.
- Treatment of other pregnancies including whether D&Cs were used for miscarriages, laparoscopy for ectopic pregnancies, etc. Were there any complications or issues such as uterine perforation, infection, cervical stenosis, etc. that might play a role in their infertility? If surgery was done, what were the intra-operative findings?

Medical History

A thorough history of the patient's as well as the partner's past and present medical conditions should be collected including previous hospitalizations, medications, allergies, and history of communicable diseases. Any hormonal disorders, including thyroid and prolactin, should be excluded given their potential impact on fertility. The preconception period is also an ideal time to maximize the health of the patient during pregnancy by optimizing any medical conditions such as diabetes or high blood pressure. Likewise, if the patient has a medical condition which is genetically transmitted, it is important to screen her partner and to consider genetic counseling prior to attempts at conception. Immunity status for Rubella and Varicella should be obtained with vaccination performed prior to conceiving if indicated.

Surgical History

Surgical procedures should be reviewed including abdominal surgeries, such as colorectal surgeries for inflammatory bowel disease, which can lead to adhesive disease that can affect future fertility [12]. As noted previously, intrauterine procedures such as curettage for spontaneous abortion can lead to adhesions and Asherman's syndrome [13, 14]. Even information about minor, prior non-gynecologic surgeries such as wisdom teeth can be helpful as it provides information about the patient's response to anesthesia.

Family History

A thorough family history should be collected, specifically focusing on (1) a history of subfertility in parents and siblings, (2) age of menopause

Table 12.1 Adapted from ACOG Preconception Carrier Screening FAQ [15]

Ethnic group	Disorder
Ashkenazi Jews	Cystic fibrosis
	Tay-Sachs disease
	Canavan disease
	Familial dysautonomia
Non-Hispanic Whites	Cystic fibrosis
African American, Mediterranean, Southeast Asian populations	Sickle cell anemia, thalassemias

in the patient's mother and if surgically induced, the indication for surgery, and (3) heritable diseases including both medical conditions and birth defects. Populations at specific risk for genetic disease should be appropriately screened at the time of the initial evaluation per recommendations made by the American College of Obstetricians and Gynecologists (Table 12.1). Of note, pre-conception testing is evolving from testing just for specific conditions such as cystic fibrosis or sickle cell to "pan-ethnic" carrier screening. Advancements in technologies now permit the rapid testing for multiple disorders with tests literally screening for hundreds of conditions without regard for ethnicity or risk factors. Major advantages of this approach include the ease of a simple blood or saliva test which may be less expensive than targeted testing and a comprehensive approach to identifying previously unscreened heritable conditions. Disadvantages include the cost of generalized screening of all patients attempting to conceive and a high screen positive rate for "non-actionable" conditions (given testing for hundreds of conditions and competition between manufacturers looking to expand the panel that they offer) that may not be clinically relevant. There is also the subsequent increased testing of partners that is recommended to confirm their status and the associated stress and cost of this process. Certainly, if a specific family condition is identified, screening should be routinely discussed for that condition. Regardless of the approach chosen, it is useful to have an established relationship with a genetic counselor to refer patients to as indicated and a general policy within your office to help determine whom and how to screen.

Social History

All patients should be asked about diet, exercise, environmental exposures, and substance use. The nutritional status of the patient should be reviewed including determination of adequate consumption of folic acid, calcium, and vitamin D. Folic acid intake is critically important to assess given its known protective impact on certain birth defects. However, up to 30% of women attempting pregnancy who are aware of the benefits of preconception folic acid may not be taking it [16]. The use of herbal preparations, vitamin supplements, or mega-vitamins should also be addressed as they may contain ingredients such as hormones or anti-inflammatory agents that may negatively impact fertility [17]. Exercise habits should also be reviewed since reproductive dysfunction has been reported to have a higher prevalence in athletes than in non-athletes. Specifically, menstrual disturbances, with amenorrhea being the most severe form, is one mechanism that is often reported, particularly in patients with a low BMI. Other mechanisms such as luteal phase defect (dysfunction of the corpus luteum) and abnormal follicular development have also been described in the literature [18, 19]. Environmental exposures at work or in the household should be addressed. For example, second-hand smoke may increase the risk of spontaneous abortion [20] and smoking should be discouraged in both the patient and her partner with a stop date agreed upon. Use of alcohol, tobacco, and recreational drug use should be assessed in both the patient and male partner [21–23]. Furthermore, chronic alcohol consumption and smoking have been shown to have a detrimental effect on male reproductive hormones and sperm quality [24]. Smoking cessation should be discussed within the context of any medical evaluation. Lastly, excessive caffeine use has been associated with both infertility and miscarriage and a recommendation for consumption of less than three cups a day should be made.

Sexual History

Coital frequency and timing should be evaluated in order to maximize the chances of conception, as couples may be engaging in intercourse which is not timed. Generally, intercourse is most likely to result in pregnancy when it occurs in the 3 days leading up to ovulation based on the survival time of sperm in the female reproductive tract [25, 26].

12.3.2 Review of Systems

In addition to the general medical history, a focused review of systems should be performed, targeting hormonal or physiologic abnormalities such as intracranial lesions and thyroid abnormalities which are closely associated with anovulation.

Headaches

Headaches are a common complaint in the outpatient setting and are benign in the majority of cases. However, headaches may reflect medical conditions and/or pituitary lesions which can negatively impact on fertility [27–30]. The features of the patient's headache should be characterized, specifically whether the pain is resolved with medication, presence of associated symptoms such as visual field disturbances, and if the headaches are new or have changed in character. Additionally, patients should be counseled that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) during ovulation or infertility treatment regimens may adversely impact ovulation and subsequent implantation [31, 32].

Visual Changes

Visual impairment is a common presenting feature of space-occupying pituitary lesions such as craniopharyngiomas or macroadenomas [33]. These pituitary lesions, if large enough, can extend out of the sella turcica and compress the optic chiasm. Although uncommon, this most frequently presents as bitemporal hemianopsia, or bilateral loss of the peripheral visual fields, and rarely total blindness due to optic atrophy. These patients typically present in an infertility consultation with abnormalities of their menstrual cycles and/or with galactorrhea.

Constitutional Symptoms and Systemic Diseases

Any significant decline in overall health and functional status or symptoms such as heat or cold intolerance should be investigated as these symptoms may suggest underlying medical conditions such as thyroid disease, diabetes, or cancer, all of which can greatly impact on both fertility and a woman's plans for conceiving.

Physical Examination

A complete physical examination should be performed at the initial visit with emphasis on the following components.

Body Mass Index

A body mass index (BMI) above or below the normal range has been associated with anovulation, oligo-ovulation, subfertility, and infertility [34]. Patients should be informed about the association between BMI and ovulation and counseled on lifestyle modifications to optimize their BMI. It is important to note that excessive weight negatively impacts fertility independent of ovulatory status. Furthermore, it is well accepted that obesity is associated with multiple high-risk obstetrical conditions which provides an independent incentive to lose weight.

Thyroid

As stated above, thyroid hormone disorders are associated with anovulation and menstrual irregularities. The thyroid gland is located in the anterior neck below the prominence of the thyroid cartilage and should be palpated for thyromegaly or nodules. Abnormalities on physical exam should be further evaluated with laboratory testing and possible imaging.

Breast

The breast examination in the fertility evaluation should focus on symmetry of the breasts and any evidence of galactorrhea as this could be indicative of a pituitary lesion. Galactorrhea is defined as active secretion of breast milk at a physiologically inappropriate time, namely other than during pregnancy or lactation. Secretions are usually white in color and occur bilaterally from hormonal stimulation of multiple ducts. Conversely, pathological discharge usually originates from a single duct and therefore is unilateral. One helpful

technique is to have the patient squeeze her breast to attempt to express any discharge. She will likely apply more pressure than the provider and, in a patient who states that she has discharge, this will show what is necessary to generate the milk (i.e., gentle pressure or significant manipulation).

Abdomen

In cases of obesity, the abdomen should be evaluated for distribution of adipose tissue. Central adiposity in addition to other signs of hypercortisolemia could be associated with Cushing's syndrome (see Skin section below). Additionally, thorough inspection for any scars indicating previous surgery that the patient may have neglected to mention should also be undertaken.

Skin

The skin should be evaluated for findings that can correlate with underlying endocrine pathology: acanthosis nigricans, abdominal striae, and hirsutism. Acanthosis nigricans is defined as hyperpigmented, velvety plaques found most commonly along the base of the neck, axilla, and the inner thighs. The formation of these lesions is thought to be triggered by hyperinsulinemia, a consequence of obesity-induced insulin resistance. Polycystic ovarian syndrome is often associated with insulin resistance. Therefore, the presence of these lesions warrants further investigation.

Abdominal striae are characterized as violaceous striations most frequently noted on the skin of the abdomen and hips [35]. They can be associated with Cushing's syndrome and therefore would warrant further evaluation for hypercortisolemia.

It is also important to assess the patient's hair growth pattern to assess for hirsutism. Hirsutism is the overgrowth of facial or body hair on women. Specifically, it can be seen as coarse, dark hair that may appear on the face, chest, lower abdomen, back, upper arms, or upper legs. Hirsutism is caused by hyperandrogenism, most commonly in the setting of polycystic ovarian syndrome when the ovaries produce excessive amounts of androgens. Hirsutism can affect up to 10% of women and its presence should also help direct further laboratory testing [36].

Gynecologic Exam

The gynecologic exam should focus on identifying anatomical abnormalities that can be a result of congenital structural anomalies or organic diseases, both of which can impact fertility. For the purposes of the infertility evaluation, the gynecologic exam should assess for the presence of clitoromegaly and structural abnormalities of the cervix, uterus, and pelvis.

Normally, in the non-erect state, the clitoris is generally 3–4 mm in width, 4–5 mm in length and partially covered by a hood of skin. Clitoromegaly, enlargement of the clitoris, is a consequence of inappropriate androgen exposure and is typically defined as a size greater than 35 mm^2 [37]. This finding on physical exam warrants further investigation about ingestion of exogenous androgens, possible in-utero exposure to androgenic substances taken by the patient's mother or an androgen-producing tumor. Physical signs of androgen excess should be correlated with laboratory testing.

Examination of the cervix should assess for cervical stenosis and structural abnormalities such as transverse ridges, cervical collars, hoods, coxcombs, pseudopolyps, cervical hypoplasia, and agenesis [38]. Cervical stenosis is the cervical abnormality most commonly associated with infertility. It decreases fertility by diminishing the mucus bridge from the vagina to the endocervix that is necessary for sperm transport. The remaining structural abnormalities are less common and can be secondary to idiopathic developmental anomalies or obstetrical trauma and surgical procedures. In utero exposure to diethylstilbestrol (DES), a medication prescribed in the 1940s to 1970s for miscarriage prevention, had been a common cause of cervical malformations. Fortunately, it is rare in younger women but should still remain on the differential for women in their forties with a cervical malformation.

The bimanual examination should assess for cervical motion tenderness as well as structural abnormalities of the uterus and adnexae. Cervical motion tenderness can be elicited by gentle lateral movement of the cervix. This finding can be associated with an active or prior pelvic infection or adhesive disease. The mechanism of this physical exam finding is that the movement of the cervix

causes movement of the adnexae as well. Therefore, in the setting of an infection or adhesions around or in the vicinity of the fallopian tubes or ovaries, sliding of the inflamed peritoneum with this test may elicit significant tenderness.

Even without pelvic adhesions, endometriosis can cause cervical motion tenderness when it involves structures attached to the cervix such as the vaginal apex, cardinal ligaments, uterosacral ligaments, and inferior aspect of the broad ligaments. The cervix may be laterally deviated as a result of ipsilateral shortening of a uterosacral ligament which has endometriosis or based on a Müllerian anomaly such as a unicornuate uterus. Nodularity of the uterosacral ligaments can often be felt on bimanual examination if endometriosis is present in that region and may be especially prominent on recto-vaginal exam.

The size and contour of the uterus should also be assessed on the bimanual exam. Notable findings such as enlargement, irregularity, asymmetry, or tenderness all warrant further investigation. Abnormalities associated with decreased fecundity include leiomyomata, adenomyosis, and Müllerian anomalies. The adnexae should also be evaluated on bimanual exam. Any abnormalities on bimanual exam should be further evaluated, typically with imaging such as ultrasound.

Diagnostic Testing

After a thorough medical history and physical examination has been performed, further testing is needed and can be divided into two categories: (1) preconception screening and (2) the infertility evaluation (Table 12.2).

Preconception Screening

Preconception screening consists of tests that should be performed on all women considering pregnancy. This includes a current Pap smear, type and screen, testing for sexually transmitted diseases (STDs), and documentation of immunity to rubella and varicella. Recommended screening for STDs includes syphilis, hepatitis B surface antigen, HIV 1 & 2, hepatitis C antibody, and RNA/DNA-based gonorrhea and chlamydia testing. Patients who are not immune to varicella or rubella should receive the appropriate vaccination at least 1 month prior to conception. Women should also be up-to-date on their tetanus- diphtheria -pertussis vaccine [39]. Furthermore, patients should receive appropriate preconception genetic screening based on their ethnicity, as outlined earlier in this chapter (Table 12.1). When indicated, additional targeted well-women health screenings, such as mammograms, should be performed during this time to maximize health and avoid delays in screening tests.

Infertility Evaluation

The infertility evaluation consists of laboratory testing in addition to those performed for preconception screening. These tests assess: (1) male infertility, (2) ovulatory function, (3) ovarian reserve. Imaging studies such as hysterosalpingography and sonohysterography are also typically performed, and if warranted from the patient's history or physical exam, hysteroscopy or laparoscopy may be indicated as well.

Male Factor

Semen analysis is the main screening tool for male infertility problems. The seminal fluid is analyzed for volume, viscosity, pH, color, presence of round cells (which may be immature sperm or red and white blood cells), and sperm concentration. In terms of sperm characteristics, motility, forward progression, and morphology are assessed. However, sperm function is not assessed in the semen analysis. Men with persistently abnormal semen analyses should be further evaluated by a reproductive urologist. Of note, semen analysis varies widely in quality between different labs and care should be taken when interpreting results.

Thyroid-Stimulating Hormone (TSH)

Hypothyroidism is a relatively common medical problem in women and can result in ovulatory dysfunction even in the presence of minimal or no symptoms. Fortunately, it is relatively easy to treat. TSH is the screening test of choice for identifying thyroid hormone abnormalities and should be drawn at the initial infertility visit. Hypothyroidism is suggested when TSH is elevated and should be repeated with a measurement of free T4 [40]. When TSH is abnormally low, it can indicate hyperthyroidism and further testing

Table 12.2 Adapted from Horowitz GM. Female infertility [61]

	Tests
Preconception screening	Current Pap smear when appropriate
	ABO, Rh factor typing
	Verify immunity with vaccination if indicated
	Rubella titer
	Varicella titer
	Appropriate genetic screening (see Table 12.1)
	Sexually transmitted diseases
	Recommended screening
	Syphilis screen (VDRL/RPR)
	Hepatitis B surface antigen (HBsAg)
	HIV 1 and 2
	Hepatitis C antibody (HCA)
	Gonorrhea (RNA/DNA-based testing)
	Chlamydia (RNA/DNA-based testing)
Infertility testing	*Semen analysis*
	Hormonal Tests
	TSH
	Prolactin
	Ovulatory function
	Basal body temperature chart (not routinely recommended)
	Mid-luteal serum progesterone
	Urinary LH surge detection
	Ovarian reserve testing options
	Day 2 or 3 FSH and Estradiol
	Clomiphene Citrate Challenge Test (CCCT)
	Anti-Müllerian Hormone
	Antral follicle count
	Imaging study options
	Transvaginal ultrasonography
	Sonohysterography
	Hysterosalpingogram
	Hysteroscopy, diagnostic or operative as indicated
	Diagnostic laparoscopy as indicated

is required. Subclinical hypothyroidism is a special issue in women attempting to conceive and, although controversial, there are recommendations for tighter control of thyroid function during peri-conception and pregnancy compared to other periods in a woman's life with the goal of a TSH being <2.5 [41].

Prolactin

Like hypothyroidism, hyperprolactinemia is a relatively common problem with many causes. It may lead to oligomenorrhea or amenorrhea, therefore causing infertility. The most common cause for hyperprolactinemia in women is a prolactin-secreting adenoma usually diagnosed with MRI after an elevated prolactin is identified on blood work. It is also important to note that thyrotropin-releasing hormone (TRH) is a potent prolactin stimulating substance and since this is increased along with TSH in hypothyroid states, prolactin levels can be elevated in women with hypothyroidism [42]. For this reason, TSH and prolactin should be drawn together at the initial evaluation to establish the correct diagnosis. Lastly a careful medication history should be taken as various medications can also contribute to an elevated prolactin.

Tests for Ovulation

Ovulatory function can often be deduced from a patient's menstrual history, specifically women with regular cycles (25–35 day intervals) and symptoms such as breast tenderness, bloating, and dysmenorrhea are likely to have ovulatory cycles. As previously noted, several hormonal abnormalities can commonly cause ovulatory dysfunction in otherwise healthy patients. Therefore, tests such as TSH and prolactin are important to obtain. Other tests such as basal body temperature charts, mid-luteal serum progesterone, and urine luteinizing hormone surge detection can provide additional information about ovulatory function.

Basal Body Temperature Charts

Basal body temperature (BBT) charts are based on progesterone-related increase of core body temperature. During the follicular phase, the BBT can fluctuate between 97.0 and 98.0°F. Progesterone levels >5 ng/mL, achieved after ovulation, raise the hypothalamic set-point for basal temperature by approximately 0.6°F. Due to the variable timing in the rise of temperature following ovulation, the stress associated with taking one's temperature every morning before getting out of bed, and the significant false-positive and false-negative rates, this modality is uncommonly recommended [43].

Serum Progesterone

Measurement of serum progesterone levels can also be used to document ovulation. Serum progesterone levels remain below 1 ng/mL during most of the follicular phase rising during the late follicular phase to 1–2 ng/mL [44]. After ovulation, progesterone is secreted from the corpus luteum and levels rise steadily until they peak approximately 7–8 days following ovulation. Typically, a serum progesterone level >3 ng/mL provides reliable evidence that ovulation has taken place but does not provide information on when it occurred [45].

There are several methods of measuring serum progesterone. Classically, it can be measured on day 21 with the assumption being that the woman has a 28-day menstrual cycle [46]. However, since normal menstrual cycles fluctuate in length, measurement of serum progesterone on day 21 may not be completely accurate. If a woman is able to estimate how long her menstrual cycles are, then she can simply obtain this test approximately 1 week before her period is due.

Urinary LH Measurements

Of the three tests described in this section, measurement of urinary LH is the only test that can predict ovulation before it occurs, therefore giving patients the ability to time intercourse. These highly accurate over-the-counter tests are designed to change color when urinary LH levels reach those associated with the mid-cycle LH surge, indicating imminent ovulation. Testing should be performed daily starting 3 days before the expected day of ovulation to ensure that ovulation is not missed. Ovulation will generally follow within 12–36 h following a positive surge with the variability reflecting the once daily testing of an ongoing process. If used for timed intercourse or intrauterine insemination (IUI), the day after the first positive test will have the highest success rate [47].

Ovarian Reserve Testing

Ovarian reserve has become an integral part of the infertility evaluation and can be assessed with several methods. It is well known that fertility declines with a woman's age due to the decrease in oocyte quantity and quality. The tests most commonly used to assess ovarian reserve include (1) Early follicular phase follicle-stimulating hormone (FSH) and estradiol levels, (2) Clomiphene Citrate Challenge Test (CCCT), (3) Anti-Müllerian hormone (AMH), and (4) Antral Follicle Count (AFC).

FSH and Estradiol

FSH is a hormone secreted by the pituitary gland and functions to recruit follicular cohorts. As ovarian reserve diminishes, the pituitary gland increases FSH production in order to compensate for fewer eggs. It has been shown that when baseline FSH levels are >10 IU/L, success with therapies including in-vitro fertilization (IVF) is greatly diminished [48]. Estradiol levels should be drawn with all basal FSH levels to demonstrate that a low FSH level is not falsely suppressed secondary to a prematurely elevated estradiol level (defined as greater than 60–80 pg/mL) [49]. To be reliable, estradiol levels can be drawn on either cycle day 2 or 3 to facilitate the process for the patient [50].

Clomiphene Citrate Challenge Test (CCCT)

Clomiphene Citrate is a selective estrogen receptor modulator (SERM) that has an antagonist effect at the hypothalamus, therefore blocking the inhibitory feedback of estrogen. This in turn leads to an increase in GnRH and therefore FSH at the level of the pituitary. The CCCT is a provocative examination designed to "unmask" those patients with a normal day 3 FSH level. With this test, a basal FSH level and estradiol are measured on cycle day 3. The patient is given clomiphene citrate 100 mg daily on cycle days 5 through 9 and the FSH level is again measured on cycle day 10. The test is considered abnormal if the day 3 FSH, day 3 estradiol or day 10 FSH levels are elevated [51].

Anti-Müllerian Hormone (AMH)

Anti-Müllerian Hormone, also known as Müllerian Inhibiting Substance (MIS) is produced by the granulosa cells of ovarian follicles and reflects the primordial follicle reserve. Unlike the Day 3 FSH test and CCCT, AMH can be measured at any point during the menstrual cycle [52, 53]. Levels less than 1.0 ng/mL are considered abnormal and are associated with poor ovarian response to gonadotropin stimulation [54, 55].

Antral Follicle Count (AFC)

The AFC is determined using transvaginal ultrasound in the early follicular phase to quantify the number of follicles between 2 and 10 mm in diameter. These antral follicles may be thought of as eggs in the pipeline reflecting overall ovarian reserve. An AFC of less than 10 has been shown to correlate with poor ovarian response to gonadotropin stimulation [56]. High antral follicle counts are often associated with PCOS.

12.3.3 Imaging Studies

Ultrasonography

Transvaginal ultrasonography is the first line imaging study for identifying structural abnormalities in the pelvis, particularly of the uterus and ovaries [57, 58]. It should be considered if a structural lesion is suspected on physical examination. However, some conditions may not be detectable, especially if the exam is limited by patient discomfort or body habitus. Transvaginal ultrasound may be considered in those women and, possibly, in all infertile women if other imaging is not available or a timed ultrasound is desired to obtain an AFC.

Sonohysterography

Sonohysterography (also known as saline infusion sonography or SIS) is an imaging test that utilizes transvaginal ultrasonography in which a fluid medium, typically saline, is instilled through the cervix to distend the uterine cavity. This allows for identification of any endometrial or intracavitary lesions such as polyps or fibroids. When used with specialized contrast media (saline with bubbles infused), sonohysterography may also be used to assess tubal patency. Similar to a hysterosalpingogram (below), both transvaginal sonography and sonohysterography provide information about the uterine myometrium and ovaries. However, an SIS may not be able to give as detailed information about the fallopian tubes as a hysterosalpingogram. As noted above, when done

early in the cycle this test may be also used to obtain an AFC. If combined with 3D ultrasound, SIS provides significant information about the uterus including possible differentiation of uterine anomalies such as a bicornuate from a septate uterus. In addition, the ultrasound probe can be used to push on structures to localize symptoms (such as pain in an endometrioma) as well as to assess sliding of the ovary or uterus alongside bowel to assess for adhesions.

Hysterosalpingography

Hysterosalpingography (HSG) is a radiographic evaluation of the uterine cavity and fallopian tubes. Contrast dye is injected through the cervix into the uterine cavity with spillage of the dye into the abdominal cavity if the fallopian tubes are patent. This test is used to diagnose intrauterine adhesions (synechia) and other intracavitary defects such as polyps and fibroids as well as Müllerian anomalies such as a septate or bicornuate uterus. Furthermore, hysterosalpingography can assess tubal patency and identify the site of obstruction if the tubes are blocked [59]. Of note, although the primary purpose of this study is not therapeutic, the media has been shown to potentially increase subsequent pregnancy rates for several months after the procedure in women [60].

Surgery

Hysteroscopy can be used for both diagnostic and therapeutic purposes in the infertile patient. Diagnostic hysteroscopy can often be performed in the office when there is suspicion for an intracavitary lesion based on the patient's history such as abnormal uterine bleeding or specific findings noted on prior sonohysterography or hysterosalpingography. Operative hysteroscopy, while potentially requiring an operative room, can be therapeutic in that the polyps or fibroids that are visualized at the time of the procedure can be immediately resected.

Diagnostic laparoscopy can be useful for some infertile women since it is the only definitive method of accurately diagnosing endometriosis and intraperitoneal adhesions. However, as fertility treatments have evolved, fewer laparoscopies are done for infertility alone since protocols such as IVF are designed to bypass many of these laparoscopic findings. Furthermore, laparoscopic treatment of Stage I Endometriosis results in only a small absolute increase in pregnancy rates. Nevertheless, in situations where higher stage endometriosis is suspected, the patient has significant pelvic pain, or IVF is not able to be performed, then laparoscopy may be an excellent option for both diagnostic and therapeutic purposes.

12.4 Treatment

There are many options for infertility treatment. Specific therapies should be selected based on the results of the patient's evaluation as described above. As many of these therapies can be extremely expensive and are not necessarily covered by medical insurance, it is ideal to begin with the least invasive and least expensive option. Although, historically, practitioners would typically proceed in a stepwise manner, patients are more frequently being offered IVF if they do not conceive with lesser therapy. The goal is to shorten the time to pregnancy while minimizing the risk of a high order multiple pregnancy [62].

12.4.1 Oral Medications

Clomiphene citrate is a selective estrogen receptor modulator that inhibits the negative feedback effect of estrogen on the hypothalamus, therefore upregulating the hypothalamic–pituitary–gonadal axis to increase the likelihood of ovulation in anovulatory women or the release of more than one egg in women who are already ovulatory. Letrozole functions as an aromatase inhibitor, decreasing the peripheral enzymatic conversion of androgens to estrogens. This overall decreases the body's estrogen level and provides feedback to the hypothalamic–pituitary–gonadal axis to increase FSH production.

When using these medications, it is important to distinguish patients who have ovulatory infertility from those with unexplained infertility. In women who are anovulatory, the goal is to achieve mono-follicular development. Letrozole is emerging as a superior choice over clomiphene in this population [63].

In women who are ovulatory and not conceiving despite releasing an egg each month, the goal is to increase their ovulatory function using a more aggressive protocol. Clomiphene citrate remains the protocol of choice over letrozole [64].

As opposed to the traditional 50 mg cycle days 5–9, the standard dosing regimen for clomiphene, when used for ovulation induction, is 100 mg orally cycle days 3–7. Furthermore, to optimize pregnancy rates, this should be combined with intrauterine insemination (IUI).

12.4.2 Ovarian Stimulation (Injectable Gonadotropins)

Controlled ovarian stimulation with gonadotropins is used to stimulate the ovaries to produce one egg in anovulatory women refractory to oral medications and more than one mature follicle per cycle in women who are infertile and not conceiving despite having regular ovulations. Multiple follicular development increases both the chances of any one egg fertilizing (and, hence, overall pregnancy rates) but also more than one egg fertilizing (increasing the risk of multiple gestations). Gonadotropin therapy is more effective than clomiphene or letrozole for ovulatory women with infertility [64]. Side effects of these medications include ovarian hyperstimulation syndrome (OHSS) and ovarian damage or torsion.

Multiple gestations typically only occur in 1–2% of naturally occurring pregnancies. With injectable gonadotropins, 20–30% of these pregnancies are associated with multiple implantations. Multiple pregnancies are associated with an increased risk of miscarriage, preterm delivery, pregnancy-induced hypertension, postpartum hemorrhage, and other maternal complications [65].

Ovarian torsion occurs in less than 2% of gonadotropin cycles and may result when an enlarged ovary twists on its vascular pedicle, thus cutting off essential blood supply. Such cases are a surgical emergency requiring de-torsion of the ovary to restore blood supply and less commonly, removal of the ovary if necrotic and unable to be saved [65].

12.4.3 Intrauterine Insemination

Intrauterine insemination (IUI) is a procedure performed in the office in which prepared sperm is placed directly into the woman's cervix or uterus through a catheter. When treating ovulatory infertile woman, IUIs are typically part of the treatment regimen to maximize pregnancy rates. There are several indications for IUI alone including the use of donor sperm, male factor such as low motility [66], coital dysfunction, cervical stenosis due to a surgical procedure such as a LEEP or cone biopsy. IUIs will not be effective in patients with tubal blockage, severe endometriosis or intraabdominal adhesions since they still require the oocyte to travel from the ovary to the uterine cavity.

12.4.4 In-Vitro Fertilization

In-vitro fertilization (IVF) is the most successful fertility intervention in any one treatment cycle for the majority of woman. It involves ovarian stimulation with injectable gonadotropins typically with the use of a gonadotropin-releasing hormone (GnRH) agonist such as Lupron, or a GnRH-antagonist such as Ganirelix or Cetrorelix to suppress the LH surge and premature ovulation. Since recombinant LH is not available, human chorionic gonadotropin (HCG) is given to mature the eggs, triggering ovulation. The oocytes are then retrieved through needle aspiration transvaginally under ultrasound guidance. The oocytes are either frozen in instances such as fertility preservation or fertilized with the prepared sperm sample and incubated. The embryos are graded using quality assessment criteria such as cell regularity, degree of fragmentation, and other microscopic characteristics [67]. Those of highest quality are selected for transfer, which is performed transcervically through a small catheter under ultrasound guidance. Procedures such as gamete intrafallopian transfer (GIFT) and zygote intrafallopian transfer (ZIFT) are essentially historical given their greater invasiveness (requiring laparoscopy) with no higher pregnancy rate. Supplemental progesterone is used to support the luteal phase since no ovulation takes place in IVF, and there may be inadequate progesterone production endogenously.

The live birth rate using IVF widely varies depending on many factors including, but not limited to, the woman's age, BMI, duration of infertility, and presence of hydrosalpinges [68]. The goal of IVF is to achieve a singleton pregnancy given the risks associated with twins or higher-order multiple gestations. Fortunately, emerging data suggests that a consecutive fresh and frozen single-embryo transfer has comparable live birth

rates to that of double-embryo transfers. There is also a significantly higher rate of multiples even with just a double-embryo transfer [69].

12.4.5 Donor Gametes and Adoption

Donor gametes (sperm or eggs) or adoption should be discussed with appropriate patients. Care should be taken to anticipate and prepare for the period of grieving or anger associated with these routes. Patients should be given time and resources to address the psychological aspects of the situation prior to pursuing either of these options [70].

12.5 Concluding Remarks

Although infertility is a relatively common medical problem, the process of evaluation and treatment can be long, emotionally taxing, and frustrating. Patients should be encouraged to seek care in a timely fashion with a provider who can attempt to expedite their treatment using modern techniques. The role of the provider is to offer support while maintaining realistic expectations based on the clinical evidence at hand. While there are many options for infertility treatment at this time, a relatively large proportion of infertility is attributed to unexplained causes. Therefore, further research is still required to better understand how infertility occurs as well as to develop innovative treatment techniques while optimizing existing ones. Fortunately, current technologies and in vitro fertilization allow a majority of patients to achieve their dream of a family.

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Fertility Preservation

Pasquale Patrizio, Emanuela Molinari, Tommaso Falcone, and Lynn M. Westphal

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13.1 Introduction

The prevalence of females being diagnosed with cancer before age 40 is about 2%. Due to advances in the treatment of many malignancies, the majority of cancer patients survive their diagnosis, with an overall 5-year survival rate greater than 80%. Consequently, fertility preservation has become an increasingly important paradigm for quality of life after cancer. Advances in assisted reproduction techniques (ART), including ovarian tissue cryopreservation and transplantation, oocyte cryopreservation, and novel ovulation induction approaches, offer concrete hopes to women at risk of being rendered sterile by their treatments.

In addition to cancer patients, fertility preservation strategies are also offered to patients exposed to chemotherapy for other medical conditions such as systemic diseases as lupus, hematological disorders such as thalassemias, multiple sclerosis, and other autoimmune conditions. Furthermore, and more recently, fertility preservation options have been offered also to women interested in delaying childbearing. Frequently referred to as "elective or social" fertility preservation, many women are mainly resorting to oocyte cryopreservation as a means to safeguard their future reproductive chances.

This chapter will first focus on fertility preservation options in cancer cases including evaluation of the ovarian reserve and the pathophysiology of chemotherapy/radiotherapy-induced gonadal toxicity, as well as the indications and the outcomes of the various techniques used for fertility preservation even in other medical indications.

Clinical Case

A.M. is affected by a severe form of beta-thalassemia requiring repeated blood transfusions and iron chelating therapy. At age 22, prior to starting conditioning chemotherapy with alkylating drugs for bone marrow transplantation, she was referred for fertility preservation and to discuss the various options.

13.2 Evaluation of the Ovarian Reserve

The age-related decline in fertility is primarily due to the relentless and progressive diminution of oocyte numbers and quality. Accurate assessment of ovarian function is a core part of an infertility evaluation. However, particularly for cancer patients, there is not too much time to complete a full assessment of the ovarian reserve. Quick indicators for ovarian reserve are Anti Mullerian Hormone (AMH) and Antral Follicle Count (AFC). A complete assessment could include also day 3 FSH and Estradiol.

13.3 Antral Follicle Count

The antral follicle count utilizes transvaginal ultrasound in the early follicular phase to determine the number of follicles between 2 and 10 mm in diameter. A low AFC is defined as fewer than 10 antral follicles has been shown to correlate with poor ovarian response to stimulation [1].

13.4 Anti-Müllerian Hormone

Anti-Müllerian hormone is produced by granulosa cells and is a reflection of the primordial follicle pool. It is a convenient test, because it can be measured at any time in the menstrual cycle [2, 3]. Although exact cut-offs for good versus poor ovarian reserve have not been established, lower levels (<1.0 ng/mL) have been correlated with poor ovarian response [4, 5]. This hormone is also used as a marker for recovery of ovarian function after completing chemotherapy/radiotherapy treatments or as a marker of decreased ovarian function in women that choose not to preserve fertility and hope to maintain their ovarian function in follow-up visits [6, 7].

13.5 Day 3 FSH and Estradiol

As the oocyte pool decreases, the FSH secreted by the pituitary will rise in response to negative feedback from the follicular pool secretion of inhibin. As demonstrated many years ago and still used today, an early follicular (basal) FSH level drawn

on cycle days 2–5 can have predictive value on fertility potential [8–10]. Estradiol levels, drawn in conjunction with basal FSH, help in a proper interpretation of the FSH levels since early rises of follicular phase estradiol (>60–80 pg/mL) falsely suppress FSH [11].

The specificity of basal FSH levels for predicting who will respond poorly to stimulation reaches 83–100%; however, the sensitivity varies widely, from 10 to 80% [12] and this is one of the main reasons why FSH levels have been supplanted by AMH testing.

13.6 Patient Population

The patient population that will seek fertility preservation for a diagnosis of cancer in the reproductive years is small, yet the impact of lost reproductive capacity is large and psychologically devastating. The overall incidence of cancer in women less than 40 years of age is 754 per 100,000 [13]. The most common malignancies in reproductive-age women are breast, Hodgkin and non-Hodgkin lymphomas, thyroid, melanoma, and cervical or uterine cancer [13]. The mainstay of treatment for many of these malignancies remains surgery, chemotherapy, and/or radiation.

Premature gonadal failure is a well-known consequence of ovarian exposure during the reproductive years to chemotherapeutic drugs and radiation therapy. In general, radiotherapy is used cautiously in children and adolescents because of its late sequelae on immature and developing tissues [14]. Pelvic radiotherapy is most commonly used to provide local disease control for solid tumors, including bladder, rectum, uterus, cervix, or vagina, all of which are more common in adult women.

13.7 Chemotherapy and Ovarian Damage

Cytotoxic drugs can destroy maturing follicles, impair follicular maturation, deplete primordial follicles, or some combination of these effects. Destruction of maturing follicles results in temporary amenorrhea, whereas destruction of primordial follicles results in permanent amenorrhea secondary to ovarian failure.

The extent of chemotherapy-induced gonadotoxicity is variable. Histologic sections of the ovary following treatment with cytotoxic drugs known to cause ovarian failure show a spectrum of changes, ranging from decreased numbers of follicles to absent follicles to fibrosis. The exact incidence of premature ovarian failure following chemotherapy is difficult to establish, because many factors contribute to ovarian toxicity. Alkylating agents and platinum complexes mechanism of action is similar: it induces DNA strand breaks that ultimately trigger apoptosis [15]. Similarly, cellular death is caused by microtubule-stabilizing agents (such as taxanes) and by DNA intercalating drugs (doxorubicin) [16]. Recent theories of chemotherapy-induced ovarian damage involve the activation of dormant follicles by chemotherapy drugs. This activation, mediated by an upregulation of PI3K/PTEN/Akt signaling pathway, leads to follicle destruction and reduction of AMH secretion. Consequently, in order to replace the dying cohort of follicles, the hypothalamic suppression of the primordial follicle pool growth is ceased, causing a rapid activation and loss of the remaining follicular cohort [17, 18].

DNA damage is also a commonly described mechanism of follicle loss as a consequence of combined radiation and chemotherapy treatment. It has been shown to originate in granulosa cells through p63 oncogene pathway [19].

Additionally, vascular damage is another mechanism of ovarian damage, as ovarian blood flow and volume are reduced shortly after chemotherapy [20].

It is important to note that menstrual dysfunction that occurs during chemotherapy is not always due to the direct toxic effects on the ovary. Severe illness, malnutrition, and general mental and physical stress can also interfere with normal function of the hypothalamic–pituitary–ovarian axis. Short-term disruption of a menstrual cycle can also be the result of destruction of growing follicles rather than primordial follicles. Destruction of all growing follicles will delay menses for at least 3 months, since it takes a primordial follicle approximately 85 days to reach the stage of ovulation. It is important to note that a normal menstrual cycle after treatment with chemotherapy is not a reliable predictor of a patient's fertility status.

13.8 Risk Factors for Gonadal Damage

The most important risk factors for gonadal damage are the age of the patient, the drug class, and cumulative dose of the drug and combination of chemo with radiotherapy. The risk related to the age of the woman is linked to the presence of smaller oocytes pool as compared to younger patients. In one study of women who had received mechlorethamine, vincristine, procarbazine, and prednisone (MOPP) for Hodgkin's disease, the subsequent amenorrhea rate was 20% for women <25 years old, compared to 45% for those ≥25 years old [21].

Additionally, cytotoxic chemotherapeutic agents are not equally gonadotoxic. Cell cycle nonspecific chemotherapeutic agents are considered to be more gonadotoxic than cell cycle specific ones (Table 13.1). Alkylating agents are among the most gonadotoxic of these cell cycle nonspecific drugs, and women who have received high-dose alkylating agent therapy are at highest risk for premature ovarian failure. Cyclophosphamide is the most commonly used gonadotoxic member of this category.

13.9 Predicting Ovarian Failure

Premature ovarian failure does not consistently occur in patients receiving multi-agent chemotherapy, regardless of age or type of chemotherapeutic agent. Most young patients with Hodgkin's disease treated with multi-agent chemotherapy and radiation to a field that does not include the ovaries will be fertile, although their fertility will begin to decrease at a younger age than matched controls [22]. Spontaneous conception can

Table 13.1 Gonadotoxic chemotherapeutic agents

Toxicity	Medication class	Medication
High	*Alkylating agents*:	Cyclophosphamide
	Chloroethylamine	Chlorambucil
	Mechlorethamine	Nitrogen mustard
	Nitrosurea	L-phenylalanine mustard
	Alkylalkane sulfonate	Carmustine
	Methylhydrazine derivative	Lomustine
		Busulfan
		Procarbazine
Intermediate	Platinum complexes Anthracyclines	Cisplatinum
		Carboplatinum
		Doxorubicin
Low	Antimetabolites	Methotrexate
	Vinca alkaloids	5-Fluorouracil
	Antibiotics	6-Mercaptopurine
		Vincristine
		Vinblastin
		Bleomycin
		Dactinomycin

occur even in after diagnosis of premature ovarian failure, such as reported in a woman who had completed 14 courses of an alkylating agent combined with pelvic irradiation for treatment of Ewing's sarcoma of the pelvis [23]. This exemplifies the difficulties in predicting the probability of ovarian failure after chemotherapy, which also makes it difficult to evaluate the efficacy of treatment aimed at preserving ovarian function.

13.10 Markers for Gonadal Damage

Ovarian reserve testing, which was discussed earlier in the chapter, should be offered both pre- and post-treatment. Basal FSH levels (if the patient has menstrual cycles), AMH, inhibin-B, and transvaginal ultrasound measured AFC are useful markers for ovarian function after chemotherapy [24].

13.11 Radiation Therapy and Ovarian Damage

Ovarian damage from radiotherapy can lead to diminished ovarian reserve and premature ovarian failure [25–33]. The ovarian follicles are remarkably vulnerable to DNA damage from ionizing radiation. Irradiation results in ovarian atrophy and reduced follicle stores [30]. As a result, serum FSH and LH levels progressively rise and estradiol levels decline within 4–8 weeks following radiation exposure. On the cellular level, irradiation of oocytes results in rapid onset of pyknosis, chromosome condensation, disruption of the nuclear envelope, and cytoplasmic vacuolization.

13.12 Risk Factors for Ovarian Damage

Cancer patients are at high risk for premature ovarian failure after treatment with pelvic or total body irradiation. The degree of ovarian damage is related to the patient's age and the total dose of radiation to the ovaries. It is generally estimated that a single dose of 5.0 Gy will cause permanent ovarian failure in over 90% of postpubertal women [34]. When looking at specific age groups, a prepubertal girl may have permanent ovarian failure if exposed to 12 Gy, but only 2 Gy may cause the same result in women over the age of 45 [29].

The dose response of the ovaries to irradiation has been demonstrated in several studies [27, 31, 33]. It is estimated that as little as 3 Gy is enough to destroy 50% of the oocyte population in young, reproductive-age women [32]. When the mean radiation dose to the ovary was 1.2 Gy, 90% of patients retained their ovarian function. When mean dose was 5.2 Gy, only 60% retained ovarian function.

Ovarian failure will occur in virtually all patients exposed to pelvic radiation at the doses necessary to treat cervical cancer (85 Gy), rectal cancer (45 Gy), or total body radiation for bone marrow transplantation (8–12 Gy exposed to the ovaries). The addition of chemotherapy to radiotherapy further decreases the dose required to induce premature ovarian failure.

Even if the ovaries are not directly in the radiation field, radiation scatter can reduce ovarian function. Given this risk, it is very important to discuss with the radiation oncologist the expected dose that will be delivered to the ovary either directly or through scatter.

13.13 Radiotherapy and Uterine Damage

Irradiation-induced uterine damage can result in impaired endometrial function and uterine blood flow [35]. Young women exposed to radiotherapy below the diaphragm are at risk of impaired development of the uterus, in addition to ovarian failure. Long-term cancer survivors treated with total body irradiation and marrow transplantation are at risk of impaired uterine growth and blood flow. Despite standard estrogen replacement, the uterine size of young girls is often reduced to 40% of the normal adult size. The younger the girl is when irradiated, the more the uterus appears to be affected.

It has been demonstrated in women treated with total body irradiation that sex steroid replacement in physiological doses significantly increases uterine volume and endometrial thickness, as well as reestablishes uterine blood flow. However, it is not known whether standard regimens of estrogen replacement therapy are sufficient to facilitate uterine growth in adolescent women treated with total body irradiation in childhood.

13.14 Pregnancy After Radiation Therapy

Pregnancies achieved by survivors of childhood cancer who have received pelvic irradiation must be considered high risk [34–36]. Radiation damage to the uterine musculature and vasculature can adversely affect pregnancy outcomes in these women. Even if the uterus is able to respond to exogenous sex steroid stimulation, and appropriate ARTs are available, pregnancy prognosis remains guarded. Common obstetric problems reported in these patients include early pregnancy loss, premature labor, and low-birth-weight infants [37, 38].

13.15 Fertility Preservation Strategies

A wide variety of strategies have been reported in an effort to preserve ovarian function and fertility in women undergoing chemotherapy and/or radiotherapy. Fertility-sparing procedures, pharmacologic options, and ARTs will be discussed (Table 13.2).

Table 13.2 Fertility preservation strategies

	Technique	Pros	Cons	Experimental vs. Established
Surgical	Fertility-preserving surgery	May be able to conceive and carry future pregnancy Less aggressive Usually covered by insurance	May leave residual disease Requires close follow-up May still require ART	Established
	Ovarian transposition	Reduces ovarian exposure to radiation	Requires surgery May still be affected by radiation (Scatter/fall into field) Can cause pain	Established
Pharmacologic	GnRH agonists	Minimal delay in treatment	Not proven Side effects similar to menopause	Experimental
Assisted reproductive technology	Embryo cryopreservation	Commonly performed, good success rates	Elevated hormone levels Time-consuming Requires a sperm source May not be covered by insurance	Established
	Oocyte cryopreservation	Good success rates, no longer experimental Does not require a sperm source	Elevated hormone levels Time-consuming May not be covered by insurance	Established
	Ovarian tissue cryopreservation	No delay in treatment Does not require a sperm source Option for prepubertal females	Requires surgery Ovarian tissue ischemia Possible reexposure to cancer cells May not be covered by insurance	Experimental
	In vitro maturation	Minimal delay in treatment Does not require a sperm source	Limited experience with this procedure	Experimental

13.16 Surgical Techniques

In general, the conventional therapy for a gynecologic malignancy consists of removal of the uterus, tubes, and ovaries. However, there are specific circumstances that may allow a more conservative surgical approach.

13.17 Cervical Cancer

Cervical cancer is typically treated with surgery or radiation therapy, depending on the stage at presentation. Those with early stage disease, Stage IA1, may be treated with cervical conization and close follow-up. Women who desire future fertility and are diagnosed with Stage IA2 and Stage IB disease may opt for a radical trachelectomy, which involves removal of the cervix, surrounding tissues, and lymph node dissection [39]. Patients treated with trachelectomy may require ART to achieve a future pregnancy, and should be aware that they are at increased risk of second-trimester loss and preterm birth after this procedure [40]. A recent study described successful fertility preservation and spontaneous pregnancies achieved with robotic trachelectomy [41].

13.18 Ovarian Cancer

Ovarian tumors classified as low malignant potential, germ cell, sex cord-stromal, or early epithelial malignancies have the potential to be treated with conservative surgery. Most ovarian cancers diagnosed in the reproductive years tend to be unilateral and are less likely to have metastasized. The surgical option most likely to succeed in removing the cancerous tissue, as well as preserve fertility potential, is unilateral oophorectomy with conservation of the remaining normal ovary and the uterus. These women should still undergo complete staging and be monitored closely by a gynecologic oncologist for possible recurrence [42].

13.19 Endometrial Cancer

Both complex endometrial hyperplasia with atypia and early stage endometrial adenocarcinoma (Stage IA1) can be treated conservatively. A hysteroscopy with dilatation and curettage, followed by high-dose progesterone therapy, is the standard treatment for those women who desire fertility preservation. Unfortunately, recurrence is common and close periodic evaluation is required to avoid progression. It is also important to stress to these patients that they should pursue child-rearing sooner rather than later, and then have a complete hysterectomy and bilateral salpingo-oophorectomy to ensure a disease-free survival.

13.20 Ovarian Transposition

Transposition of the ovaries out of the field of radiation appears to help maintain ovarian function in patients scheduled to undergo gonadotoxic radiation therapy. This technique can be utilized for gynecologic, colon, rectal, and anal cancers. Transposition of the ovaries has been reported to reduce the radiation dose to each ovary by approximately 90–95% compared to ovaries left in their original location [38].

There are two techniques available, lateral and medial transposition. Lateral transposition appears to be more effective than medial transposition. A compilation of ten case reports and a small series showed an ovarian failure rate of 14% after lateral transposition compared to 50% after medial transposition [43].

Ovarian transposition can be performed by either laparotomy or laparoscopy. When surgery is required for the treatment of cervical cancer or during staging and treatment of ovarian cancer, lateral ovarian transposition can be performed simultaneously. However, if a surgical procedure is not required for treatment, the transposition can easily be performed as an outpatient procedure. The ovaries have a tendency to migrate back to their original position, so it is recommended to complete the procedure immediately prior to the initiation of radiation therapy [33, 44, 45].

Most ovaries will maintain function if they are transposed at least 3 cm from the upper edge of the field [46]. It has been shown that approximately 80% of women undergoing laparoscopic ovarian transposition will maintain ovarian function after radiation therapy for various indications [47].

Ovarian failure following transposition can occur because of several different mechanisms. Ovarian failure may result if the ovaries are not moved far enough out of the radiation field. Another reason for failure would be ovarian migration back

to their original position. Ovarian failure following transposition may also be due to compromised ovarian blood flow from surgical technique or radiation injury to the vascular pedicle [48].

Pregnancies have been reported after ovarian transposition. Some have occurred spontaneously, but others required reversal of the procedure.

13.21 Pharmacologic Protection

13.21.1 Gonadotropin-Releasing Hormone Agonists

The ideal approach to decrease or eliminate gonadal damage from chemotherapy is pharmacologic. The patient can take a medication and proceed with her cancer treatment without undergoing an invasive procedure. The critical step in the development of such a drug is an understanding on how chemotherapy actually causes ovarian follicle destruction. The impact, as stated earlier, depends not only on the type of chemotherapeutic agents but on age, ovarian reserve, dose, and duration of treatment. The unique feature of chemotherapy induced gonadal damage is the predilection towards damage of the primordial follicle, which consists of non-growing cells. Growing follicles are immediately impacted resulting in amenorrhea. Chemotherapeutic agents can directly cause apoptosis of follicles, with the dividing granulosa cells being particularly susceptible to damage [49–51]. This latter phenomenon leads to the theory of "follicle burn out" [49]. Since growing follicles have a direct effect in dampening the initiation of primordial follicle growth, the immediate and complete loss of growing follicles causes an accelerated recruitment of primordial follicles and a decrease in the total ovarian follicular reserve. In addition to these effects chemotherapy can cause stromal fibrosis and damage to intra-ovarian vessels. The ideal drug would impede these effects. Drugs that act on apoptotic pathways such as sphingosine-1-phosphate or drugs that impede follicle activation pathways such as AMH would be ideal. Most are in pre-clinical trials and not available clinically. While testing these drugs it is important not to interfere with the efficacy of the cancer treatment. The only drug clinically available for use in patients undergoing gonado-toxic treatment is Gonadotropin-releasing hormone agonists (GnRHa).

Protection of gonadal function is more than just preservation of fertility. Many aspects of quality of life are related to gonadal function. Hypogonadal symptoms such as hot flashes, insomnia, vaginal dryness, dyspareunia, and impaired sexual function are equally important. Ovarian failure is associated with osteoporosis, cardiovascular disease, and neurocognitive decline. Therefore drugs that prevent chemotherapeutic damage can be efficacious in maintaining an estrogenic environment and quality of life without necessarily protecting fertility.

It is unclear how GnRHa can impede the gonadotoxic effects of chemotherapy. Its effect on suppressing the pituitary gonadotropin secretion is well described. This aspect of the drug cannot be solely responsible for its observed effects as primordial follicle activation is independent of gonadotropins. It may be acting on impeding follicle recruitment by different mechanisms [49]. GnRHa are thought to decrease vascularity at the level of the ovary, thereby reducing the concentration of chemotherapy acting directly on the ovary [49].

The use of GnRHa during chemotherapy is still a controversial and considered experimental. In some circumstances such as preventing the severe menstrual bleeding associated with some chemotherapeutic drugs it is quite effective. GnRHa can be useful for preservation of gonadal function to alleviate hypogonadal consequences. It seems to be more effective when used in conjunction with chemotherapy in breast cancer patients than lymphoma patients [52–54]. This may be due to the temporal relationship of the diagnosis and initiation of treatment in breast cancer patients which is often delayed until after surgery as compared with lymphoma patients which is often immediate. A review of 14 previously published meta-analyses evaluating RCTs on this subject showed mixed results [55]. The majority showed a favorable impact on gonadal protection but others did not. This is most probably the result of the heterogeneous population of patients with different cancers and different chemotherapy protocols.

Additionally, GnRHa are often beneficial as adjuvant treatment in combination with chemotherapy for a subset of patients. Certainly it is clear that it is not deleterious to chemotherapy outcomes. Breast cancer patients, including those with estrogen receptor positive tumors, who

received GnRHa co-treatment had increased or no impact on disease free survival and overall survival compared to chemotherapy alone [52, 53]. In the Prevention of Early Menopause Study [52], a trend towards a higher rate of disease free survival in those individuals treated with GnRHa was observed, as well as a statistically significant higher rate of overall survival in this group compared to those treated with chemotherapy alone [52]. Similarly, in the Lambertini et al. study, a trend towards improved 5-year disease free survival was observed in the GnRHa group versus controls [53].

The impact of GnRHa on improving fertility potential is less clear. It is especially difficult because spontaneous pregnancy rates in women after breast cancer treatment are high enough to make clinical studies difficult to interpret. To date the ASRM recommends use of GnRHa in concert with other fertility preservation methods for patients who desire future pregnancies [56]. The use of GnRHa does not impede the use of other strategies for fertility preservation [55]. Additionally, the National Comprehensive Cancer Network and the St. Gallen International Expert Consensus panel guidelines support the use of GnRHa for the prevention of ovarian failure secondary to gonadotoxic chemotherapy [42]. For individuals who have completed childbearing but are still far from menopause, GnRHa can be considered with the goal of preserving of ovarian function.

13.22 Assisted Reproductive Technology

The application of ART for patients interested in fertility preservation depends on multiple factors, such as the type of cancer, treatment planned, time until treatment will start, and presence of a partner. There are multiple options available to the patient; some are considered established practices and others are experimental techniques. The overall goal is to preserve embryos, oocytes, or ovarian tissue for these women prior to treatment, so they may have options to reproduce in the future.

13.23 Embryo Cryopreservation

For postpubertal patients who have a committed male partner, embryo cryopreservation is an established technique for fertility preservation [56]. The age of the patient, number, stage and quality of the frozen embryos mainly determine the likelihood of success with embryo cryopreservation. The chances of a live birth from a cryopreserved embryo in a woman under the age of 40 years old are 28.5–38.7%. In general, the post-thaw survival rate of embryos ranges between 76 and 93%, and the clinical pregnancy rate is 37.5–62.5% [57].

A typical IVF cycle for fertility preservation can be done in a few weeks from start to finish; with traditional protocols, the time constraint has sometimes been dependent on where the patient is in her menstrual cycle. Some centers have offered natural cycle-IVF for breast cancer patients. During this process, a single oocyte is aspirated during a woman's spontaneous menstrual cycle. Unfortunately, cancellation rates are high and the pregnancy rates are very low for this protocol (7.2% per cycle and 15.8% per embryo transfer) [58, 59].

Most centers will use mild ovarian stimulation with a GnRH antagonist to prevent ovulation [60]. A new protocol in the starting time of ovarian stimulation has been reported. In those patients who present in the luteal phase of the menstrual cycle, a GnRH antagonist can be started immediately to help down-regulate LH and initiate luteolysis. Ovarian stimulation is started at the same time, thereby reducing the time to retrieval to less than 2 weeks. Reports in the literature have identified similar dosage requirements, numbers of oocytes retrieved, and fertilization rates in women who started in the luteal phase compared to those who started at in the follicular phase of their menstrual cycle [60, 61].

13.24 Oocyte Cryopreservation

Postpubertal female patients who do not have a male partner or do not wish to fertilize their eggs have the possibility to cryopreserve their oocytes for future use. The option of oocyte cryopreservation is no longer considered an experimental technique by the ASRM [62]. Freezing gametes, rather than embryos, also avoid ethical and legal considerations of embryo storage and disposal, which is of concern for some patients. The greatest concern about utilizing oocyte cryopreservation is that the success rate in the past was significantly lower than with embryo

cryopreservation. Early studies reported a low survival, fertilization, and pregnancy rate with thawed oocytes [63].

The structural complexity of the oocyte is most likely responsible for the reduced success rate in oocyte cryopreservation. Unlike fertilized embryos, the subcellular organelles in oocytes are far more complex and more sensitive to thermal injury [64, 65]. Improvements in the cryopreservation technique have led to significant improvements in the overall outcome of oocyte cryopreservation. The advent of vitrification for cryopreservation, rather than the slow-freeze protocol, has reduced the damage caused from ice-crystal formation and subsequent cellular damage [66]. Recent reports have seen survival rates after a thaw of 75–86%, fertilization rates of 77%, and live birth rates of 38% [67]. In those pregnancies that have resulted from oocyte cryopreservation, there appears to be no increase in chromosomal abnormalities, birth defects, or developmental deficits [68].

How many oocytes should be cryopreserved to have a good chance for future reproductive success? A survey of the literature on oocyte vitrification reported about 5% live birth rate per vitrified oocyte in women under the age of 36 years, meaning that on average, one live birth should be expected for about 20 vitrified oocytes [69] Other reports suggest live births with as little as 8–10 frozen-thawed oocytes [70].

13.25 Tamoxifen and Letrozole

There has been some concern that the high estrogen levels obtained during ovarian stimulation may decrease long-term survival for breast cancer patients. In those women with hormone-sensitive tumors, stimulation with tamoxifen, a nonsteroidal antiestrogen, or letrozole, an aromatase inhibitor, may be beneficial.

In a manner similar to clomiphene citrate, tamoxifen (40–60 mg) is started on day 2 or 3 of the cycle and given daily for 5–12 days. Letrozole has more recently been utilized as an ovulation induction agent as well. Adding letrozole at doses of 2.5 mg or 5 mg during a standard gonadotropin stimulation protocol has been shown to lower total serum estradiol levels [71].

To date, ovarian stimulation for fertility preservation has not been associated with an increase in breast cancer recurrence rates [71, 72].

13.25.1 Unconventional Stimulations Protocols

Studies on ovarian follicle development of large domestic species such as sheep and cattle demonstrated how follicular growth proceeds in waves [73]. Likewise in human, some investigators have documented the growth of non-atretic follicles during the luteal phase [74, 75]. This observation paved the way to a new stimulation protocol, aimed at collecting oocytes during both the follicular and luteal phase [76]. Recently, Ubaldi and collaborators demonstrated the non-inferiority of collecting oocytes during the luteal as compared to follicular phase in terms of maturity, fertilization, and development rate [77]. Therefore, the possibility to harvest oocytes during both phases of the cycle is a suitable tool for fertility preservation patients as it allows to maximize the number of gametes cryopreserved without having to repeat stimulation cycles and consequently delay cancer therapies.

13.26 Cryopreservation of Ovarian Tissue

Ovarian tissue cryopreservation is still considered an experimental procedure to preserve fertility in women who cannot delay oncological treatments to undergo ovarian stimulation necessary to create embryos or oocytes for cryopreservation [78]. It is also the only option currently available to prepubertal females. It involves removal of small strips of ovarian cortical tissue and freezing it as an avascular graft, in an effort to save thousands of primordial follicles for future use. When the patient is in remission, the ovarian tissue can then be transplanted back to the ovary or to the patient's subcutaneous tissue. It is also possible that one day the primordial follicles can be matured in vitro.

One of the concerns about regrafting tissue back to the patient is the theoretical risk of reintroducing cancer cells. This concern may limit its use in malignancies that have a high chance of involving the ovaries, including leukemias and potentially advanced stages breast cancers.

An additional limitation to the procedure is the loss of a large fraction of follicles during the initial ischemia that occurs after transplantation [79–81]. Previous work indicated that while loss due to freezing is relatively small, up to two-thirds of follicles are lost subsequent to transplantation.

The return of ovarian function and the occurrence of many pregnancies, both spontaneous and after IVF have been reported in patients after orthotopic transplantation [82–85]. It is difficult to estimate the pregnancy rate after ovarian transplantation because the number of cases reported is still low and the follow-up of women who received the re-grafting is still incomplete; however, a pregnancy rate of about 25% has been reported by the groups with the largest experience [86]. In some patients, this does not exclude the possibility that ovarian function resumed from areas in the ovary that had not been removed and reimplanted.

13.27 In Vitro Maturation

In vitro maturation is another potential modality for obtaining oocytes with little or no ovarian stimulation. In the context of fertility preservation for cancer patients, this approach appears most effective in patients who undergo ovarian tissue freezing, where few oocytes can be harvested from visible follicles on the ovarian strips. In addition, IVM can be attractive for patients who have multiple follicles (for example, polycystic ovaries) some of which will inevitably provide immature oocytes at the time of the retrieval. Patients with a clear contraindication to ovarian stimulation can also benefit from IVM. In these instances, an ultrasound is performed on day 6–8 of the cycle and human chorionic gonadotropin (hCG) is given to help increase oocyte maturity at the time of retrieval. Oocyte retrieval is scheduled 36 h later and immature oocytes are obtained and incubated in special culture media. If the oocytes mature in 24 h, as determined by extrusion of the first polar body, either the oocytes or embryos (if fertilization is attempted) can be cryopreserved.

This procedure is significantly less successful than those mentioned earlier. To date, over 300 live births have resulted from this procedure; however, significantly fewer embryos will be obtained per cycle and the chance of implantation, pregnancy, and live birth is lower than conventional IVF [87].

In vitro maturation of primordial follicles obtained from frozen thawed ovarian cortical strips is not yet possible. Contrary to in vitro maturation of oocytes from antral follicles, which requires days to mature, in vitro maturation of oocytes derived from primordial follicles would require months.

13.28 Whole Ovary Cryopreservation

One of the major limitations of ovarian tissue freezing is the result of ischemia-induced damage to the tissue, which consequently impacts the viability of the primordial follicles within the cortex. Recently, it has been suggested that freezing of the whole ovary instead of ovarian cortex could be used as an option for fertility preservation [88]. By harvesting the ovary and maintaining its vascular anastomosis, there are higher chances of follicle pool survival. Moreover, the entire follicular pool will be transplanted as opposed to a portion of it. Clearly, there are still several obstacles to this procedure, such as the complex microsurgery needed for transplantation and the technical limitations of the cryopreservation procedures [89]. If from one side the surgical limitations have been overcome by some, resulting in successful pregnancies in animals and in human [90–93], cryopreservation of an entire organ still represents a major challenge. Technically, it requires the penetration of an adequate amount of cryoprotectant into a rather complex tissue such as the ovary. Although no attempts of frozen/thawed human whole ovary transplantation have been performed to date, promising results have been reported for experimental models with large animals such as ewes and sheep [94, 95].

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Ovarian Reserve Testing

Paula Amato

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14.1 Introduction

The general purpose of ovarian reserve testing is to assess the quantity and quality of the remaining oocytes in an attempt to predict reproductive potential. Ovarian reserve testing should be performed in women older than 35 years who have not conceived after 6 months of attempting pregnancy (or women less than 35 who have not conceived after 1 year) and women at higher risk of diminished ovarian reserve, such as those with a history of cancer or other medical condition treated with gonadotoxic therapy and/or pelvic irradiation, or women who have had ovarian surgery for endometriomas.

Available tests for ovarian reserve include biochemical markers, i.e., FSH, estradiol, AMH, and inhibin B and ovarian ultrasound imaging, i.e., antral follicle count and ovarian volume [1] (Fig. 14.1). For general obstetrician-gynecologists, the most appropriate ovarian reserve screening tests to use in practice are basal FSH plus estradiol levels or anti-Müllerian hormone (AMH) levels. An antral follicle count (AFC) may also be useful if there is an indication to perform transvaginal ultrasonography. These screening tests are better predictors of oocyte yield from ovarian stimulation during in vitro fertilization (IVF) than rate of pregnancy. Low ovarian response to stimulation, usually defined as fewer than three to five developing follicles during an IVF cycle, is an indicator of a poor reproductive outcome. It is important to recognize, however, that a poor result from ovarian reserve testing does not signify an absolute inability to conceive and should not be the sole criteria considered to limit or deny access to infertility treatment. Although these tests are used to assess oocyte quantity and quality, the best surrogate marker for oocyte quality is age. At this time, ovarian reserve testing results cannot be extrapolated to predict the likelihood of spontaneous conception.

		Poor Response to Ovarian Stimulation		Nonpregnancy*				
Test	Cutpoint	Sensitivity	Specificity	Sensitivity	Specificity	Reliability	Advantages	Limitations
FSH (international units/L)	10–20	10–80	83–100	7–58	43–100	Limited	Widespread use	Reliability Low sensitivity
AMH (ng/mL)	0.2–0.7	40–97	78–92	†	†	Good	Reliability	Limit of detectability Two commercial assays Does not predict nonpregnancy
AFC (n)	3–10	9–73	73–100	8–33	64–100	Good	Reliability Widespread use	Low sensitivity
Inhibin B (pg/mL)	40–45	40–80	64–90	†	–	Limited	–	Reliability Does not predict nonpregnancy
CCCT, day 10 FSH (international units/L)	10–22	35–98	68–98	23–61	67–100	Limited	Higher sensitivity than basal FSH	Reliability Limited additional value to basal FSH Requires drug administration

Abbreviations: AFC, antral follicle count; AMH, antimüllerian hormone; CCCT, clomiphene citrate challenge test; FSH, follicle-stimulating hormone.
Note: Laboratories ELISA.
*Failure to conceive
†Insufficient evidence
Testing and interpreting measures of ovarian reserve: a committee opinion. Practise Committee of the American Society for reproductiveMedicine. Fertile Steril 2012;98:1407-15.

Fig. 14.1 Available tests for ovarian reserve include biochemical markers, i.e., FSH, estradiol, AMH, and inhibin B and ovarian ultrasound imaging, i.e., antral follicle count and ovarian volume

As women age oocytes decrease in quality and quantity and do not regenerate. The number of human oocytes in a female peaks at six to seven million during fetal life around midgestation, followed by profound atresia. Approximately one to two million oocytes are present at birth, 300,000–500,000 at the start of puberty, and 1000 at 51 years of age, which is the average age of menopause in the USA [2]. Factors such as genetics, lifestyle, environment, and medical issues including endometriosis, ovarian surgery, chemotherapy, and radiation can influence the quantity and quality of a woman's oocytes [1] (Fig. 14.2). Cross-sectional studies suggest that fertility declines before the onset of the premenopausal transition.

The goal of ovarian reserve testing is to add more prognostic information to the counseling and planning process so as to help couples chose among treatment options. Ovarian reserve tests should not be the sole criteria used to deny patients access to assisted reproductive technology or other treatments. Evidence of decreased ovarian reserve does not necessarily equate with inability to conceive.

In women from the general population, with no known history of infertility, who are attempting to conceive naturally, cumulative probability of pregnancy has been shown to decrease with age [3]. Cross-sectional studies have shown that chronological age is correlated with ovarian reserve, as measured by the size of the follicle pool in histologic studies of ovaries. Chronological age is strongly associated with other biomarkers of ovarian reserve including antral follicle count, anti-Müllerian hormone (AMH) levels, and early follicular phase follicle stimulating hormone (FSH) levels. Chronological age is an excellent predictor of fertility among infertile women undergoing assisted reproduction [4].

Existing research on ovarian reserve testing is often confusing because of heterogeneity among

- Advanced reproductive age (older than 35 years)
- Family history of early menopause
- Genetic conditions (eg, 45, X mosaicism)
- *FMR1* (Fragile X) premutation carrier
- Conditions that can cause ovarian injury (eg, endometriosis, pelvic infection)
- Previous ovarian surgery (eg, for endometriomas)
- Oophorectomy
- History of cancer treated with gonadotoxic therapy or pelvic irradiation
- History of medical conditions treated with gonadotoxic therapies
- Smoking

Data from Testing and interpreting measures of ovarian reserve:a committee opinion. Practice Committee of the American Society for Reproductive Medicine. Fertile Steril 2012;98:1407–15; Gurtcheff SE, Klein NA_Diminished ovarian reserve and infertility. Clin Obstet Gynecol 2011;54:666–74; te Velde ER, Pearson PL Thevariability of female reproductive ageing. Hum Reprod Update 200;8:141–54; *and* Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. ESHRE working group on poor Ovarian Response Definition. HUM Repord 2011;26:1616–24.

Fig. 14.2 Risk factors for diminished ovarian reserve

tested populations (the general population, infertility patients of all ages, infertility patients more than 35 years old, etc.). No single result is definitive, since findings must be interpreted in context and should be repeated or supplemented as appropriate. This chapter will discuss the application of ovarian reserve tests in evaluating fertility.

Clinical Case

A 38-year-old nulligravid female and her male partner present with a 3 year history of infertility. She has regular cycles. A hysterosalpingogram shows a normal uterine cavity and bilateral patent tubes. Her AMH level is 0.7 ng/mL. Day 3 FSH and estradiol levels are 11 IU/L and 20 pg/dL, respectively. Her partner had a semen analysis which showed normal semen parameters. The couple has failed three cycles of controlled ovarian stimulation using clomiphene citrate in combination with intrauterine insemination (IUI). How would you counsel this patient regarding her treatment options and chance of pregnancy success?

14.2 Basic Principles of Screening Tests

The purpose of using ovarian reserve testing as a screening test is to identify infertility patients at risk for decreased ovarian reserve, who are likely to exhibit a poor response to gonadotropin stimulation and to have a lesser chance of achieving pregnancy with IVF. Good screening tests have validity as measured by sensitivity and specificity. A valid test correctly categorizes persons who have disease as test positive (highly sensitive) and those without disease as test negative (highly specific).

For clinical purposes, specificity is the test characteristic that should be optimized to decrease false positives, or wrongly categorizing patients with normal ovarian reserve as having decreased ovarian reserve (DOR). Graphically, the sensitivity and specificity of different cut-points of a diagnostic test can be plotted as receiver operating characteristic (ROC) curves.

Positive predictive value (PPV) and negative predictive value (NPV) are screening test characteristics that change with the prevalence of disease (DOR) in the study population. The PPV is the probability that a woman who tests positive truly has DOR. The NPV is the probability that a woman who tests negative has normal ovarian reserve. Ovarian reserve testing is most useful in identifying DOR in women at high risk for DOR. Ovarian reserve testing in women at low risk for DOR will yield a larger number of false-positive results (lower PPV).

14.3 A Shortened Menstrual Cycle

As the ovary ages, the size of the follicle pool declines. Fewer follicles result in less production of AMH and inhibin. Because of lower inhibin levels, FSH rises prematurely or more rapidly leading to elevated early follicular phase serum FSH levels. Premature and rapid follicular growth results in elevated early follicular phase estradiol levels and a shortened follicular phase and overall shortened menstrual cycle. A short menstrual cycle length is associated with a lower probability of conceiving naturally or following IVF [5]. The cutoff value to define "short" cycle length varies by study ranging from 25 to 26 days.

14.4 Biochemical Markers of Ovarian Response

14.4.1 Basal Follicle Stimulating Hormone

Follicle stimulating hormone is released by the pituitary gland in response to gonadotropin-releasing hormone from the hypothalamus and is subject to negative feedback from estradiol and inhibin B. In the setting of a smaller follicular cohort and decreased estradiol and inhibin B levels, an increase in pituitary FSH secretion occurs, which can be identified as an elevated early follicular phase FSH level. This higher FSH level stimulates rapid ovarian follicular growth, which results in higher estradiol levels as well as a shorter follicular phase and menstrual cycle.

FSH is typically measured by immunoassay on cycle day 3. The basal FSH level can vary, so a single FSH value has limited reliability. Moreover, there is variability among different FSH assays. Although basal FSH is commonly used to assess ovarian reserve, and high values (>10–20 IU/L)

are associated with diminished ovarian reserve and poor response to ovarian stimulation, the test is not predictive of failure to conceive [6]. If FSH values are consistently elevated, a poor reproductive prognosis is likely; in contrast, a single elevated FSH value in women younger than 40 years predicts a lower oocyte yield during IVF but does not predict the rate of pregnancy [7].

Early follicular phase FSH levels have not been a sensitive test for nonpregnancy, suggesting that an elevated FSH is an excellent predictor of nonpregnancy following ART, but a normal level is not predictive of pregnancy. The value of serum or urinary FSH levels as predictors of reproductive potential in the general population has not been determined. Testing is cycle day specific (cycle days 2–4), limiting flexibility.

Women having an abnormally elevated FSH value will have DOR. The PPV of FSH for poor response to ovarian stimulation or failure to conceive is higher in older women. Limited evidence suggests that women with fluctuating FSH levels should not wait for the ideal cycle, wherein the FSH concentration is normal, to undergo IVF stimulation [8].

FSH is a late marker of dwindling ovarian function. With AMH and AFC demonstrating better predictive value for ovarian response than FSH, these are more likely to be the tests of choice. It remains unknown whether high FSH levels in women of reproductive age predict an earlier onset of menopause.

14.5 Basal Estradiol

Estradiol levels vary over the course of a menstrual cycle, peaking in both the late follicular and midluteal phases. As ovarian reserve declines, the follicular phase shortens because of decreasing feedback inhibition by follicles recruited during the previous cycle. As a result, an elevated day 3 estradiol level could reflect diminishing ovarian reserve.

Estradiol is released from the ovary during follicular development. The estradiol level is usually low (<50 pg/mL) on days 2–4 of the menstrual cycle. An elevated value (>60–80 pg/mL) in the early follicular phase can indicate reproductive aging and hastened oocyte development. Through central negative feedback, a high estradiol level can suppress an elevated FSH concentration into the normal range. The value of obtaining an estradiol level is that it allows the correct interpretation of a normal basal FSH level. Basal estradiol has low predictive accuracy for poor ovarian response and failure to conceive and, therefore, this test should not be used in isolation to assess ovarian reserve [9].

14.6 Anti-Müllerian Hormone

AMH is a homodimeric glycopeptide that is produced predominantly by granulosa cells. AMH is believed to downregulate FSH-mediated folliculogenesis. AMH expression is highest in secondary, preantral, and small antral follicles. AMH seems to have a role in selecting the dominant follicle in addition to generally mediating preantral follicular recruitment. AMH levels start undergoing a log-linear decline approximately 15 years prior to menopause and drop to very low levels approximately 5 years before menopause [10].

The anti-Müllerian hormone concentration is fairly stable within and between menstrual cycles [11]. As the number of ovarian follicles decreases with age, a concomitant decrease in AMH levels occurs, which reflects this age-related oocyte depletion [12]. Although an undetectable AMH level suggests diminished ovarian reserve and can identify individuals at risk of poor ovarian response to stimulation, undetectable and low AMH levels (0.2–0.7 ng/mL DSL ELISA) are not predictive of failure to conceive [13]. AMH levels may allow treatment to be tailored to each individual. Lower AMH levels are associated with reduced ovarian response to stimulation and high levels are associated with a brisk ovarian response to stimulation [13]. Although the AMH level is a good predictor of oocyte quantity, it may not provide information about egg quality. Young women with low AMH levels may have a reduced number of oocytes, but normal age-appropriate oocyte quality [14].

One limitation of AMH level testing is the variability of results between the available assays. In clinical practice, individual AMH level test results must be interpreted based on the normal range of the assay used [15]. AMH level testing is a useful screening test in women at high risk of diminished ovarian reserve and in women undergoing IVF [16, 17].

The nonpregnancy predictive value of a low AMH value appears to increase if older women

at risk for ovarian aging are tested. The use of AMH as a routine screening tool for DOR in a low-risk population is not recommended.

AMH level testing may be valuable in assessing ovarian reserve in young women with cancer before and after chemotherapy [18]. AMH may enable assessment of ovarian reserve before and after ovarian surgery and for women at high risk of primary ovarian insufficiency. AMH level testing may in future provide an accurate method of predicting the reproductive lifespan and the timing of menopause [19].

AMH has the advantage over FSH in that AMH levels remain relatively stable over the menstrual cycle, thus measurement does not need to be cycle day specific. A recent meta-analysis of earlier studies showed no significant association between AMH, modeled as a continuous variable, and pregnancy following ART [20]. However, more recent studies of larger sizes, modeling AMH using cutoff values, have shown lower odds of pregnancy and live birth following ART among women with low AMH levels [21–24].

High AMH values are associated with polycystic ovary syndrome (PCOS) and may identify women at risk for OHSS. It is believed that AMH remains a valid assay even when ovarian suppression occurs through oral contraceptives, although age-specific AMH percentiles decrease by 11% with oral contraceptives. [25].

14.7 Inhibin B

Inhibin B is a glycoprotein hormone that is secreted primarily by preantral and antral follicles. The serum concentration of inhibin B decreases with the age-related decrease in the number of oocytes. Inhibin B has central negative feedback that controls FSH secretion. Therefore, a decrease in inhibin B levels leads to increased pituitary FSH secretion and higher early follicular FSH levels.

Inhibin B levels exhibit high intra-cycle variability [16]. Inhibin B levels also vary significantly between menstrual cycles [16]. Inhibin B levels are a late finding for diminished ovarian reserve and typically start falling around 4 years prior to menopause [10] and are thus suboptimal. Inhibin levels are measured by immunoassay. Inhibin B is typically measured on the third day of the menstrual cycle. Inhibin B has limited sensitivity and specificity. This marker does not reliably predict a poor response to ovarian stimulation and thus, is not a recommended test.

14.8 Clomiphene Citrate Challenge Test

Clomiphene is a selective estrogen receptor modulator (SERM) that inhibits negative feedback inhibition by estradiol on the hypothalamus thereby increasing FSH secretion, which enhances follicular recruitment. Clomiphene can be used for ovulation induction and superovulation.

The clomiphene citrate challenge test is performed by measuring serum FSH on cycle day 3, administering 100 mg clomiphene citrate daily on cycle days 5–9, and again measuring serum FSH on cycle day 10. An elevated FSH level on day 10 of the CCCT is suggestive of diminished ovarian reserve. However, cycle-to-cycle variability in ovarian biomarkers limits the reliability of this provocative test [26]. The stimulated FSH level on cycle day 10 of the CCCT is predictive of poor ovarian response but is not predictive of failure to conceive [27]. Compared with the basal FSH level and the antral follicle count, the cycle-day-10 FSH level does not improve the prediction for poor ovarian response [27]. In studies comparing the test performance of basal (cycle day 3) and stimulated (cycle day 10) FSH values, stimulated FSH levels have higher sensitivity but lower specificity than basal FSH concentrations [27].

In summary, basal measure of FSH may be preferable to the CCCT, unless one is using the test to purposely increase sensitivity. It is unclear if the CCCT confers any benefit over basal FSH alone, and it is less cost-effective. The CCCT may have a role in helping to discriminate normal ovarian reserve from poor ovarian reserve in patients with potentially borderline function.

14.9 Home Fertility Tests

Available home fertility tests use a urine sample to assess the FSH level on cycle day 3. The tests are marketed directly to consumers. The limitations of these tests include misinterpretation of

instructions and results and the unavailability of a medical professional to interpret and explain the results [1]. Although these tests are used commonly by women at low risk of diminished ovarian reserve, the results may provide false reassurance or raise unnecessary concern.

14.10 Ultrasound Evaluation of Ovarian Reserve

14.10.1 Antral Follicle Count

The antral follicle count records the number of visible ovarian follicles (2–10 mm mean diameter) that are observed during transvaginal ultrasonography in the early follicular phase (cycle days 2–5). The number of antral follicles correlates with the quantity of remaining follicles and with the ovarian response during controlled ovarian stimulation. Good intercycle and interobserver reliability has been demonstrated [16]. A low antral follicle count is considered three to six total antral follicles and is associated with poor response to ovarian situation during IVF, but it does not reliably predict failure to conceive; in a meta-analysis, a low antral follicle count was a mean of 5.2 (2.11 SD) total antral follicles [28]. When AFC was compared to age, basal FSH, basal estradiol, AMH, inhibin B, and ovarian volume, antral follicle count, and AMH were the most significant predictors of poor response to ovarian stimulation but were not predictive of failure to conceive [29].

Low AFC cutpoints are highly specific for predicting poor ovarian response, but have lower sensitivity [28]. The high specificity of a low AFC makes the test useful for predicting poor ovarian response and treatment failure, but its clinical utility is limited by its low sensitivity. Inter- and intra-observer variability also may be limiting. There is debate regarding the effect of oral contraceptives on the measurement of antral follicle count.

14.11 Ovarian Volume

The calculation of ovarian volume requires ovarian measurements in three planes and the use of the formula for the volume of an ellipsoid: $D1 \times D2 \times D3 \times 0.52$. Mean ovarian volume, the average volume calculated for both ovaries from the same individual, is the value used to assess ovarian reserve. With age, changes in ovarian volume are concordant with the age-related decrease in ovarian follicles.

Several studies have demonstrated that low ovarian volume, typically <3 mL, predicts poor response to ovarian stimulation with high specificity and a wide range of sensitivity [16]. In general, ovarian volume has been a poor predictor of pregnancy.

The generalizability to patients with ovarian pathology is limited. Ovarian volume may vary in response to normal physiologic changes and coexisting medical conditions (such as endometriomas). Exogenous hormones can decrease ovarian volume. For these reasons, AFC is believed to be a better marker for ovarian reserve.

14.12 Combined Ovarian Reserve Tests

AMH and AFC are the most accurate predictors, but combinations of a few tests are only slightly better than a single test. Models of combined ovarian reserve tests do not significantly improve the ability to predict poor reproductive outcomes over a single ovarian reserve test [29]. Furthermore, the use of multiple ovarian reserve tests may increase the expense of screening. Further research is needed to determine an optimal combination of tests.

14.13 Repetitive Testing

Repetitive testing of biomarkers of ovarian reserve to assess reproductive potential appears to be of little benefit. In general, hormonal biomarkers do not appear to fluctuate greatly between cycles [30]. However, intercycle variability does appear to increase with age, suggesting that repetitive testing may be valuable among older women to rule out DOR. Fluctuations in biomarker values reflect diminished ovarian reserve. However, within a given individual, the probability of conceiving in a given ART treatment cycle does not appear to correlate with the cycle-specific biomarker level [8, 31].

14.14 Conclusions

The primary goal of ovarian reserve testing is to identify women at risk of decreased ovarian reserve, with a secondary goal of individualizing treatment strategies for each woman. Although these may predict ovarian response to infertility treatment, they do not reliably predict failure to conceive.

Generally, women of the same age with higher FSH levels seem to have lower fecundability. Younger women with elevated FSH levels often have much better fecundability than older women with comparably elevated FSH and age can be a better predictor of outcome than FSH. The assay in general has suboptimal sensitivity for both ovarian response and pregnancy rates, as reflected by receiver-operator curves. AMH and AFC have a better balance of sensitivity and specificity than FSH. AMH and AFC seem to be emerging as the best approaches to procreative testing [1] (Fig. 14.3). These measures can also be used to predict hyperstimulation.

No ovarian reserve test should be used as a sole criterion for the use of ART. Combined tests do not consistently improve the ability to predict ovarian response. Combined testing is unlikely to be cost-effective. Though some ovarian reserve tests appear better than others in predicting ovarian response to stimulation, most are limited at best in predicting pregnancy, and this predictive value is highly dependent on patient demographics within a study. The number of false-positive test results will increase when screening tests for DOR are used in low-risk populations.

In summary, biomarkers of ovarian reserve are associated with natural and treatment-related fertility. However, controversy remains as to their ability to predict reproductive potential. Cutoff values vary tremendously in the literature. For infertile women undergoing ART treatment,

Fig. 14.3 AMH and AFC seem to be emerging as the best approaches to procreative testing

Test	Details
FSH plus estradiol	• Serum level on cycle day 2–3 • Variation between cycles possible • High FSH value is associated with poor response to ovarian stimulation • Does not predict failure to conceive
AMH	• No specific timing for the test • Stable value within and between menstrual cycles • Low AMH value is associated with poor response to ovarian stimulation • Does not predict failure to conceive
AFC	• Number of visible follicles (2–1 0 mm) during transvaginal ultrasound • Performed on cycle days 2–5 • Number of antral follicles correlates with ovarian response to stimulation • Does not predict failure to conceive

Abbreviations: AFC, antral follicle count; AMH, antimOllerian hormone; FSH, follicle-stimulatin hormone.

these biomarkers tend to be highly specific but not sensitive for cycle failure (nonpregnancy). Biomarkers of ovarian reserve are being used as fertility tests in the general population. The value of these biomarkers as predictors will likely depend on the study population, with the highest predictive value likely to be observed in women at risk for ovarian aging (older reproductive age women). Among the laboratory biomarkers, AMH appears to have the most promise as a measure of reproductive potential; however, studies are especially limited in the general population. Further studies are needed to determine test characteristics in the prediction of natural fertility or infertility in the general population.

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Recurrent Early Pregnancy Loss

Paul R. Brezina and William H. Kutteh

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15.1 Introduction

Recurrent early pregnancy loss is a profound personal tragedy to couples seeking parenthood and a formidable clinical challenge to their physician. When to evaluate a couple and what constitutes a complete evaluation has recently been clarified in a committee opinion from the American Society for Reproductive Medicine (ASRM) [1].

While spontaneous abortion occurs in approximately 15% of clinically diagnosed pregnancies of reproductive aged women, recurrent pregnancy loss occurs in about 1–2% of this same population [2]. Great strides have been made in characterizing the incidence and diversity of this heterogeneous disorder and a definite cause of pregnancy loss can be established in over half of couples after a thorough evaluation [3, 4]. A complete evaluation will include investigations into genetic, endocrinologic, anatomic, immunologic, and iatrogenic causes. The occurrence of recurrent pregnancy losses may induce significant emotional distress and in some cases intensive supportive care may be necessary. Successful outcomes will occur in over two-thirds of all couples [3, 5].

Clinical Case

A 32-year-old G3P0030 female presents for a consultation with a chief complaint of "I cant hold a pregnancy." The patient states she easily conceives but has not progressed passed 9 weeks gestation in any of these pregnancies. In all three pregnancies, the patient has had a documented live intrauterine pregnancy with reassuring fetal cardiac activity at 6–7 weeks and then loss of fetal heart beat prior to the 9th week gestation. She has undergone one spontaneous miscarriage and two dilation and curettage (D&C) procedures. Genetic evaluation was not performed on the products of conception (POC) in any of these pregnancies.

- Past Medical History: Noncontributory
- Past Obstetric History:
  - One Spontaneous miscarriage
  - Two D&C for miscarriage, no karyotype obtained
- Past Gynecologic History
  - Menarche at age 13
  - Regular cycles every 28 days
  - No dysmenorrhea or bleeding irregularities
  - No history of abnormal pap smears or sexually transmitted diseases
- Past Surgical History
  - TWO D&C
- Medications: prenatal vitamins
- Allergies: No known allergies
- Social History
  - The patient is a third grade teacher
  - She denies alcohol, tobacco, or drug use

15.2 Evaluation and Treatment of the Patient

The following text will review how to approach the diagnostic and therapeutic approach to treating the patient described above. Firstly, recurrent pregnancy loss (RPL) will be defined and prevalence described. Following this, a detailed description of the various contributing factors of RPL and appropriate diagnostic and therapeutic strategies will be reviewed. A summary overview of a standard RPL workup is outlined in Fig. 15.1. In addition, a novel algorithm for the evaluation of pregnancy loss is outlined in Fig. 15.2. The chapter outline will be the following with the goal of a systematic and comprehensive discussion and review of the current medical literature for RPL.

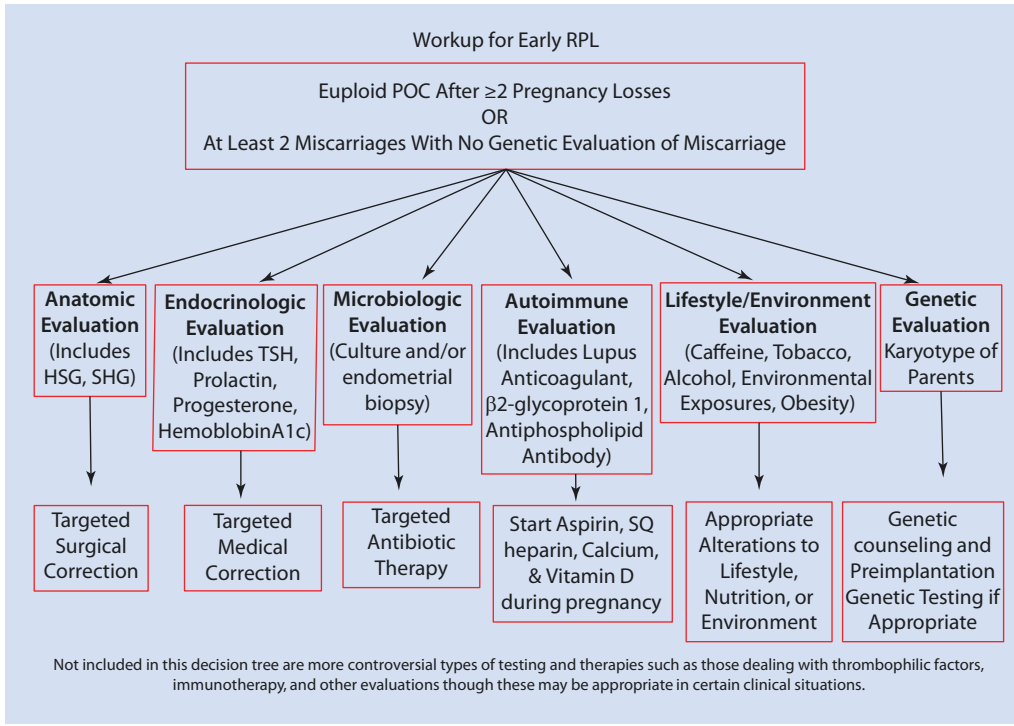

Fig. 15.1 Workup for early RPL. This figure outlines an algorithm for the full workup of early RPL. *Arrows* are provided that guide the reader through various outcomes possible during the RPL evaluation and appropriate "next steps" in diagnostic and therapeutic management

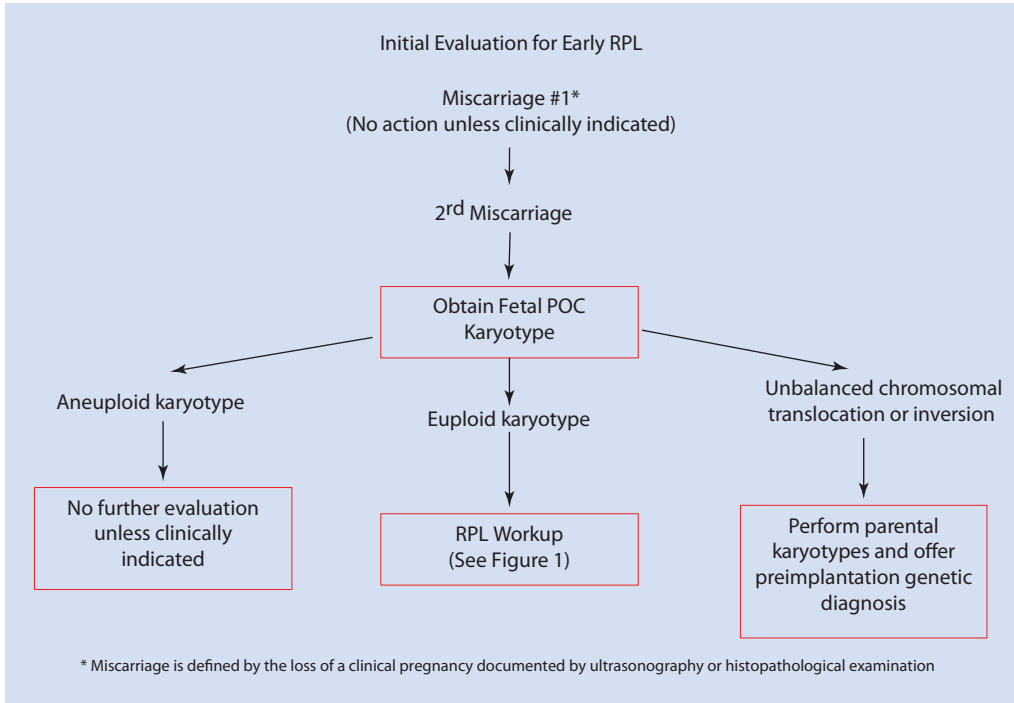

Fig. 15.2 Initial evaluation for Early RPL. This figure outlines an algorithm for the initial evaluation of early RPL. *Arrows* are provided that guide the reader through various outcomes possible during the RPL evaluation and appropriate "next steps" in diagnostic management

15.3 Definition of Pregnancy Loss

The traditional definition of recurrent pregnancy loss (RPL) included those couples with three or more spontaneous, consecutive pregnancy losses. Ectopic and molar pregnancies are not included. The ASRM has defined RPL as "a distinct disorder defined by 2 or more failed clinical pregnancies" [6, 7]. For purposes of determining if an evaluation for RPL is appropriate, pregnancy "is defined as a clinical pregnancy documented by ultrasonography or histopathological examination" [6, 7]. Several studies have recently indicated that the risk of recurrent miscarriage after two successive losses is similar to the risk of miscarriage in women after three successive losses; thus, it is reasonable to start an evaluation after two or more consecutive spontaneous miscarriages to determine the etiology of their pregnancy loss, especially when the woman is older than 35 years of age, or when the couple has had difficulty conceiving [8].

Those couples with primary recurrent pregnancy loss have never had a previous viable infant, while those with secondary recurrent loss have previously delivered a pregnancy beyond 20 weeks and then suffered subsequent losses. Tertiary recurrent loss refers to those women who have multiple miscarriages interspersed with normal pregnancies.

15.4 Recurrence Risk

The main concerns of couples with recurrent miscarriage when they present to our Recurrent Pregnancy Loss Center are to find the cause and to establish the risk of recurrence. In a first pregnancy, the overall risk of loss of a clinically recognized pregnancy loss is 15% [9, 10]. The true risk of early pregnancy loss, however, is estimated to be around 50% because of the high rate of losses that occur before the first missed menstrual period [11]. Furthermore, as women age, this rate likely rises due to chromosomal errors introduced through meiotic nondisjunction errors during oocyte maturation. Studies that evaluated the frequency of pregnancy loss, based on highly sensitive tests for quantitative hCG, indicated that the total clinical and preclinical losses in women aged 20–30 is approximately 25%, while the loss rate in women aged 40 or more is at least double that figure [9–11]. The ability to predict the risk of recurrence is influenced by several factors including maternal age, parental and fetal karyotypes, the gestational age at which prior losses occurred, and the presence of various maternal laboratory findings [9, 12–17].

15.5 Etiologies, Diagnosis, and Treatment of Recurrent Pregnancy Loss

15.5.1 Introduction

Traditionally, the chief causes of RPL have been thought to be due to embryonic chromosomal abnormalities, maternal anatomic abnormalities such as a uterine septum, luteal phase defects, and antiphospholipid antibodies. Other factors such as infection and hypercoagulable state have also been considered but to a somewhat lesser degree.

When to initiate an RPL workup has been a source of recent debate. Classically, conducting a workup for RPL was recommended after three miscarriages. Recent data does not necessarily support this traditional evaluation protocol [18, 19]. The evaluation of healthy women after a single loss is usually not recommended as this a relatively common, sporadic event. However, the risk of another pregnancy loss after two miscarriages is only slightly lower (24–29%) than that of women with three or more spontaneous abortions (31–33%) [10]. Therefore, evaluation and treatment can reasonably be started after two consecutive miscarriages [3, 6, 7, 11]. Furthermore, additional testing such as chromosomal testing of the products of conception from a second miscarriage may confer a cost savings measure [18, 19]. Based on available data, we outline a new strategy for the workup of RPL (Figs. 15.1 and 15.2).

An evaluation of an RPL patient should always include a complete history, including documentation of prior pregnancies, any pathologic tests that were performed on prior miscarriages, any evidence of chronic or acute infections or diseases, any recent physical or emotional trauma, history of cramping or bleeding with a previous miscarriage, any family history of pregnancy loss, and any previous gynecologic surgery or complicating factor. A summary of the diagnosis and management of recurrent pregnancy loss includes an investigation of genetic, endocrinologic,

Table 15.1 Diagnosis and management of recurrent pregnancy loss

Etiology	Diagnostic evaluation	Therapy
Genetic	Karyotype partners	Genetic counseling
	Karyotype POC[a]	Donor gametes, PGD[b]
Anatomic[e]	Hysterosalpingogram	Septum transection
	Hysteroscopy	Myomectomy
	Sonohysterography	Lysis of adhesions
	Transvaginal 3D US[c]	
Endocrinologic	Midluteal progesterone	Progesterone
	TSH	Levothyroxine
	Prolactin	Bromocriptine, dostinex
	HgBalC	Metformin
Immunologic	Lupus anticoagulant	Aspirin
	Antiphospholipid antibodies	Heparin + Aspirin
	Anti β2 glycoprotein	
	Antithyroid antibodies (?)	Levothyroxine
Microbiologic[d]	Mycoplasma/ureaplasma	Antibiotics
Psychologic	Interview	Support groups
Iatrogenic	Tobacco, alcohol use, obesity	Eliminate consumption
	Exposure to toxins, chemicals	Eliminate exposure

[a]Products of conception
[b]Preimplantation genetic diagnosis
[c]Transvaginal three-dimensional ultrasound
[d]Indications for testing and treatment are not clearly established
[e]Only one of these diagnostic tests needed

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anatomic, immunologic, microbiologic, and iatrogenic causes (Table 15.1).

We outline a proposed algorithm for the evaluation and treatment of RPL (Figs. 15.1 and 15.2). Under this new schema, no diagnostic/therapeutic action is recommended following one miscarriage unless clinically indicated, such as in the case of a submucosal myoma. A fetal karyotype is recommended after the second miscarriage. Products of conception (POC) to send for karyotype may be obtained from early nonviable pregnancies either via traditional D+C. POC may be sent for traditional karyotype or, as we recommend, be sent for 23 chromosome pair microarray evaluation.

The results of this POC karyotype guide further evaluation. If the POC are found to be aneuploid, no further evaluation or treatment is recommended at that juncture because the cause for the loss is known, though all future early miscarriages should also be subject to karyotypic evaluation. If an unbalanced chromosomal translocation or inversion is identified in the fetal POC, then the workup would focus on performing parental karyotypes and offering appropriate therapeutic options such as preimplantation genetic testing. If the fetal POC are found to be chromosomally normal, then a full RPL workup is to be done. If the fetal POC karyotypes have not been performed, then we recommend a full RPL workup after at least two miscarriages. The full RPL workup is outlined in Fig. 15.1.

What constitutes a full RPL is a topic of debate. We recommend including an anatomic

evaluation, endocrinologic evaluation, testing for autoimmune factors, evaluating lifestyle and environmental factors, microbiologic factors and obtaining parental karyotypes if the karyotypic status of prior POC is unknown. Not included in this evaluation are more controversial types of testing and therapies such as those dealing with thrombophilic factors, immunotherapy, and other evaluations though these may be appropriate in certain clinical situations. Below, we describe the physiologic background, diagnostic approaches, and therapy of the various components of our proposed RPL workup. In addition, we will address other more controversial proposed etiologies of RPL.

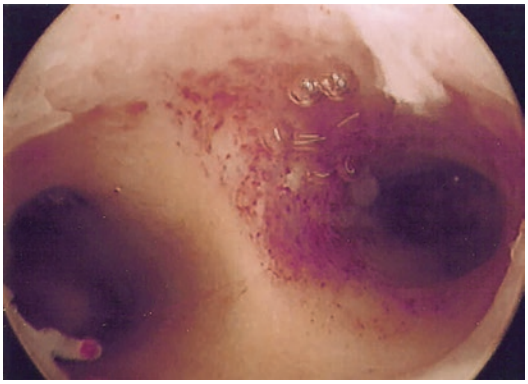

Fig. 15.3 Uterine septum at hysteroscopy. This picture shows uterine septum as visualized during hysteroscopy

15.6 Anatomic Causes of RPL

Anatomic causes of RPL are typically diagnosed using hysterosalpingography (HSG) or sonohysterography. Hysteroscopy, laparoscopy, or magnetic resonance imaging (MRI) can supplement these tests as needed. Recently, transvaginal three-dimensional ultrasonography has been introduced and has allowed an accurate and non-invasive diagnosis of congenital uterine anomalies. The treatment of congenital and acquired uterine anomalies often involves corrective surgery.

15.6.1 Congenital Malformations

Congenital malformations of the reproductive tract result from failure to complete bilateral duct elongation, fusion, canalization, or septal resorption of the Müllerian ducts. Müllerian anomalies were found in 8–10% of women with three or more consecutive spontaneous abortions who underwent hysterosalpingography or hysteroscopic examination of their uteri [3, 4, 20]. Inadequate vascularity compromising the developing placenta and reduced intraluminal volume have been theorized as possible mechanisms leading to pregnancy loss.

The most common congenital abnormality associated with pregnancy loss is the septate uterus (Fig. 15.3). The spontaneous abortion rate is high, averaging about 65% of pregnancies in some studies [21]. A septum is primarily composed of fibromuscular tissue that is poorly vascularized. This lack of vascularization may compromise decidual and placental growth. Alternatively, an uterine septum may impair fetal growth as a result of reduced endometrial capacity or a distorted endometrial cavity [21]. Uncontrolled studies suggest that resection of the uterine septum results in higher delivery rates than in women without treatment. Other congenital abnormalities such as uterine didelphys, bicornuate, and unicornuate uterus are more frequently associated with later trimester losses or preterm delivery.

15.6.2 Intrauterine Adhesions

Intrauterine trauma resulting from endometrial curettage or endometritis is associated with a risk for the development of adhesions. Intrauterine adhesions (synechiae) are an acquired uterine defect that has been associated with recurrent miscarriage. The severity of adhesions may range from minimal to complete ablation of the endometrial cavity. The term Asherman's syndrome is often used to describe intrauterine adhesions associated with oligo or amenorrhea. These adhesions are thought to interfere with the normal placentation and are treated with hysteroscopic resection. The insertion of an intrauterine balloon catheter for one week is recommended after resection of synechiae by some physicians to help prevent reformation of adhesions. During this time antibiotic prophylaxis with doxycycline (100 mg twice a day) is given to prevent endometritis. Patients may also be given estrogen and progestin for 1 month.

15.6.3 Intrauterine Masses

Intrauterine cavity abnormalities, such as submucosal leiomyomas and polyps, can contribute to pregnancy loss. Depending on the leiomyoma size and location, it may partially obliterate or alter the contour of the intrauterine cavity, providing a poorly vascularized endometrium for implantation or otherwise compromising placental development. Uterine leiomyomas and polyps may also act like an IUD, causing subacute endometritis (Figs. 15.4 and 15.5). Until recently, it was felt that only submucous leiomyomas should be surgically removed prior to subsequent attempts at pregnancy. However, several recent studies investigating the implantation rate in women undergoing in vitro fertilization have clearly demonstrated decreased implantation with intramural leiomyomas in the range of 30 mm [22]. Minimally invasive surgery is the method of choice to correct the uterine septum as well as to remove fibroids, adhesions, and polyps [23].

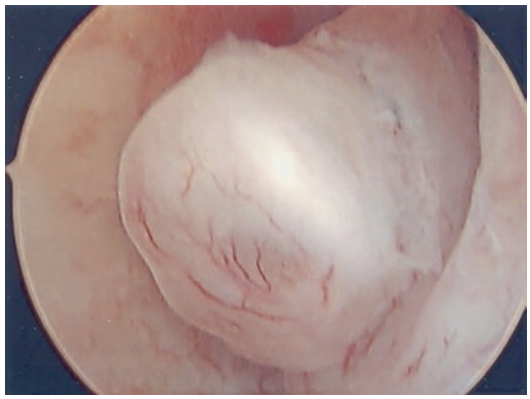

Fig. 15.4 Endometrial Polyp at Hysteroscopy. This picture shows an endometrial polyp as visualized during hysteroscopy

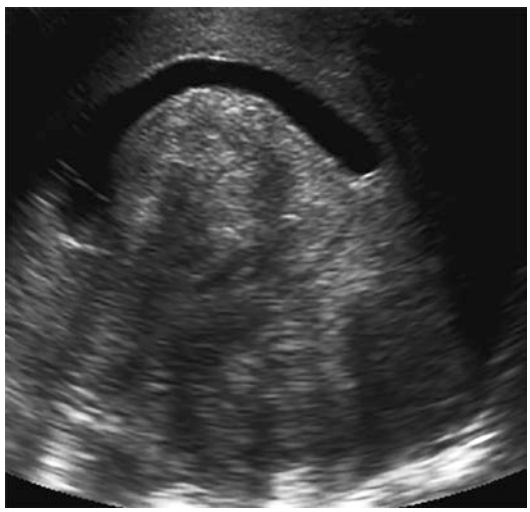

Fig. 15.5 Submucosal uterine myoma on saline infusion sonogram. This picture shows a submucosal myoma as visualized during saline infusion sonogram

15.6.4 Incompetence Cervix

Cervical incompetence can be considered as an acquired uterine anomaly that is associated with RPL. The diagnosis of cervical incompetence is based on the presence of painless cervical dilation resulting in the inability of the uterine cervix to retain a pregnancy. Cervical incompetence commonly causes pregnancy loss in the second, rather than first, trimester [24]. It may be associated with congenital uterine abnormalities such as septate or bicornuate uterus. Rarely, it may be congenital following in-utero exposure to Diethylstilbestrol. It is postulated that most cases occur as a result of surgical trauma to the cervix from conization, loop electrosurgical excision procedures, dilation of the cervix during pregnancy termination, or obstetric lacerations.

15.7 Endocrinologic Causes of RPL

Endocrine factors may contribute to 8–12% of recurrent pregnancy loss. Therefore, an endocrinologic evaluation is a critical component of the RPL workup [25].

15.7.1 Luteal Phase Deficiency

Maintenance of early pregnancy depends on the production of progesterone by the corpus luteum. Between 7 and 9 weeks of gestation the developing placenta takes over the progesterone production. Luteal phase deficiency (LPD) is defined as an inability of the corpus luteum to secrete progesterone in high enough amounts or for too short a duration. The preponderance of evidence suggests that LPD is a preovulatory event most likely linked to an alteration in the preovulatory estrogen stimulation which may indicate poor oocyte quality and a poorly functioning corpus

luteum [26, 27]. Classically, the diagnosis is based upon results of endometrial biopsy, though this is currently not recommended as a diagnostic modality. Most authors advocate the measurement of serum progesterone levels in the luteal phase for the diagnosis of LPD with levels below 10 ng/ml considered abnormal [28]. However, progesterone levels are subject to large fluctuations because of pulsatile release of the LH hormone. Moreover, there is a lack of correlation between serum levels of progesterone and endometrial histology [29]. While conflicting data exist, a recent Cochrane review evaluating 15 trials concluded that there was a benefit to the routine administration of progesterone to all women with a history of RPL [30, 31]. Progesterone is available either as intravaginal suppositories (50–100 mg twice daily starting the third day after LH surge and continuing for 8–10 weeks) or as intramuscular injections (50 mg IM daily).

15.7.2 Untreated Hypothyroidism

Untreated hypothyroidism may increase the risk of miscarriage. A study of over 700 patients with recurrent pregnancy loss identified 7.6% with hypothyroidism [32]. Hypothyroidism is easily diagnosed with a sensitive TSH test and patients should be treated to become euthyroid (defined for the purposes of RPL as between 1.0 and 2.5 uIU/mL) before attempting a next pregnancy [1, 6, 30, 33]. The exact value at which to treat subclinical hypothyroidism is somewhat controversial, however, with some professional societies not recommending treatment with TSH levels less than 4 uIU/mL [34]. It has also been suggested that thyroid antibodies are elevated in women with recurrent pregnancy loss. A retrospective study of 700 patients with recurrent pregnancy loss demonstrated that 158 women had antithyroid antibodies but only 23 of those women had clinical hypothyroidism on the basis of an abnormal TSH value [35]. The presence of antithyroid antibodies may imply abnormal T-cell function, and therefore, more of an immune dysfunction rather than an endocrine disorder may be responsible for the pregnancy losses. The Endocrine Society recommends that patients with RPL be treated to keep a TSH level of between 1.0 and 2.5 uIU/mL in the first trimester [33]. For TSH levels found to be between 2.5–10 mIU/mL, a starting levothyroxine dose of at least 50 μg/d is recommended [33].

15.7.3 Abnormal Glucose Metabolism

Patients with poorly controlled diabetes are known to have an increased risk of spontaneous miscarriage, which is reduced to normal spontaneous loss rates when women are euglycemic preconceptually [36]. Testing for fasting insulin and glucose is simple and treatment with insulin-sensitizing agents can reduce the risk of recurrent miscarriage [37]. More recently, determining the average load of blood glucose through testing of hemoglobin A1C has become an increasingly utilized modality to evaluate insulin resistance [6, 7]. Because there is strong evidence that obesity and/or insulin resistance are associated with an increased risk of miscarriage, weight reduction in obese women is a first step in the treatment. Metformin seems to improve pregnancy outcome, but the evidence for this treatment is limited to a few cohort studies. Metformin is a Category B medication in the first trimester of pregnancy and appears to be safe. Other endocrine abnormalities, such as thyroid disorders and diabetes, should be corrected prior to conception.

15.7.4 Hyperprolactinemia

Normal circulating levels of prolactin may play an important role in maintaining early pregnancy Data from animal studies suggest that elevated prolactin levels may adversely affect corpus luteal function; however, this concept has not been proven in humans [38]. A recent study of 64 hyperprolactinemic women showed that bromocriptine therapy was associated with a higher rate of successful pregnancy and that prolactin levels were significantly higher in women who miscarried [39].

15.7.5 Diminished Ovarian Reserve

Follicle stimulating hormone (FSH) is thought to be a marker of the number of follicles available for recruitment on any given menstrual cycle. Therefore, elevated levels of FSH in the early follicular phase of the menstrual cycle are

representative of diminished ovarian reserve; a condition in which there a low number of follicular units available for recruitment. More recently, other markers, such as decreased anti-Müllerian hormone, have been introduced to identify diminished ovarian reserve. Although the frequency of elevated day 3 FSH levels in women with recurrent miscarriage is similar to the frequency in the infertile population, the prognosis of recurrent miscarriages is worsened with increased day 3 FSH levels [40]. Although no treatment is available, testing may be helpful in women over the age of 35 with recurrent pregnancy loss, and appropriate counseling should follow.

15.8 Autoimmune/Thrombotic Factors as the Cause of RPL

15.8.1 Immunologic Disorders

Autoimmune Factors: Maternal Response to Self

In some instances, there is a failure in normal control mechanisms that prevent an immune reaction against self, resulting in an autoimmune response [41]. Autoantibodies to phospholipids, thyroid antigens, nuclear antigens, and others have been investigated as possible causes for pregnancy loss [32]. Antiphospholipid antibodies include the lupus anticoagulant, anti-beta 2 glycoprotein I antibodies, and anticardiolipin antibodies. There is still controversy concerning testing for other phospholipids, but an increasing number of studies suggest that antibodies to phosphatidylserine are also associated with pregnancy loss [42]. Women with systemic lupus erythematosus and aPL have increased risks for miscarriage compared to those with lupus and negative aPL [43].

Antiphospholipid Antibody Syndrome (APS):

APS is an autoimmune condition characterized by the production of moderate to high levels of antiphospholipid antibodies (APS) and certain clinical features [44] (Table 15.2). The presence of antiphospholipid antibodies (anticardiolipin and lupus anticoagulant) during pregnancy is a major risk factor for adverse pregnancy outcome [45]. In large meta-analysis of studies of couples with recurrent abortion, the incidence of antiphospholipid antibody syndrome was between 15 and 20% compared to about 5% in nonpregnant women without a history of obstetrical complications [46, 47].

Several mechanisms have been proposed by which antiphospholipid antibodies (aPl) might mediate pregnancy loss. Classically, it was believed that aPl antibodies induced thromboses in vessels surrounding the placental-maternal unit, resulting in placental infarction and fetal death. However, recent data suggests that the primary

15

Table 15.2 Clinical and laboratory characteristics of antiphospholipid antibody syndrome

Clinical	Laboratory
Pregnancy morbidity	IgG aCL[a]
≥1 unexplained death at ≥10 weeks or	IgM aCL[a]
Delivery at ≤34 weeks with severe PIH or	Positive lupus anticoagulant test
Three or more losses before 10 weeks	IgG anti β2 Glycoprotein 1[a]
Thrombosis	IgG anti β2 Glycoprotein 1[a]
Venous	
Arterial, including stroke	

Modified from Miyakis et al. [40]
Patients should have at least one clinical and one laboratory feature at some time in the course of their disease. Laboratory tests should be positive on at least two occasions
GPL IgG phospholipid units, *MPL* IgM phospholipid units, *PIH* pregnancy induced hypertension
[a]≥99th percentile

mechanism by which aPl antibodies lead to miscarriage may be via a deleterious effect conferred directly on trophoblastic cells and/or endothelial cells [48, 49]. aPl antibodies can interact with cultured human vascular endothelial cells with resultant injury and/or activation [49]. Furthermore, aPl have been demonstrated to inhibit secretion of human placental chorionic gonadotropin and to inhibit the expression of trophoblast cell adhesion molecules (alpha 1 and alpha 5 integrins, E and VE cadherins). These mechanisms could explain RPL secondary to aPl antibodies early in the first trimester [46].

Antiphospholipid antibody syndrome (APS) is treated with a combination of low dose heparin (5000–10,000 units subcutaneously every 12 h) and low dose aspirin (81 mg PO daily) appears to be effective and may reduce pregnancy loss by 54% in women with APS [41, 50]. Aspirin alone does not appear to reduce miscarriage rates [51]. Unfractionated heparin is preferred to low molecular weight heparin (LMWH) based on available data [52].

Treatment with steroids is not recommended based on current evidence [46, 50]. Aspirin should be started preconceptually and heparin should be started after the first positive pregnancy test [49]. Treatment should be continued until the time of delivery because these women are at an increased risk for thrombosis. Post-partum thromboprophylaxis is reasonable for a short interval to prevent thrombosis when the risk is high [47]. The adverse reactions associated with heparin include bleeding, thrombocytopenia, and osteoporosis with fracture. Calcium (600 mg twice daily) with added vitamin D supplementation (400 IU daily) and weight–bearing exercise are encouraged to decrease the risk of osteoporosis. In any pregnant woman starting on heparin, the platelet count should be monitored weekly for the first 2 weeks after initiation, and after any dosage change. Women with APS should consider avoiding the use of estrogen-containing oral contraceptives in the future.

Immunotherapy

Immunotherapy for alloimmune disorders is based on the hypothesis that spontaneous abortion occurs due to a failure of maternal immunological adaptation to the developing conceptus resulting in a form of transplantation rejection. Although some randomized double blinded studies have shown an increase with therapies such as paternal leukocyte immunization, trophoblast immune infusion, intravenous intralipid therapy, and immunoglobulin infusion in successful pregnancy outcomes, other have not confirmed these results [53–56]. A Cochrane review of 19 trials of various forms of immunotherapy did not show significant differences between treatment and control groups [57]. There is currently insufficient evidence to recommend the use of these therapies for RPL. Testing for Th-1 and Th-2 profiles, parental HLA-profiles, alloantibodies, NK cells, antiparental cytotoxic antibodies or embryotoxic factor assessment are currently not clinically justified.

Antinuclear Antibodies

Approximately 10–15% of all women will have detectable antinuclear antibodies regardless of their history of pregnancy loss. Their chance of successful pregnancy outcome is not dependent on the presence or absence of antinuclear antibodies. Treatments such as steroids have been shown to increase the maternal and fetal complications without benefiting livebirths [58]. Thus, routine testing and treatment for antinuclear antibodies is not indicated.

Lifestyle issues in RPL

Microbiologic Causes of RPL

Certain infectious agents have been identified more frequently in cultures from women who have had spontaneous pregnancy losses [59]. These include *Ureaplasma urealyticum*, *Mycoplasma hominis,* and *Chlamydia*. Increasing evidence is accumulating that chronic endometritis is more frequently identified in women with RPL and that directed antibiotic therapy may improve outcomes. It is important to be aware that none of these pathogens have been causally linked to RPL. Because of the association with sporadic pregnancy losses, the ease of diagnosis, and the increasing evidence for the role of infection in RPL some clinicians will test women with RPL and treat for the appropriate pathogen in both parents.

Appropriate antibiotic therapy should be instituted in both parents when cervical infections are identified. Infections with *Mycoplasma*, *Ureaplasma*, and *Chlamydia* are treated with doxycycline 100 mg twice daily by mouth for 14 days. For those that fail treatment based on a test of cure culture, the options are to extend treatment of both partners to 30 days or to use ofloxacin 300 mg daily for 14 days for both partners.

15.8.2 Thrombotic Disorders

Thrombophilias are thought to be responsible for more than half of maternal venous thromboembolisms in pregnancy, however ACOG recommends that only patients with a personal or family history of thromboembolic events should be tested [60]. The association of inherited and acquired thrombophilias with adverse pregnancy outcome is still being investigated but current evidence suggests a limited role [61]. The recommended evaluations for patients with a personal or strong family history of thrombosis are:

(a) **Factor V Leiden** screening with activated protein C resistance using a second generation coagulation assay is probably the most cost-effective approach. Patients with a low APC resistance ratio (<2.0) should then be genotyped for the factor V Leiden mutation.
(b) **Prothrombin G20210A** gene mutation using PCR.
(c) **Antithrombin activity** with normal levels between 75–130%.
(d) **Protein S activity** with normal levels between 60–145%.
(e) **Protein C activity** with normal levels between 75–150%.

15.9 Genetic Factors as the Cause of RPL

There are a variety of genetic factors that may result in failure of a pregnancy to develop. These include aneuploidy (the gain or loss of a chromosome), chromosomal imbalances as a result of parentally harbored translocations or inversions, deletions or duplications of genetic information within chromosomes, and single-gene mutations. Broadly, genetic factors may be divided into embryonic errors derived from known parental chromosomal abnormalities and embryonic errors that arise de novo in apparently chromosomally normal parents [11].

15.9.1 Parental Chromosomal Disorders

Parental chromosome anomalies occur in 3–5% of couples with RPL as opposed to 0.7% in the general population. These include **translocations**, **inversions**, and the relatively rare **ring chromosomes**. Balanced translocations are the most common chromosomal abnormalities contributing to recurrent pregnancy loss [11]. In recurrent pregnancy loss, this abnormality is found more frequently in the female partner at a ratio of 2:1 up to 3:1 (female:male). In addition to genetic errors resulting from the parental balanced translocation, recent data from preimplantation genetic testing has shown that embryos resulting from parents harboring a balanced reciprocal translocation also have rates of unrelated chromosomal aneuploidy at rates exceeding 35% [62].

Translocations are where chromosome segments are exchanged between nonhomologous chromosomes. There are two main types of translocation: reciprocal and Robertsonian. Robertsonian translocations involve two acrocentric chromosomes (numbers 13, 14, 15, 21, 22) that fuse near the centromere region with loss of the short arms. Studies indicate that when the Robertsonian translocation is maternal, there is a greater risk that the fetus will exhibit an unbalanced phenotype [63]. In reciprocal translocations, the type of rearrangement results from breakage of nonhomologous chromosomes, with reciprocal exchange of the broken-off segments [64]. Balanced reciprocal translocations are thought to directly contribute to both infertility and recurrent pregnancy loss (RPL) [65].

Parental balanced chromosomal errors such as translocations or inversions are diagnosed through karyotyping of couples. Chromosomal abnormality in one of the parents can be found in upto 3–5% of couples who experience multiple spontaneous abortions [3]. If no fetal POC are available and the couple has a history of at least two fetal losses, we recommend obtaining parental karyotypes. The treatment of parental balanced chromosomal translocations/inversions using comprehensive chromosomal screening coupled with trophectoderm biopsy in preimplantation genetic testing has been recommended by the Society of Obstetricians and Gynecologists of Canada because it is associated with favorable outcomes [66].

15.9.2 Recurrent Aneuploidy

The first chromosomally abnormal abortus was documented in 1961, and since then a large body of data on the chromosomal status of spontaneous abortuses has accumulated. The overall

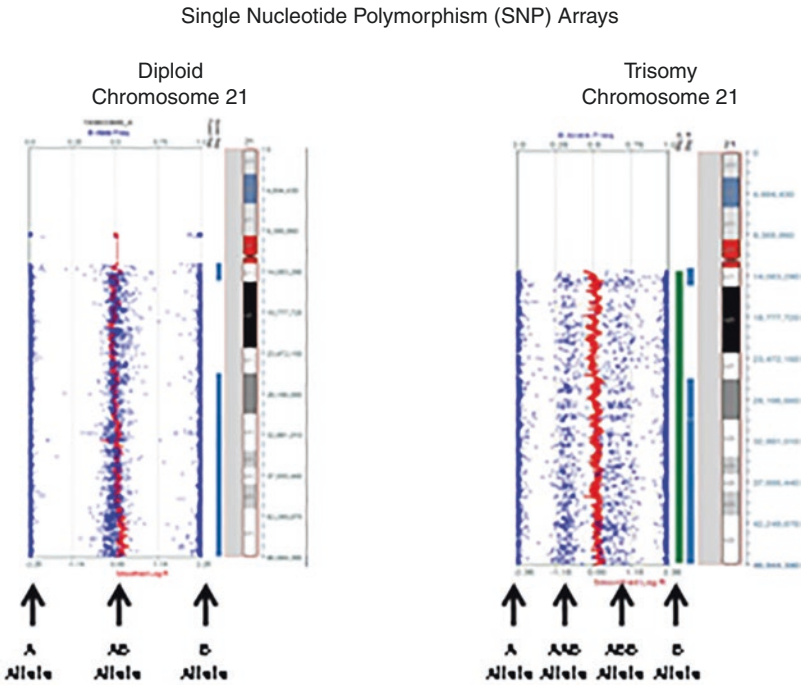

Fig. 15.6 Single Nucleotide Polymorphism (SNP) arrays. In this figure a diploid chromosome (chromosome 21 on *left*) and a trisomy chromosome (chromosome 21 on *right*) can be seen on SNP array. Note the presence of the A, B, and heterozygote AB band in the diploid sample and the two heterozygote bands (AAB and ABB) associated with the trisomy sample

frequency of chromosome abnormalities in spontaneous abortions is at least 50% [17, 67–70]. Of these abnormalities, most are numerical: 52% are trisomies, 29% are monosomy 45, X, 16% are triploidies, 6% are tetraploidies, and 4% are structural rearrangements [71]. Evidence suggests that some couples are at risk for conceptions complicated by recurrent aneuploidy. Empirically, the birth of a trisomic infant places a woman at an approximately 1% increased risk for a subsequent trisomic conceptus [72]. Germline mosaicism has been reported in recurrent cases of Down syndrome and may also be responsible for recurrent aneuploidy in some couples [73]. The vast majority of embryonic aneuploidy is thought to be a result of maternal meiotic nondisjunction during oocyte development though abnormalities arising from the sperm component are possible, especially in couples with male factor infertility.

Due to the high rate of aneuploidy known to be associated with pregnancy loss, genetic evaluation of the products of conception (POC) is increasingly common to determine the cause of RPL in cases of missed abortion [11]. In such cases an endometrial biopsy or sample taken at the time of D&C may be sent for chromosomal karyotypic evaluation. Traditionally, these evaluations have been performed using G-banded karyotyping [74]. This technology required live cells be cultured and evaluated. Many studies in RPL patients from POC using G-banded karyotyping yielded a disproportionately large number of 46XX results. Many of these normal 46XX results are thought to be the result of maternal rather than fetal cells, a phenomenon termed maternal cell contamination (MCC) [74].

Current technologies including single nucleotide polymorphism (SNP) array and next generation genomic sequencing (NGS) are capable of producing a POC Karyotype with a smaller sample cell population than is required with G-banded karyotyping [74] (Fig. 15.6). Additionally, these technologies are capable of differentiating placental versus maternal cell lines through comparison of the sample DNA to a maternal blood sample [11]. Therefore, SNP array and NGS are capable of ruling out MCC even when 46XX results are obtained. For this reason, POC testing is recommended to be performed using an NGS or SNP array platform capable of ruling out MCC (Fig. 15.7).

There is significant debate in the professional community as to how prevalent aneuploidy is among RPL embryos. Emerging data suggest that RPL patients may have lower rates of embryonic aneuploidy in first trimester miscarriages as

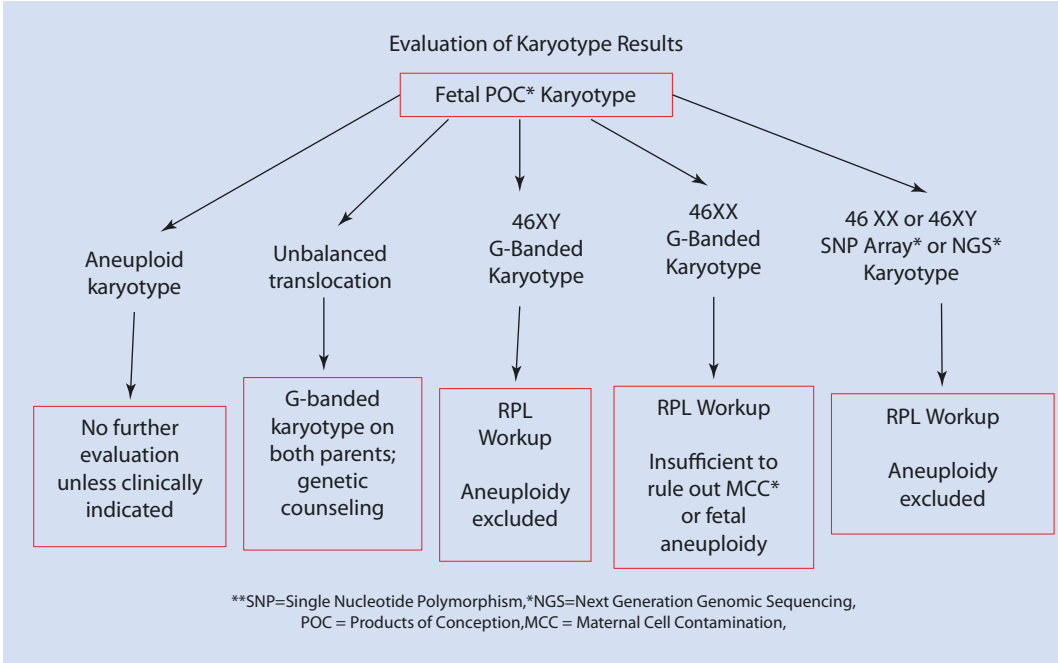

Fig. 15.7 Evaluation of karyotype results. This figure outlines an algorithm for the evaluation of karyotypes obtained from products of conception obtained from a failed pregnancy. Note the difference in clinical management based on the technology used to perform the genetic analysis

compared to all women. For example, a study evaluating 4873 embryos via single nucleotide polymorphism (SNP) microarray showed the rate of aneuploidy found using trophectoderm biopsy at the blastocyst stage in RPL was significantly lower (32%) than the rate of aneuploidy found with cleavage stage biopsy (61%) [11, 75]. Therefore, it is possible that couples with a diagnosis of RPL may produce embryos with aneuploidy at a high rate. It is less clear, however, what percentage of these aneuploid embryos progress to the blastocyst stage. Other data from small studies suggest that the rate of aneuploidy in embryos in RPL is higher than 65% [75–77]. Therefore, the true rate of embryonic aneuploidy in couples with a diagnosis of RPL is currently a topic of great debate among reproductive endocrinologists.

15.9.3 Chromosomal Deletions and Duplications

In contrast to aneuploidy which is the gain or loss of an entire chromosome, chromosomal deletions and duplications of discrete regions of chromosomes in euploid embryos are also possible. The impact of these regions of deletions and duplications is still unclear. Small deletions and duplications are also known as copy number variations and have been shown to be extremely common and are present in most phenotypically normal adults. Recently however, many of these copy number variations have been linked to a variety of medical disorders. The impact of larger deletions and duplications is ill defined as it relates to embryo development. However, certain deletions and duplications in which a critical area of a chromosome is significantly altered may certainly lead to significant medical disorders or arrest of a developing embryo.

15.9.4 Developmental Errors in Euploid Embryos

Another cause of first trimester miscarriage appears to be failure of chromosomally normal embryos to develop properly. Much as a women with Müllerian agenesis are chromosomally normal but have a separate developmental abnormality, serious developmental abnormalities that involve vital structures may result in euploid embryos. Small studies have suggested that the

rates of significant anatomic abnormalities in chromosomally normal embryos taken from first trimester miscarriages in women with RPL may exceed 25% [76].

15.9.5 Preimplantation Genetic Testing

Genetic causes of RPL may be subdivided into embryo abnormalities that are the result of known parental abnormalities (such as parental balanced translocations or inversions) and embryo aneuploidy in parents believed to be chromosomally normal. Preimplantation genetic testing is a technology that is designed to minimize the effects of these and other embryonic genetic abnormalities [78]. Preimplantation genetic testing is accomplished by performing an in vitro fertilization (IVF) cycle, removing a cell(s) from the resultant embryos or oocytes, evaluating this cell for genetic abnormalities, and using the results to determine which embryos are ideal for uterine transfer. When a known parental genetic abnormality is identified, as in parental balanced translocations, this practice is referred to as preimplantation genetic diagnosis or PGD. When this process is executed to determine which embryos are aneuploid in parents believed to be chromosomally normal, the procedure is referred to as preimplantation genetic screening or PGS.

PGD for structural aberrations such as translocations and inversions is a generally accepted utilization of preimplantation genetic testing. In contrast, PGS is far more controversial. A 2007 publication in the New England Journal of Medicine by Mastenbroek et al. showed no benefit to PGS [79]. This was followed by major medical societies discouraging the routine use of PGS [80]. Since this time, newer technologies, such as microarrays, have been introduced that are capable of evaluating the ploidy status of all 23 pairs of chromosomes instead of the 9–14 pairs of chromosomes evaluated with older fluorescence in situ hybridization (FISH) technologies [81]. Additionally, performing embryo biopsy at the blastocyst, as opposed to the cleavage stage, seems to confer superior pregnancy rates [80–83].

Recent data evaluating pregnancy rates in RPL patients using 23 chromosome microarrays are encouraging [84, 85]. PGS as a treatment modality is currently widely utilized. Of 27,630 PGT IVF cycles reported over the past 10 years to the ESHRE PGD Consortium, collecting data from around the globe, 61% (n = 16,806) were performed for PGS [81]. Despite this high rate of utilization, however, large and well-conducted randomized controlled trials are necessary to firmly establish the efficacy of PGS and define which patient populations may benefit from these technologies. Furthermore, PGS is far from a full proof technology in determining the ploidy status of an embryo. Embryo mosaicism, the presence of more than one cell line within the same embryo, has been shown to be as high as 50% in cleavage stage embryos and as high as 10% in blastocysts [75, 86]. Therefore, the cell taken at the time of embryo biopsy may not always be representative of the genetic composition of the embryo. Furthermore, technical limitations such as failure to successfully amplify genomic DNA, genomic contamination, and the possibility for human error may be another source of diagnostic error. It is vital that providers explain to patients contemplating utilizing preimplantation genetic testing the risks, benefits, and alternatives of the technology in detail. PGS may be a viable option for couples with recurrent pregnancy loss shown to be resultant from embryo aneuploidy to reduce the incidence of future miscarriage.

15.10 Lifestyle Issues and Environmental Toxins

Couples experiencing recurrent pregnancy losses are often concerned those toxins within the environment may have contributed to their reproductive difficulty. It is important that health care providers, counseling patients about exposures to substances in the environment, have current and accurate information in order to respond to these concerns.

15.10.1 Cigarette Smoking

Cigarette smoking reduces fertility and increases the rate of spontaneous abortion. The data evaluating smoking and miscarriage are extensive and involve approximately 100,000 subjects. The studies suggest a clinically significant detrimental effect of cigarette smoking that is dose dependent, with a relative risk for miscarriage among

moderate smokers (10–20 cigarettes a day) being 1.1–1.3 [87]. Patients should be aggressively counseled to stop cigarette smoking prior to attempting pregnancy.

15.10.2 Alcohol Consumption

Alcohol consumption associated with a risk of spontaneous abortion [88]. The minimum threshold dose for significantly increasing the risk of first trimester miscarriage appears to be two or more alcoholic drinks per week [89, 90]. When personal habits, cigarette smoking and alcohol are utilized in the same individual, the risk of pregnancy loss may increase fourfold. Couples should be counseled concerning these habits and strongly encouraged to discontinue these prior to attempting subsequent conception [91].

15.10.3 Obesity

Obesity, defined as a body mass index over 30, has been associated with an increased risk of miscarriage. Obesity (BMI > 30 kg/m^2) has been shown to be an independent risk factor for first trimester miscarriage [26]. The association is strongest in women with BMI > 40. The etiology of this phenomenon is unclear. However, many studies have linked obesity to a generalized increase in systemic inflammatory responses [92].

15.10.4 Caffeine Intake

Several studies have shown that caffeine in excess of 300 mg/day (3 cups of coffee per day) is associated with a modest increase in spontaneous abortion, but it is not clear if this relationship is causal [93].

15.10.5 Ionizing Radiation

The studies of atomic bomb survivors in Japan showed that in utero exposure to high-dose radiation increased the risk of spontaneous abortions, premature deliveries, and stillbirths [94]. Diagnostic X-rays in the first trimester delivering less than 5 rads are not teratogenic [95]. Large doses (360–500 rads) used in therapeutic radiation, however, induce abortion in offspring exposed in utero in the majority of cases. Adverse effects of chronic low-dose radiation on reproduction have not been identified in humans [87].

15.11 Outcome

The treatment of RPL should be directed at the cause. Given the good outcome for most couples with unexplained recurrent abortion in the absence of treatment, it is difficult to recommend unproven therapies, especially if they are invasive and expensive. Explanation and appropriate emotional support are possibly the two most important aspects of therapy. In fact, in one study, antenatal counseling and psychological support for couples with recurrent abortion and no abnormal findings resulted in a pregnancy success rate of 86% compared with a success rate of 33% for women who were given no specific antenatal care [21].

In approximately 60% of all cases of recurrent pregnancy loss, a complete evaluation will reveal a possible etiology [3, 4]. Abnormal findings during the evaluation should be corrected prior to attempting any subsequent pregnancy. If no cause can be found, the majority of couples will eventually have a successful pregnancy outcome with supportive therapy alone [96]. Once a pregnancy occurs, the patient should be monitored closely with evaluation of quantitative hCG levels at least twice and documentation of adequate progesterone levels. Early sonography should be scheduled and any encouraging results should be communicated to the couple. In women with a history of RPL, the presence of a normal embryonic heart rate between 6 and 8 gestational weeks that is confirmed with repeat sonography in one week is associated with a live birth rate of 82% [97].

Couples who have experienced RPL want to know what caused the miscarriage. Unexplained reproductive failure can lead to anger, guilt, and depression. Anger may be directed toward their physician for not being able to solve their reproductive problems. Feelings of grief and guilt following an early loss are often as intense as those following a stillbirth and parents experience a grief reaction similar to those associated with the death of an adult. The couple should be assured that exercise, intercourse, and dietary indiscretions do not cause miscarriage. Any questions or

concerns that the couple may have about personal habits should be discussed.

Thankfully, the prognosis for women with RPL to eventually deliver with medical therapy is quite good. A recent study evaluating 987 women with RPL found that the chances of achieving a live birth within 5 years of initial physician consultation was in excess of 80% for women under the age of 30 and approximately 60–70% for women ages 31–40 [5].

Women who suffer RPL have already begun to prepare for their baby, both emotionally and physically, as compared to couples with infertility who have never conceived. When a miscarriage occurs, a couple may have great difficulty informing friends or family about the loss. Feelings of hopelessness may continue long after the loss. Patients may continue to grieve and have episodes of depression on the expected due date or the date of the pregnancy loss. Participation in support groups or referral for grief counseling may be beneficial in many cases (SHARE, Pregnancy and Infant Loss Support, Inc., ► www.nationalshareoffice.com).

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Ovulation Induction

Togas Tulandi and Hélène S. Weibel

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16.1 Introduction

About a third of women with infertility are found to have an ovulatory disorder. It often presents as menstrual irregularity from irregular menstrual cycles to complete amenorrhea. The most common underlying condition is polycystic ovarian syndrome (PCOS). A wide range of interventions are available for ovulation induction, starting from lifestyle changes to medications and historically surgery. Oral ovulation-inducing agents include selective estrogen receptor modulators and aromatase inhibitors. If unsuccessful, the next line of treatment is gonadotropin injection.

This chapter reviews the classification of ovulatory disorders, the indications for ovulation induction and the treatments, including their success rates and associated complications.

Clinical Case

A 29-year-old woman presents with a history of infertility. Her menstrual cycle length is typically 45–60 days. Her investigation shows mildly elevated testosterone, and normal FSH, thyroid studies, and serum prolactin. A tubal evaluation and semen analysis was normal.

16.2 Clinical Presentation and Classification of Ovulatory Disorders

The diagnosis of ovulatory disorder is often made with a good menstrual history. Oligomenorrhea or amenorrhea is both suggestive of ovulatory disorders. Tests to detect ovulation include basal body temperature measurement, mid-luteal serum level of progesterone, and documentation of ovulation by serial ultrasonography examinations.

Ovulatory disorders are classified by the World Health Organization as WHO Group I, II, and III.

16.2.1 WHO Group I: Hypogonadotropic Hypogonadal Anovulation

This group includes women with low to normal endogenous gonadotropins levels as well as low serum estradiol level. It is usually related to hypothalamic dysfunction resulting in inconsistent GnRH secretion. About 5–10% of anovulatory women are classified in this category. Examples of conditions presenting as hypogonadotropic hypogonadal anovulation are anorexia nervosa, excessive exercise, excessive weight loss, stress, and hypothalamic conditions such as Kallmann syndrome.

16.2.2 WHO Group II: Eugonadotropic Euestrogenic Anovulation

This group includes women with normal level of endogenous gonadotropins, sometimes elevated luteinizing hormone (LH) and normal estradiol level. Most of anovulatory women fall into this category. Examples of conditions include polycystic ovarian syndrome (PCOS, Fig. 16.1) or less commonly, late onset congenital adrenal hyperplasia (CAH).

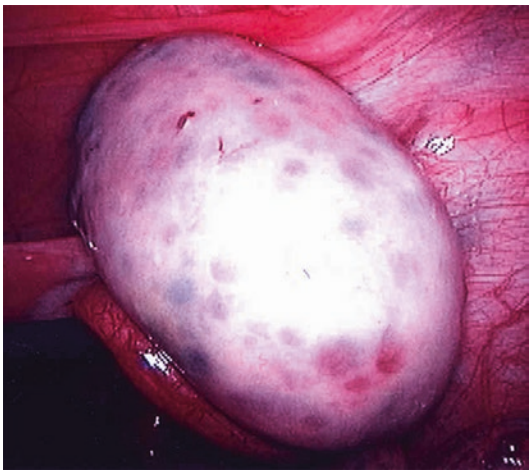

Fig. 16.1 Laparoscopic image of polycystic ovaries after transvaginal oocyte collection

16.2.3 WHO Group III: Hypergonadotropic Anovulation

This group includes women with elevated level of gonadotropins such as follicle stimulating hormone (FSH) and low or normal estradiol level. Typically, it is associated with amenorrhea, and accounts for about 10–20% of ovulatory disorders. Examples include idiopathic premature

ovarian failure (POF), as well as POF related to underlying conditions, such as Turner Syndrome and Fragile X carriers. Iatrogenic POF after treatment with gonadotoxic substances such as chemotherapy or pelvic radiation therapy is also included in this group.

An additional cause of anovulation that is not included in the above classification, but is sometimes considered a fourth group, is hyperprolactinemia. High prolactin level inhibits gonadotropin secretion and results in oligo or amenorrhea. Investigations and treatment of hyperprolactinemia is discussed in a different chapter.

16.3 Treatments for Ovulatory Disorders

Various options are available for the treatment of ovulatory disorders. The choice of treatment depends on the underlying cause of anovulation. A thorough history and physical examination of both partners should be first performed to rule out any underlying pathologies and determine the best course of action. As a general rule, the least invasive options with fewer side effects are used first and more invasive treatments with higher risks kept as a last resort. Interventions can be as simple as weight loss and exercise, followed by oral agents such as clomiphene citrate or aromatase inhibitors, with or without insulin-sensitizing agents. More aggressive therapies include gonadotropins injections. With the availability of in-vitro fertilization, surgery such as laparoscopic ovarian drilling or wedge resection has become outdated.

16.4 Lifestyle and Exercise

Metabolic syndrome and high body mass index is associated with anovulation, often due to polycystic ovarian syndrome (PCOS). PCOS is associated with insulin resistance, hyperandrogenism, oligo or amenorrhea and polycystic ovarian morphology on ultrasound. It is a very common cause of secondary amenorrhea in reproductive age female. There is good evidence that in overweight patient, lifestyle modifications including diet and exercise leading to weight loss can lead to spontaneous return of ovulation. The serum levels of testosterone and insulin decrease after weight loss in obese-PCOS women [1]. Clinical signs of hyperandrogenism such as hirsutism and acne subsequently improve [2]. In fact, weight loss by diet and exercise alone can lead to resumption of ovulation in overweight patients presenting with PCOS [3].

Another important aspect to consider in such patients is pregnancy-related morbidity including gestational diabetes, preeclampsia, hypertension, cesarean delivery and postpartum weight retention. Fetal and child morbidity and mortality are also more prevalent due to increased stillbirth, prematurity, congenital anomalies, macrosomia leading to possible birth injury and childhood obesity. Due to obesity, these patients might have difficulties with anesthesia during labor and increased wound infection. Initiation and sustainment of breastfeeding is also less likely in obese mothers [4].

The first line of treatment for overweight anovulatory women desiring pregnancy is therefore weight loss and exercise. It often leads to spontaneous resumption of ovulation and provides future mothers with the opportunity for a healthier pregnancy with less risk of complications.

16.5 Clomiphene Citrate

Clomiphene citrate is the first agent used for ovulation induction, first described in the 1950s [5]. It is given orally and cleared through the liver and then excreted in the stool. About 85% of a dose is eliminated after 6 days, however traces can remain in the circulation for much longer.

16.5.1 Pharmacology and Mechanism of Action

It is a selective estrogen receptor modulator (SERM) which acts as an agonist or antagonist on the estrogen receptors, depending on the target tissue. The currently manufactured product is a mixture of two isomers, in an approximate 3:2 ratio of enclomiphene and zuclomiphene. Enclomiphene seems to be the most potent isomer of the two, and the one responsible for the ovulation induction effect. It is usually cleared more rapidly than zuclomiphene, which does not seem to have any clinical relevance [6].

The mechanism of action of clomiphene citrate is believed to happen at the level of the hypothalamus, where it binds to the estrogen receptors and

depletes its concentration by interfering with the normal replenishing mechanism. This depletion of estrogen receptor is viewed by the hypothalamus as low circulating estrogen. It then triggers an alteration in GnRH pulsatility resulting in an increase of circulating gonadotropins stimulating the ovary. The subsequent increase in circulating levels of FSH and LH stimulates folliculogenesis and ovulation. It is expected to occur 5–12 days after clomiphene citrate administration [6].

16.5.2 Dosage and Administration

The usual starting dose of clomiphene citrate is 50–100 mg orally every day, for 5 days, starting on day 2–5 of the menstrual or induced cycle. The standard effective dose of CC ranges from 50 to 250 mg/days, although doses in excess of 100 mg/days are not recommended by the US Food and Drug Administration (FDA) and seem to add little to clinical pregnancy rates [6]. Response can be evaluated by ultrasound examination around day 10 of the cycle to view and measure the developing follicle. Urinary LH kits can also be used at midcycle to detect the presence of ovulation. A spontaneous menses at the expected timing of the cycle is also indicative of ovulation.

16.5.3 Side Effects and Risks

In general, clomiphene citrate is well tolerated. Common side effects include mood swings and hot flushes, but are rarely persistent or severe enough to discontinue the treatment. These side effects are temporary and short lived. Visual symptoms such as blurred or double vision, scotomata, and light sensitivity are rare and reversible. Yet, there have been reports of persistent visual symptoms and severe complication such as optic neuropathy [7]. If such visual disturbances occur, CC should be discontinued. Other less specific side effects include pelvic discomfort, breast tenderness, and nausea, observed in 2–5% of patients treated with clomiphene citrate [6].

Treatment with CC is associated with the risks of multiple pregnancies and rarely ovarian hyperstimulation syndrome (OHSS). Multiple pregnancies are due to multifollicular development, and usually results in twin pregnancies. The rate of twin pregnancies is around 8% in anovulatory women and 2.6–7.4% in those with unexplained infertility. The rate of high order multiple pregnancy is much lower (0.08–1.1%) [6]. Ovarian hyperstimulation syndrome rarely occurs with CC. There is no good evidence that the use of clomiphene citrate per se increases the risk of miscarriage, congenital malformations, or ovarian cancer [6].

However, clomiphene citrate can have a negative effect on estrogen responsive tissue such as the endometrium and the cervix. It can result in a thin endometrium and luteal phase defect [8]. Ovulation trigger with hCG or progesterone supplementation may improve the luteal phase. Strategies to avoid a thin endometrium include starting clomiphene citrate on day 1 of the cycle, lowering the dose to 25 mg daily, or supplementation with exogenous estrogen near the time of ovulation [9].

16.5.4 Effectiveness

About 75–80% of patients with PCOS will ovulate with clomiphene citrate treatment. The conception rate per cycle in ovulatory women after clomiphene citrate treatment is up to 22% [10]. Over a half of the patients will ovulate with 50 mg daily dose. Those who do not ovulate with 50 mg may ovulate at higher doses using a step-up regimen with doses escalation by 50 mg with each anovulatory cycle (22% with 100 mg, 12% with 150 mg, 7% with 200 mg, and 5% with 250 mg). Higher doses sometimes required in patients with increased BMI [6].

16.6 Aromatase Inhibitors

Aromatase inhibitors were first developed to lower estrogen levels in the context of breast cancer treatments. The application of this medication for ovulation induction in WHO Group II anovulatory patients, mainly in PCOS was first described in 2001 [11].

16.6.1 Pharmacology and Mechanism of Action

This compound is a cytochrome P450 inhibitor of the aromatase enzyme complex, which results in downregulation of estrogen production. Lower

circulating estrogen levels inhibit the negative feedback loop to the hypothalamus, which results in stronger GnRH pulses release. This further stimulates the pituitary gland to produce more FSH, which induces development of follicles in the ovaries.

The lack of depletion of estrogen receptors by aromatase inhibitors offers a few potential advantages over clomiphene citrate. First, because the estrogen receptors are intact at the level of the hypothalamus, the normal negative feedback loop is also intact. As the growing dominant follicle produces more estrogen, it leads to normal atresia of the smaller follicles and produces monofollicular growth and lower risk of multiple pregnancy. Aromatase inhibitors have less anti-estrogenic effect on the endometrium and cervix than clomiphene citrate [12].

The most commonly use aromatase inhibitor for ovulation induction is its third generation, such as letrozole. It has a relatively short half life of 45 h, offering the advantage of being cleared from the system rapidly, often even before conception occur, therefore limiting the exposure of the potential early pregnancy [13].

16.6.2 Dosage and Administration

Letrozole is given for 5 days starting on day 3–7 of the menstrual cycle. The dose used varies from 2.5 to 7.5 mg orally per day, administration of a single dose of 20 mg on day 3 of the menstrual cycle has also been described [13]. It appears that the optimal dose is 5 mg daily for 5 days [14].

16.6.3 Side Effects and Risks

Despite growing evidence for its effectiveness and safety, FDA and Health Canada do not approve its use for ovulation induction. The concern about congenital malformations in offspring born after letrozole treatment is unfounded [3, 12, 15]. In general, side effects of letrozole use for ovulation induction are mild and limited. These include hot flushes, dizziness, and fatigue [3].

The risk of multiple pregnancy with letrozole is lower than that with clomiphene citrate. In a randomized trial, the multiple pregnancy rate after letrozole was 3.4% versus 7.4% in the clomiphene citrate group [3].

16.6.4 Effectiveness

In a recent randomized study, the cumulative live birth rates were significantly higher at 27.5% in the letrozole group and 19.1% in the clomiphene group (relative risk [RR] 1.44, 95% CI 1.10–1.87). The cumulative ovulation rate was also higher with letrozole, at 62% versus 48% with clomiphene citrate (RR 1.28, 95% CI 1.19–1.37) [16]. A Cochrane review on the subject reported a higher live birth rate with letrozole (275 per 1000) than with clomiphene (188 per 1000) and a higher clinical pregnancy rate (262 per 1000 for letrozole and 202 per 1000 for clomiphene). The miscarriage rates were similar between the two groups (123 per 1000 for letrozole and 134 per 1000 for clomiphene). There was no case of OHSS reported in any of the above-mentioned study [12].

Letrozole is as effective as and maybe more effective than clomiphene citrate for ovulation induction in PCOS patients. However, patients should be informed of the risks and benefits of both options, and be aware that letrozole use is off label for such indication.

16.7 Gonadotropins

Exogenous gonadotropins were first derived from the urine of menopausal women, and their initial clinical use was in the 1950s. Today, purified versions of these gonadotropins are available, as well as recombinant forms [17]. These compounds consist of either FSH or LH alone, or a combination of both and could be administered intramuscularly or subcutaneously.

16.7.1 Pharmacology and Mechanism of Action

Gonadotropins act by directly stimulating the FSH and LH receptors on the granulosa and theca cells of the ovary, resulting in proliferation of one or more follicles. Ovulation induction with gonadotropin is usually accompanied by ovulation trigger with hCG. HCG and LH share the same alpha-subunit, making hCG able to bind to LH receptor and to mimic the endogenous LH surge. Since recombinant and human hCG is readily available, easy to administer, less expensive and requires smaller doses than recombinant LH, it is the most common

used compound to trigger ovulation. GnRH agonist could also be used to trigger ovulation, using the "flare effect" of gonadotropins at the initial use to mimic the LH surge. However, this would not be efficacious in women with hypothalamic amenorrhea who have an endogenous low LH and FSH.

Gonadotropins are effective agents for ovulation induction in WHO Group I (hypogonadotropic hypogonadal anovulation) by supplementing lack of FSH and LH to the HPO axis. In such patients, choosing a preparation containing LH, or adding recombinant LH to the regimen is important, as there is no endogenous production of LH. LH stimulation of the theca cells will produce androgens, which will then be used as a substrate for granulosa cells to produce the estrogen necessary for proper follicular maturation. Gonadotropins are also effective in WHO Group II (eugonadotropic euestrogenic anovulation patients) by augmenting the level of endogenous FSH and LH present.

16.7.2 Dosage and Administration

Gonadotropin is usually started between day 2 and 5 of the menstrual or progesterone-induced cycle. In women with hypothalamic anovulation, the treatment can be started any time. A baseline ultrasound examination should be first done to exclude other ovarian pathology and to evaluate the endometrial thickness, especially in anovulatory PCOS patients. Dosage is based on patient's age, ovarian reserve, and previous response to gonadotropins, but is usually started at a relatively low dose, and increased as needed according to the patient's response.

Typical starting doses are between 37.5 and 75 IU per day. Ultrasound examination is performed after 4–5 days of injection and then every 1–3 days depending on each individual's response. When a mature follicle has developed, usually around size 16–18 mm, exogenous hCG is given to trigger ovulation. Response to gonadotropins can also be followed with estradiol level. It is usually not measured routinely, but can be helpful in cases of atypical or prolonged responses to gonadotropins. Estradiol concentrations generally range between 150 and 300 pg/mL per dominant follicles [18].

To reduce the occurrence of multiple pregnancy, ovulation should not be triggered if multiples follicles are present. Ideally, hCG is given when no more than two mature follicles of 16–18 mm are observed. HCG is administered in a single intramuscular or subcutaneous injection of 5000–10,000 IU. Recombinant hCG is given at a dose of 250 mg subcutaneously, which corresponds to 6000–7000 IU of human hCG. As ovulation can be expected between 24 and 48 h after the hCG injection, intercourse or intrauterine insemination is usually scheduled 24–36 h after trigger [18].

16.7.3 Side Effects and Risks

The most common complication of gonadotropin treatment is multiple pregnancy. It occurs in up to 20% of gonadotropin-induced pregnancies. If numerous follicles are growing, cancellation or conversion to IVF with elective single blastocyst transfer can be offered. In case of high order multiple pregnancies, fetal reduction could be offered. Other risk of gonadotropin treatment is ovarian hyperstimulation syndrome. In the presence of multiple developing follicles, the cycle could be cancelled, converted to IVF with or without agonist trigger and freeze all embryos, or use a lower dose of hCG for trigger [18].

Minor and common side effects include injection-site reaction such as erythema and discomfort. These are usually self-limited and spontaneously resolve. There have been concerns that ovulation induction may be associated with an increased risk for ovarian cancer mainly borderline tumor and breast cancer. Results of studies on this subject have been mixed [18].

16.7.4 Effectiveness

Overall pregnancy rate after ovulation induction with gonadotropins is in the range of 15–20% per cycle. This is dependent on the underlying pathology and individual prognosis factors including age. Women who are obese or insulin resistant may require increased amounts of gonadotropins per cycle to achieve ovulation [18].

16.8 Combination of Gonadotropins and Oral Agents

The addition of clomiphene citrate or letrozole to gonadotropins for ovulation induction in insemination cycle has been used in some centers. It is

associated with reduced total dose of gonadotropins needed to achieve ovulation and the duration of treatment [19–21]. Because lower doses of gonadotropins are needed, patients achieve a reduced rate of monofollicular growth, and less cancellation for ovarian hyperstimulation [20]. In addition, the clinical pregnancy rate appears similar when the combination of oral agents and gonadotropins for ovulation induction is compared to gonadotropin alone [19].

16.9 Insulin-Sensitizing Agents

PCOS is associated with obesity, insulin resistance, and hyperinsulinemia. For this reason, insulin-sensitizing agents have been used to treat ovulatory dysfunction in these patients. The most commonly used compound is a biguanide, metformin, which improves peripheral sensitivity to insulin through stimulated glucose uptake by the tissues. As first line treatment, metformin alone offers no advantage over clomiphene citrate [22]. Combined metformin and clomiphene citrate may increase clinical pregnancy rates, but not live birth rates [23]. Yet, in clomiphene citrate resistant patients, the addition of metformin improves ovulation rate, clinical pregnancy rates but not live birth rates.

Gonadotropins in this specific population may offer an improved live birth rate over adding metformin to clomiphene citrate [22]. Current evidence shows that the rate of spontaneous abortion in PCOS patient does not seem to be reduced with addition of metformin [22]. ESHRE/ASRM guidelines state that "metformin appears to be useful in patients with normal BMI who have infertility due to anovulatory PCOS" and that "metformin, in combination with clomiphene citrate, is the treatment of choice in clomiphene-resistant patients with anovulatory PCOS" [24].

Side effects of metformin include nausea, vomiting, diarrhea, flatulence, GI upset, and rarely lactic acidosis. Metformin is in FDA category B if continued during pregnancy, which implies that "animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women." Clinical evidence of the use of metformin in first trimester of pregnancy is reassuring, as there does not seem to be an increase in congenital malformation in offspring [22].

16.10 Pulsatile GnRH and Dopamine Agonist

Pulsatile GnRHa can be administered to women with WHO Group I. However, its use is not practical and is rarely used today. Women with hyperprolactinemia can be treated with dopamine agonists. Although ovulation will resume, dopamine agonists are not considered ovulation-inducing agents.

16.11 Laparoscopic Ovarian Drilling or Wedge Resection

Ovarian surgery for ovulation induction in PCOS patient includes ovarian wedge resection and ovarian drilling by diathermy or laser [25]. This intervention is believed to be effective due to the destruction of the ovarian androgen-producing tissue. A fall in serum LH and androgen levels has been demonstrated after ovarian drilling, as well as an increase in serum FSH levels. It results in a change from the adverse androgen-dominant intrafollicular milieu to an estrogenic one, which restores the normal hormonal environment by correcting the ovarian-pituitary feedback mechanism. Consequently, both these local and systemic effects promote follicular recruitment, maturation and ovulation [26].

Potential complications of this procedure include the usual surgical and anesthetic risks, as well as post-operative formation of adhesion and diminution of the ovarian reserve by destruction of the healthy follicular pool. In order to limit the latter two, laparoscopic ovarian drilling is often preferred to wedge resection. The typical indication for surgical treatment is clomiphene citrate resistance in patients with anovulatory PCOS. Given the availabilities of other effective methods of ovulation induction and in vitro fertilization for such patients without the risk of affecting the ovarian reserve, surgery should be used very sparingly.

In any event, the procedure is effective and usually results in monofollicular ovulation. Pregnancy rates and live birth rates are comparable when compared to gonadotropin use for CC-resistant patients; however, the multiple pregnancy rate is significantly higher with gonadotropins [10]. The procedure may be best suited for patients in whom frequent ultrasound monitoring is impractical, or in milieu with limited

Table 16.1 Treatment approaches to infertility secondary to anovulation from PCOS

	Intervention	Advantages	Disadvantages
First line	Lifestyle changes including weight control and exercise	– Low cost – Low risk of complications during treatments and pregnancy – Better response to ovulation induction – No increase in risk of multiple pregnancy	– None
	Ovulation induction with oral agents clomiphene citrate or letrozole (off-label in North America)	– Low cost – Easy administration – Limited monitoring	– Side effects – Risk of multiple pregnancy
Second line	Ovulation induction with gonadotropins	– Efficacious when first line treatment fail	– Higher cost – Close ultrasound monitoring required – Administered by injection – High risk of multiple pregnancy – Side effects
	Laparoscopic ovarian drilling	– One time procedure needed – No increase in risk of multiple pregnancy – No monitoring required afterwards	– Surgical risks – High cost – Risk of damaging ovarian reserve
Third line	In vitro fertilization	– High pregnancy rates – Risk of multiple pregnancy can be controlled by elective single embryo transfer	– Risks of procedure (including OHSS) – High cost

resources [10]. In our institution, laparoscopic ovarian drilling or ovarian wedge resection is not performed anymore.

16.12 Conclusion

Anovulation is a common cause of infertility and usually presents as menstrual irregularity. A complete evaluation of the couple is mandatory to exclude systemic illnesses and other infertility factors. The first line treatment for ovulatory disorder should include lifestyle changes if applicable followed by treatment with ovulatory-inducing agents according to the underlying pathology in a stepwise approach (Table 16.1). If conventional treatment is unsuccessful, patient can be treated with controlled ovarian hyperstimulation and in vitro fertilization.

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Assisted Reproductive Technology: Clinical Aspects

Erica B. Mahany and Yolanda R. Smith

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17.1 Introduction

Clinical Case

A 29-year-old nulligravida woman with 2 years of primary infertility presents to your office with her husband. She has regular menstrual cycles, and she denies any symptoms of androgen excess. Her only notable medical history is a hospital admission at the age of 20 for pelvic inflammatory disease. Her surgical history is negative. Her husband is a healthy 30-year-old male with no significant medical history. He conceived a pregnancy with a prior partner 5 years ago. Initial evaluation showed normal ovarian reserve testing and normal semen analysis, although hysterosalpingogram showed bilateral hydrosalpinx without spill bilaterally.

17.1.1 Diagnostic Criteria

A thorough history is usually the key in determining the cause of infertility during a new patient evaluation. It is important to assess the previous fertility history of each partner. Clues to the diagnosis of tubal factor infertility include a history of pelvic inflammatory disease, history of ectopic pregnancy, or one or more male partners who have conceived pregnancies with other partners. A hysterosalpingogram with bilaterally obstructed fallopian tubes confirms this diagnosis. Clues to the diagnosis of severe male factor infertility include secondary infertility in a female patient, where the only new variable is a different partner. Semen analysis results with a total motile count of <10 million (after processing) or normal morphology <4% is associated with poor fertilization [1, 2], and IVF is indicated in these situations.

It is important to understand which individuals or couples would most benefit from Assisted Reproductive Technology (ART). Of note, the term "ART" has historically been used to describe all treatments involving the handling of sperm and oocytes, although currently more than 99% of ART procedures are in vitro fertilization (IVF) procedures. ART does not include ovulation induction or intrauterine insemination, and the other techniques such as zygote intrafallopian transfer or gamete intrafallopian transfer are rarely used today [3]. The term "IVF" will be used throughout the rest of this chapter. IVF is the only treatment for individuals or couples with severe male factor infertility or severe tubal factor infertility. In addition, there are other situations in which IVF is the first-line treatment, such as women or men who have undergone a sterilization procedure, single or homosexual partnered males using a gestational carrier, and any individual or couple using donor oocytes or previously frozen oocytes. IVF is also recommended for patients with other infertility diagnoses if other treatment options are not successful.

Some patients meet criteria for a fertility preservation procedure, which involves harvesting oocytes or embryos for later use. This may be performed prior to fertility-threatening treatments expected to harm gametes, for example, as in treatment for cancer. This is also gaining acceptance for purposes of deferred childbearing. In patients with a planned fertility-threatening treatment, the process can be expedited, requiring approximately 2–3 weeks [4, 5].

17.1.2 Prevalence

It is estimated that 15% of all couples suffer from infertility, and of those, only about half seek medical treatment [6]. In 2002, the United States Departments of Health and Human Services conducted a survey to estimate the fertility, family planning, and reproductive health indicators among females aged 15–44 in the USA. The study found that the most common encounter for infertility was for advice alone (6.1% of women), followed by medical help to prevent miscarriage (5.5% of women), testing on either partner (4.8%), ovulation drugs (3.8%), and insemination (1.1%). Although it is difficult to ascertain the denominator for patients where IVF has been recommended, in this survey only 0.3% of women had ever undergone ART [6]. It is likely that more patients would benefit from IVF, although lower rates of IVF utilization have been correlated with a lack of insurance coverage, and decreased availability of physicians providing this service. Despite these social factors, approximately 190,000 cycles of in vitro fertilization (IVF) are performed each year in the USA, resulting in the

birth of over 61,000 babies annually (over 1% of all children born in the USA each year) [7].

The development of IVF, based on an understanding of reproductive physiology, was a monumental discovery that has changed the course of human history. In 1969, Drs. Patrick Steptoe and Robert Edwards successfully matured human oocytes and fertilized them by spermatozoa. Through their efforts, in 1978, the first human birth from IVF was achieved in England. John and Lesley Brown had 9 years of infertility secondary to bilateral fallopian tube obstruction. Dr. Steptoe retrieved a single mature oocyte laparoscopically from one of Lesley's ovaries during a natural cycle. In 2010, 32 years later, Dr. Edwards was awarded the Nobel Prize for Physiology or Medicine [8, 9].

Around that same time, across the Atlantic, Drs. Howard and Georgeanna Jones were pioneering IVF in the USA. Their first patients all had tubal factor infertility. In December of 1981, Elizabeth Jones Carr was the first baby born through IVF in the USA [10, 11].

Far-reaching advances in laboratory techniques and culture conditions have been made since then. Although the first IVF cycles were performed with natural cycles, stimulating the ovaries with exogenous gonadotropins (pituitary hormones that cause follicular growth and maturation) has long been the standard to increase the number of oocytes retrieved and to improve pregnancy rates [10, 11]. Gonadotropins may come from the urine of menopausal women, or they may be recombinant. This process is referred to as "controlled ovarian hyperstimulation," which is the administration of hormone medications that stimulate the ovaries to produce multiple oocytes. It is sometimes called enhanced follicular recruitment or superovulation. Although the first IVF procedures were performed laparoscopically, procedures are now performed vaginally under ultrasound guidance, followed by transcervical embryo transfer, and indications have expanded beyond tubal factor infertility.

17.2 Evaluation Prior to IVF

Before starting IVF, each patient is evaluated to help maximize chances for a healthy pregnancy. The patient's detailed past medical, surgical, family, and social history is reviewed, and any special considerations are followed up as appropriate. Chronic diseases should be well controlled, and weight should be optimized. In addition, smokers should undergo smoking cessation [12]. If review of the history reveals something that could affect the patient or the pregnancy, a preconception consultation with Maternal Fetal Medicine may be warranted to discuss the risks involved in becoming pregnant.

In general, the patient should be screened for conditions that could affect the health of the pregnancy (Table 17.1 provides an example of a typical evaluation prior to IVF). The patient's blood type should be confirmed, and she should be screened for immunity to rubella and varicella. If her blood type is Rh negative, she will be counseled on the indications for and benefits of RhoGAM in pregnancy. If she does not have immunity to rubella or varicella, she may receive a vaccine prior to pregnancy to help prevent

Table 17.1 Standard evaluation prior to in vitro fertilization

Female evaluation	Workup
Ovarian reserve testing	Anti-Müllerian hormone Day 3 follicle stimulating hormone, Estradiol Antral follicle count
Preconception (preventative) testing	Thyroid stimulating hormone Type and screen Rubella IgG, Varicella IgG Complete blood count Pap test
Uterine cavity evaluation	Saline infusion sonography or hysteroscopy Hysterosalpingogram (2nd line)
Infectious disease testing	Gonorrhea/chlamydia polymerase chain reaction Rapid plasma reagin Hepatitis B surface antigen Hepatitis C antibody Human immunodeficiency virus antigen antibody

Partner evaluation (if applicable)	Workup
Infectious disease testing	Same as above
Sperm testing (if planning sperm from male partner)	Semen analysis

vertical infection during pregnancy. In addition, the patient and her partner (if applicable) should be tested for hepatitis B and C, human immunodeficiency virus (HIV), gonorrhea, chlamydia, and syphilis. A careful family history and review of ethnic background will also inform whether additional tests such as cystic fibrosis and hemoglobin electrophoresis (for sickle trait or thalassemias) may be recommended [12].

There are other tests that, if abnormal, would significantly affect various aspects of the IVF process.

17.2.1 Ovarian Reserve Testing

Ovarian reserve testing is typically performed to estimate oocyte quantity and the expected response to an IVF cycle. An anti-Müllerian hormone (AMH) level is drawn, and in most patients, a cycle day 3 follicle stimulating hormone (FSH) and estradiol are drawn as well. Exceptions to drawing "day 3" labs may include young patients with a high AMH and fertility preservation patients undergoing an expedited cycle. Measuring an antral follicle count with transvaginal ultrasound also gives an assessment of ovarian reserve. A selection of all or some of these tests assists with dosing of gonadotropins and protocol selection.

17.2.2 Sperm Testing (If Applicable)

If sperm are coming from a male partner, a recent semen analysis is indicated to assess whether intracytoplasmic sperm injection (ICSI) is necessary and whether a sperm extraction technique may be needed.

17.2.3 Uterine Evaluation

If an embryo transfer is anticipated, a cavity evaluation is performed. The best options include a saline infusion sonogram ("SIS," which involves injecting sterile saline into the uterus under ultrasonographic guidance) and hysteroscopy (using a small lighted scope while using a distension medium such as normal saline to look directly at the uterine cavity). Hysterosalpingogram ("HSG," which is a procedure where radio-opaque dye is injected under fluoroscopy to evaluate the fallopian tubes and uterine cavity) is also accepted in some centers, although HSG has a low sensitivity (50%) and low positive predictive value (30%) compared to the other two options [13]. The uterine cavity evaluation sometimes reveals pathology, such as intrauterine polyps, fibroids, septae, or retained products of conception. Although the benefit of optimizing the uterine cavity requires further study, this is generally considered standard practice prior to IVF. Many offices also perform a "mock," or practice, embryo transfer prior to the actual embryo transfer in order to anticipate any difficulties and increase the chances for a non-traumatic embryo transfer [12].

17.2.4 Optimizing IVF Outcomes

The reason for such extensive, and sometimes costly, evaluation prior to IVF is that there are many aspects of a patient's health and readiness for IVF that can be optimized prior to IVF. For example, if a patient is noted to have a hydrosalpinx, her pregnancy rates during an IVF cycle will be significantly higher if she has a salpingectomy or a tubal transection prior to embryo transfer [14, 15]. This minor surgery can usually be accomplished laparoscopically.

Suboptimal thyroid function has been shown to have deleterious effects on pregnancies. Overt hypothyroidism is characterized by elevated thyroid stimulating hormone (TSH), a decreased free T4, and clinical findings such as fatigue, constipation, cold intolerance, muscle cramps, weight gain, dry skin, hair loss, and prolonged deep tendon reflexes. There are strong data that overt hypothyroidism decreases fertility, presumably due to ovulatory dysfunction, and thyroid hormone should be administered prior to pregnancy to normalize the thyroid axis [16–20]. In addition, poorly controlled overt hypothyroidism has been shown to cause neurodevelopmental delay in the fetus [17], as well as approximately double the risk of miscarriage [21], low birth weight, stillbirth, preeclampsia, and heart failure [16].

Subclinical hypothyroidism, which is characterized by an abnormally high serum TSH concentration with free T4 levels within the normal reference range [16], is more controversial. The data are inconclusive regarding whether subclinical hypothyroidism increases the risk for

infertility, miscarriage, or fetal effects. There are limited data regarding treatment of subclinical hypothyroidism. Some studies show improved IVF outcomes when subclinical hypothyroidism is treated prior to IVF [22, 23]. Although this question is still unanswered, both the Endocrine Society and the American Society for Reproductive Medicine recommend a TSH level of less than 2.5 milli-international units per liter prior to conception [18, 24].

17.3 Process of IVF

In general, IVF involves surgically removing oocytes from a woman's ovaries, combining them with sperm in the laboratory, and transferring embryo(s) into the uterus or donating them to another woman. The process of IVF is a highly coordinated, time-intensive process that involves weeks of preparation as well as flexibility by patients and providers, as the exact trajectory of each patient and their readiness for an oocyte retrieval cannot be predicted ahead of time. The next few paragraphs will describe the IVF process in detail. ◘ Figure 17.1 provides a timeline of a typical IVF cycle.

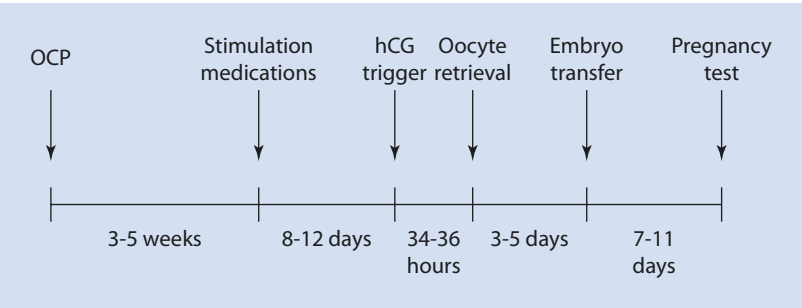
17.3.1 Controlled Ovarian Hyperstimulation

Gonadotropins

Although the first successful human IVF cycle utilized a natural menstrual cycle, subsequently higher pregnancy rates were achieved when ovarian stimulation with gonadotropins was utilized. Exogenous follicle stimulating hormone (FSH) causes growth of follicles, upregulates aromatase, and prevents the physiologic decrease in FSH in a natural cycle when the dominant follicle is selected. This allows for multi-follicular development during controlled ovarian hyperstimulation. Luteinizing hormone (LH) acts on the theca cells to increase testosterone production by the ovary, which becomes the substrate for estradiol by the granulosa cells in the developing follicles. LH is also the signal by the pituitary to cause luteinization with large amounts of progesterone from the corpus luteum. Recent data have shown that variations in the relative proportions of FSH and LH may have a substantial impact on the outcomes of ovarian stimulation, with an optimal LH: FSH ratio of 0.30: 0.60 [25]. Monitoring the response to ovarian stimulation is accomplished with a combination of transvaginal ultrasonography, serum estradiol levels, and occasionally progesterone levels.

Cycle Timing

There are various ways to begin an IVF cycle, and these will be described. Oral contraceptive pills (OCPs) are often used prior to stimulation to attenuate the FSH rise and induce a more homogenous follicular cohort, as well as prolong the FSH-responsive window and prevent the occurrence of spontaneous LH surges [26]. In addition, in low responder patients, suppression with OCPs has been shown to improve the response [27], however, patients over the age of 35 who undergo ovarian suppression with OCPs may require a longer duration of stimulation with gonadotropins [26]. OCPs may also provide more flexibility with the timing of appointments for both patients and providers. In addition, some clinics batch cycles, or plan the stimulation start so procedures are likely to occur when staffing is optimal. If women are unable to take OCPs due to a preexisting contraindication (such as migraine headache with aura) or intolerance related to side effects, an



◘ **Fig. 17.1** An example of a typical IVF timeline

IVF cycle may be started with menses. If a woman is amenorrheic or oligomenorrheic, menses may be induced with progesterone withdrawal. In addition, fertility preservation patients often have cancer treatments scheduled and need to start stimulation as soon as possible. There are data that the timing of cycle start does not affect outcomes, even when gonadotropins are started in the luteal phase [4, 5].

Ovulation Prevention

The goal of ovarian stimulation for IVF is eventually to harvest a cohort of mature oocytes before the patient ovulates. If ovulation occurs, the oocytes cannot be retrieved, and the cycle must be cancelled. When a patient undergoes controlled ovarian hyperstimulation, the estradiol levels usually far surpass the estradiol threshold that would cause an LH surge under physiologic conditions. Prior to the use of gonadotropin releasing hormone (GnRH) agonists to combat this issue, more than one-quarter of stimulation cycles were cancelled because of a premature LH surge and ovulation [28]. There are three standard IVF protocols to physiologically prevent or delay an LH surge.

Gonadotropin Releasing Hormone Agonists

GnRH agonists initially stimulate LH and FSH release and within 2 weeks will suppress gonadotropins [28], due to downregulation of gonadotropin releasing hormone receptors at the level of the pituitary. Once suppression has occurred, the ovaries can be stimulated with exogenous gonadotropins. In the USA, the most popular GnRH agonist is leuprolide acetate, given subcutaneously at doses of 0.25–1.0 mg/day.

Microdose, or "Flare," Protocols

Microdose Lupron is a smaller dose of the GnRH agonist leuprolide acetate (40–50 μg twice daily). The goal is less pituitary suppression in the beginning of the cycle, thus causing a rise in the patient's endogenous gonadotropins to complement the exogenous gonadotropin injections. Over time, it causes suppression of the GnRH receptors at the level of the pituitary, preventing an LH surge and premature ovulation. This protocol has comparable pregnancy rates when comparing cycles of patients with similar baseline characteristics [29, 30].

Gonadotropin Releasing Hormone Antagonists

GnRH antagonists are the most recent development in ovulation prevention. Their main advantage is that they directly antagonize the GnRH receptors at the level of the pituitary and have an immediate effect. The usual dose is 250 μg/day. A multicenter IVF trial compared the GnRH agonist protocol with the GnRH antagonist protocol, and found a mean duration of 19 days of injections with the GnRH agonist, as time is needed to get beyond the flare effect, compared to only 4 days of injections with the GnRH antagonist [31], which is typically initiated after the start of stimulation medications.

Ovulation Trigger

Once a cohort of mature follicles is present, urinary human chorionic gonadotropin (hCG, 5000–10,000 international units), or recombinant hCG (250–500 μg), is typically administered to mimic the LH surge and complete oocyte maturation. Oocyte retrieval is performed prior to ovulation, 34–36 h after the hCG injection. Urinary and recombinant hCG products are equivalent [32]. HCG, however, has a relatively long half-life and remains elevated in the serum for 6 days [33]; for this reason, it may exacerbate symptoms of ovarian hyperstimulation syndrome in patients at risk. Alternately, a GnRH agonist trigger may be used in patients on an antagonist protocol to cause an endogenous LH surge. In patients on a GnRH agonist protocol, there are limited data to suggest the use of recombinant LH to trigger ovulation in patients at risk of ovarian hyperstimulation syndrome (OHSS). This is appealing due to the shorter duration (approximately 34 h) [33], although more data are needed to determine the optimal dose of recombinant LH [34].

17.3.2 Luteal Phase Hormonal Support

Similar to a natural cycle, the luteal phase of an IVF cycle requires progesterone [35]. Aspiration of granulosa cells during collection of the oocytes reduces the capacity of the ovaries to produce progesterone. Agonist and antagonist cycles change the milieu and therefore, progesterone supplementation is the standard of care. A recent Cochrane review showed no difference in route of

progesterone administration and pregnancy outcomes [36]. Combined supplementation with estradiol is not well studied, but is often employed. There are some data to suggest that supplemental estradiol, in particular vaginal estradiol, may improve outcomes [37]. In patients who conceive, hormonal supplementation typically continues until 7–10 weeks gestation.

17.3.3 Embryo Transfer

Embryos may be transferred into the uterus anytime during preimplantation development, however, the transfer of one or two embryos on day 3 or day 5 is standard. In general, transfer of a single day 5 blastocyst has been proposed as a way of minimizing the risk of high-order multiple pregnancies while maintaining satisfactory pregnancy rates. Blastocyst transfer has several potential advantages. Fewer embryos survive to day 5, and abnormal embryos are less likely to survive, increasing the chances that a normal embryo is transferred. Delaying the embryo transfer to day 5 after fertilization also allows for more detailed examination of embryo morphology (Fig. 17.2); the timing more closely mimics the timing for implantation in a natural spontaneous pregnancy cycle, where preimplantation embryos enter the uterus on day 4 or day 5 following fertilization [38]. A day 5 transfer has some disadvantages, however, as in some patients none of the day 3 embryos will grow to blastocysts on day 5 and they may have nothing to transfer. In a reputable lab, this is more reflective of embryo quality than the laboratory milieu, but the possibility of having no embryos to transfer is a risk, and appropriate counseling is necessary, especially in older women or poor prognosis patients.

Balancing the risks and benefits of multiple-embryo transfer remains one of the most vexing problems in the field. The transfer of more than one embryo increases the chance of pregnancy but also increases the risk of multiple gestation. The American Society for Reproductive Medicine (ASRM) recommends that no more than two embryos be transferred to women under 35 years of age who have a favorable prognosis for pregnancy (Table 17.2) [39]. In particular, the ASRM states that elective single embryo transfer (eSET) is most appropriate for patients with a

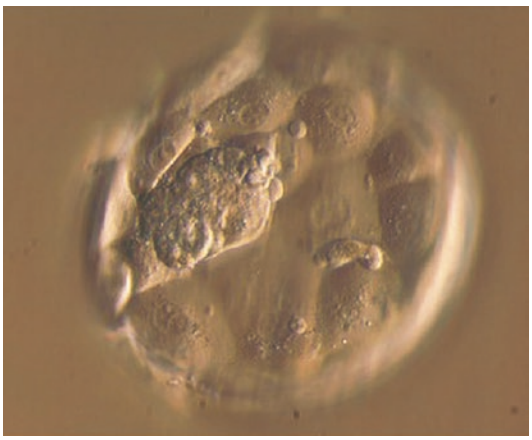


Fig. 17.2 Expanded blastocyst on day 5 of development. The inner cell mass (which ultimately develops into the fetus) is apparent (reproduced with permission from Steinkampf MP, Malizia BA. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

Table 17.2 Recommended limits on the number of embryos to transfer [40]

Prognosis	<35 years	35–37 years	38–40 years	41–42 years
Cleavage stage				
Favorable	1–2	2	3	5
All others	2	3	4	5
Blastocysts				
Favorable	1	2	2	3
All others	2	2	3	3

Favorable = first cycle of IVF, good embryo quality, excess embryos available for cryopreservation, or previous successful IVF cycle

good prognosis: age less than 35 years, more than one top-quality embryo available for transfer, first or second treatment cycle, previous successful IVF, and recipients of embryos from donated oocytes. Women aged 35–40 may be considered for eSET if they have high quality blastocysts available for transfer [40]. A randomized, controlled trial comparing elective single-embryo transfer and subsequent frozen embryo transfer vs. double-embryo transfer showed comparable live birth rates and a substantially lower rate of multiple gestations following eSET [41]. Several meta-analyses have demonstrated similar findings [42–44]. However, barriers exist to the widespread acceptance of eSET, including financial issues and lack of insurance coverage for costly IVF treatments [40].

Embryo Transfer Technique

Embryo transfer is the final, and arguably the most important, step in IVF. The basic steps of an embryo transfer include placing a speculum in the vagina, inserting a catheter into the uterus, and injecting the embryo near the uterine fundus. The optimal technique for embryo transfer has been studied, as there is large variation in techniques. Cervical flushing has not been shown to improve pregnancy rates [45]. By contrast, ultrasound guidance seems to maximize the chance for a successful embryo transfer, likely by allowing for an atraumatic transfer 1.5–2.0 cm from the fundus. Soft catheters seem to improve outcomes compared to stiff catheters [46]. There is controversy about whether a single lumen catheter (embryo pre-loaded into the catheter) or a double lumen catheter (embryo loaded after correct placement of catheter) is superior, although there are no strong data to suggest the superiority of either [47].

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Transfer of Previously Frozen Embryos

An embryo transfer cycle of previously frozen embryos has significant advantages compared to a controlled ovarian hyperstimulation cycle, including decreased risk of OHSS, ability to have genetic information for the embryos, and some data suggest better pregnancy outcomes. Because the ovaries are not hyperstimulated, the risk of ovarian hyperstimulation syndrome is essentially negligible, which is especially important for patients with polycystic ovarian syndrome or patients who have a robust response to controlled ovarian hyperstimulation. In addition, couples that elect PGS or PGD on embryos will be able to have this information prior to a transfer if embryos are frozen. Although some clinics are able to offer trophectoderm biopsy and transfer during the same (fresh) cycle, this requires proximity to a genetics lab and is not widely available.

Practically speaking, this type of cycle may be considered a uterine preparation cycle. Once the embryos have been created, they remain in cryopreservation until the patient desires to use them. Typically, the patient's hypothalamic–pituitary–ovarian axis is suppressed with oral contraceptive pills (OCPs) and/or leuprolide acetate. As with fresh IVF cycles, non-OCP start cycles are possible as well. Estrogen is given to grow the uterine lining. When the lining is adequately thick (studies support >6 mm [48]), progesterone is administered, and the number of days corresponds to the stage at which the embryos were cryopreserved, as this is when the endometrium and embryo will be chronologically in sync. Then, the embryo(s) is(are) transferred. A pregnancy test is performed 7–11 days later, corresponding to the time of a missed period. Approximately 97% of embryos survive the thawing process, and the pregnancy rates are not worse compared to fresh IVF cycles [49].

In fact, recent data have suggested that vitrified-warmed embryo transfer cycles may have better outcomes compared to fresh embryo transfer cycles, such as higher implantation rates, higher day 14 beta hCG levels per implantation, lower ectopic pregnancy rates, higher live birth rates per transfer, and higher infant birth weight [50]. Other studies have shown significantly higher ongoing pregnancy rates and clinical pregnancy rates compared to fresh cycles [51]. These findings may be due to better endometrial receptivity and placentation in vitrified-warmed cycles.

There are also disadvantages of transferring previously frozen embryos, not the least of which is timing. Most patients who have suffered from infertility want to become pregnant as soon as possible, and choosing to freeze all embryos may result in a delay of several weeks. Another disadvantage is the small chance that the embryo may not survive the thaw, although survival rates have been improved with vitrification protocols [51].

17.3.4 Special Considerations

After the decision is made to proceed with in vitro fertilization, there are still several important considerations.

Fertilization Methods

A decision must be made whether to fertilize the oocytes with conventional IVF (putting sperm and oocytes in close proximity in the laboratory) or intracytoplasmic sperm injection (ICSI, which is a micromanipulation procedure in which a single sperm is injected directly into an oocyte to attempt fertilization). ICSI is the treatment of choice for male factor infertility, as it may overcome the negative effects of abnormal semen characteristics and sperm quality on fertilization. For couples that have conceived together previously, conventional in vitro fertilization is usually appropriate. For individuals and couples who do not fit into these categories, the IVF lab may provide guidance.

Reports on the risk of birth defects associated with ICSI, compared to conventional insemination, have yielded conflicting results. The largest study to date has suggested that ICSI is associated with an increased risk of congenital anomalies [52]. Whether the association is due to the ICSI procedure itself, or to inherent sperm defects, has yet to be determined. Although the relative risk of ICSI is increased compared to conventional IVF (1.57, 95% CI 1.3–1.9, vs. 1.07, 95% CI 0.90–1.26), the absolute risk is low. The estimated risk for congenital anomalies in all ART pregnancies is estimated to be 4.3% [53], compared to 3.0% in the general population. Notably, studies have shown that couples with infertility have a higher risk of birth defects, even in the absence of ART [52].

ICSI has also been implicated in other effects on the offspring. In particular, an early report suggested that children conceived with ICSI had an increased risk of developmental delay, however, more recent data have not detected any differences in the development of children born after ICSI, conventional IVF, or natural conception [54]. The prevalence of sex chromosome abnormalities in children conceived via ICSI is higher than observed the general IVF population, but the absolute difference between the two groups is small (0.8–1.0% in ICSI offspring vs. 0.2% in the general IVF population) [55]. The reason for this is unclear—it may result from the ICSI procedure itself, or it may reflect a direct paternal effect. Men with sperm problems are more likely themselves to have genetic abnormalities and often produce sperm with abnormal chromosomes. More studies are needed to clarify these areas of ambiguity, although given the low absolute risk of these issues, most patients are willing to accept the risk.

Embryo Genetic Testing

With the advent of new technologies in the laboratory, genetic testing of the embryos is becoming more common. It is often used for aneuploidy screening, single gene disorders, chromosomal abnormalities such as translocations, mitochondrial disorders, and gender selection in sex-chromosome linked disorders [56]. If patients desire preimplantation genetic testing, the outer layer of a mammalian blastocyst, also called the trophectoderm, may be biopsied on day 5 or 6 following the oocyte retrieval. Although a day 3 biopsy can technically be performed, day 5 or day 6 biopsy is the standard of care due to better sensitivity and specificity [57].

Following embryo biopsy, these cells can be sent for Preimplantation Genetic Screening (PGS) testing, which is the process of removing one or more cells from an embryo to test for a normal number of chromosomes, or Preimplantation Genetic Diagnosis (PGD) testing, which is a similar process but testing for an allele that is associated with a particular disease. PGS provides chromosomal information about the embryos: whether all are present, and whether there are deletions, duplications, or translocations. Pregnancy rates are higher when a euploid embryo is transferred [58, 59]. PGD provides information regarding the presence or absence of a particular disease-associated allele in each embryo that was biopsied. The specific mutation must be known ahead of time, and the testing platform must be built prior to proceeding with controlled ovarian hyperstimulation. There are long-term data that these technologies are safe [60], and they are helping patients to make informed choices prior to the embryo transfer. A day 5 or 6 biopsy usually necessitates cryopreservation of embryos following the biopsy, and waiting for the results before proceeding with a vitrified-warmed blastocyst transfer cycle.

Third Party Reproduction

Third Party Reproduction, which is the use of oocytes, sperm, or embryos that have been donated by a third person (donor) to enable an infertile individual or couple (intended recipient) to become a parent (or parents), is another area that has grown considerably in the recent years. Third party reproduction also includes the use of a gestational carrier. Patients who are entering into third party reproduction need additional testing and counseling, including a psychological evaluation and a consultation with a lawyer.

Oocyte Donation

The first pregnancy achieved with oocyte donation was reported in 1984. Since then, there has been increasing use of this technology to help patients conceive. Oocyte donors are identified, through an agency or a known donor. Through IVF, oocytes are obtained from the donor, fertilized with sperm, and transferred into the recipient's uterus.

Indications for Oocyte Donation

Oocyte donation is often used for women with ovarian insufficiency, which may be due to previous chemotherapy, radiation, surgery, age-related factors, congenital absence of the ovaries, or idiopathic ovarian insufficiency. It may also be used for women who prefer not to pass on a known genetic condition, or for patients whose families have a genetic condition where a mutation cannot be identified. Oocyte donation is becoming increasingly common for women who have not conceived after multiple cycles of IVF when oocyte quantity and/or quality seem to be the putative factor [61].

Evaluation of the Oocyte Donor

Donors, both anonymous and known, are screened for eligibility with extensive testing according to ASRM guidelines. Preferably, they are between the ages of 21 and 34. They complete an extensive questionnaire regarding their medical and family history in detail, as well as their sexual history, substance abuse history, history of family disease, and psychological history. In the USA, the Federal Drug Administration (FDA) requires screening for communicable disease. A donor is ineligible if screening or testing identifies a risk factor or communicable disease. If chosen, they are informed and educated about the process [61].

Simultaneously, the recipient patient or couple also undergoes evaluation comparable to patients undergoing IVF, in addition to a psychological evaluation. If all parties pass their evaluations, the oocyte donor undergoes controlled ovarian hyperstimulation. The recipient's uterus is typically prepared with exogenous estrogen and progesterone to receive the embryo(s), which can require significant coordination if a fresh embryo transfer is desired. The use of donor oocyte banks (similar to sperm banks) circumvents this issue, as it eliminates the need for coordination of timing between donor and recipient. Many regimens for endometrial preparation have been described, and successful pregnancies have also been reported in natural cycles where donor oocytes were used to create the embryos. The use of donor oocytes for IVF consistently results in high pregnancy rates when young, healthy, fertile women donate their oocytes, with pregnancy rates from 51 to 58% per IVF cycle [62, 7].

Sperm Donation

Artificial insemination using donor sperm has been practiced since the early 1900s, and non-ART applications of donor sperm usually involve intrauterine insemination. Sperm donation may also be used in the context of IVF. Current FDA and ASRM guidelines recommend that in any case of therapeutic sperm donation, the sperm be quarantined for 6 months before being used, as this decreases the risk of transmission of communicable diseases such as human immunodeficiency virus [61].

Indications for Sperm Donation

Currently, therapeutic donor sperm is appropriate when the male partner has severe abnormalities in semen parameters that would preclude a pregnancy. IVF has allowed couples that previously would have needed donor sperm for insemination to conceive with IVF with their own sperm, although there are situations in which sperm donation is still needed in the context of IVF. In heterosexual couples where the male has azoospermia despite invasive sperm retrieval methods, sperm donation is indicated. In addition, sperm donation may be indicated if the male partner is a carrier of a genetic disease that he does not want to pass on, or if a disease runs in his

family for which a particular mutation has not been found [61]. Single women and same sex female couples also benefit from sperm donation, and this may be performed in the context of IVF if there is another reason for IVF (genetic testing desired, tubal factor, etc.).

Evaluation of the Sperm Donor

Per ASRM guidelines, anonymous sperm donors should be between 18 and 40 years old. The donor may be known or anonymous. The ASRM recommends that all donors be tested for communicable diseases, although the FDA requires that only anonymous donors be tested. Similar to oocyte donors, a thorough medical history is reviewed, with focus on communicable diseases, genetic issues, or psychological factors that would preclude them from being donors. A semen analysis is performed and a test sample will evaluate the post-freezing/thawing parameters. The sperm donor is again screened for communicable diseases 6 months after the semen sample is frozen to ensure that the results of screening are negative [61].

Embryo Donation

Embryo donation, which is a procedure that enables embryos created by couples that underwent IVF previously to be transferred to infertile patients, may be considered a special form of adoption. Indications include untreatable infertility that involves both partners or a single woman, recurrent pregnancy loss thought to be related to embryonic factors, and genetic disorders affecting one or both partners. The evaluation process is similar to recipients of oocyte or sperm donation, as well as a uterine cavity evaluation prior to the transfer cycle. The treatment cycle is quite similar to an embryo transfer cycle of previously frozen embryos (see above). Pregnancy following embryo donation depends on a number of factors, such as the quality of the embryos that were frozen, the age of the woman who provided the oocytes, and the number of embryos transferred [61]. One important consideration is that these are typically embryos from infertile couples, which may theoretically affect success rates, although SART recently reported a live birth rate of 36.4% for donated embryos in 2014 [7]. Embryo donation is a controversial process with both ethical and legal issues, and informed consent and counseling are very important [61].

Gestational Carriers

A gestational carrier is a woman who carries a pregnancy for another person. It is both medically and emotionally complex, and involves legal and ethical issues as well. The initial indication for a gestational carrier was a woman who had normally functioning ovaries, but who lacked a uterus, either due to congenital absence or surgical removal. Indications have expanded to patients with recurrent pregnancy loss thought to be due to uterine factor, patients with untreatable uterine scar tissue, or patients with a medical contraindication to pregnancy [61].

Controversies

There are a number of controversial issues that are brought to light by third party reproduction, such as directed donor oocyte programs, financial compensation for oocyte donation and gestational carriers, and the methods used to recruit these individuals. Laws differ between and among states, and some laws are much more favorable for allowing women to be gestational carriers. Legal counsel is encouraged to ensure that rights of the intended parents and the gamete donor or gestational carrier are clearly defined, not the least of which is regarding parentage and financial obligations. Consultation with an attorney well-versed in reproductive law within a patient's individual state is advised. In addition, laws may change, and anonymity may not be guaranteed in the future [61].

Fertility Preservation

Fertility preservation, which involves harvesting oocytes or embryos for later use, may be performed to protect some gametes in the face of fertility-threatening treatments, for example, as in treatment for cancer, or for purposes of deferred childbearing. In patients with a planned fertility-threatening treatment, the process can be expedited, requiring approximately 2–3 weeks [4, 5]. The process involves controlled ovarian hyperstimulation with oocyte retrieval. Previously, embryo cryopreservation was recommended instead of oocyte cryopreservation, which was considered experimental. In 2012, however, the ASRM clearly stated, "the evidence indicates that oocyte vitrification and warming should no longer be considered experimental" [63]. Women with fertility-threatening diagnoses and treatment plans may now choose whether to cryopreserve mature oocytes or embryos created with partner or donor sperm. There are data that the process of a fertility

preservation cycle does not affect survival, which is especially important for counseling of patients with hormonally responsive cancers [64].

In addition, more women are choosing to undergo oocyte cryopreservation due to deferred or delayed childbearing. It is known that the quantity and quality of oocytes diminishes as a woman ages, although for each individual this "set point" may be different. Population studies indicate that the most cost effective time to cryopreserve oocytes is 37 years of age [65], although many women are presenting for consultation earlier than this. There are limitations to this technology, however, as there are no guidelines on the number of oocytes to cryopreserve in order to predict a viable pregnancy. In addition, some women ultimately desire to have more than one child, and the number of oocytes needed for this goal is also not yet known.

Additional technological limitations include that currently only mature oocytes may be cryopreserved. Ongoing research is exploring in vitro maturation of immature oocytes, but this is not yet a reliable technology. Ovarian tissue cryopreservation is also an experimental technology being performed for fertility preservation. Hopefully in the near future, there will be more non-experimental options for women who present for fertility preservation, either for medical or deferred childbearing indications.

17.4 IVF Outcomes

17.4.1 Success Rates

Although many endpoints have been used to measure IVF success rates, most patients are interested in the chance of obtaining a healthy infant that they can bring home from the hospital after delivery. This statistic takes time to accumulate, however, and is reflective of a fertility clinic's success in years past as well as its selection of patients.

The Society for Assisted Reproductive Technology (SART) has shifted its focus recently to reflect healthy outcomes. Table 17.3 highlights the most recent National Data Summary statistics for fresh cycles, cycles of previously frozen embryos, as well as fresh donor oocyte cycles. There is also a renewed focus on singleton gestation as an outcome measure. The recent SART report is useful in that it notes the available data as well as limitations in the reporting process, such as cancelled cycles and delay to having outcome data (for example, in embryo banking and fertility preservation). In the USA, these data are collected on an annual basis, and reports are available to the public [7].

17.4.2 Potential Adverse Outcomes

Ovarian Hyperstimulation Syndrome

The most serious side effect of controlled ovarian hyperstimulation is ovarian hyperstimulation syndrome (OHSS), which is an exaggerated response to ovulation induction. It is generally a self-limiting disorder that resolves spontaneously within a few days, but may persist longer in conception cycles. Symptoms can include increased ovarian size, nausea, vomiting, bloating, accumulation of fluid in the abdomen, breathing difficulties, hemoconcentration, kidney and liver problems, and in the most severe cases, venous thromboembolic disease (including in the upper extremities), kidney failure, and death. The

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Table 17.3 Society for assisted reproductive technology 2014 live birth rates per intended retrieval[a] [8]

Oocyte source	Age of woman				
	<35	35–37	38–40	41–42	>42
Fresh, patient's own oocytes	42.1%	32.5%	20.5%	9.8%	3.0%
Frozen, patient's own oocytes	45.0%	39.0%	28.2%	18.7%	7.5%
Fresh, donor oocytes	53.5% (all ages)				

[a]The primary outcome is the outcome for the first embryo transfer following an oocyte retrieval (fresh or frozen) within a year of the oocyte retrieval cycle start

severe cases only affect a very small percentage of women undergoing IVF (0.1–3.0% or fewer), and the most severe cases affect an even smaller percentage. OHSS occurs at two general time points during a cycle: early (1–5 days after oocyte retrieval, usually as a result of the hCG trigger), and late (10–15 days after oocyte retrieval due to placental hCG if pregnancy occurs). The relative risk of severe complications is higher if pregnancy occurs, which is why occasionally a transfer is not performed in patients at high risk. Treatment is usually supportive, and involves fluid and electrolyte management, anti-emetics, and occasionally anticoagulation [66, 67].

Cancer

Some reports have suggested an increased risk of cancer in patients who use fertility medications, however, these reports should be interpreted with caution, because most studies do not take into account that several cancers (ovarian, breast, and endometrial, for example) are more common in nulliparous patients [68]. More research is needed to examine the long-term effect that fertility medications may have on cancer rates, although to date there are no compelling data.

Risks to Pregnant Woman

Pregnancies that occur through IVF are associated with an increased risk of certain complications [53]. Some of these risks may be related to the older age of women undergoing IVF, and some may also be related to the underlying cause of infertility. Some risks may also be due to the IVF procedure, although it is difficult to parse out these differences with the available data. Multiple gestation also imposes additional risks of pregnancy, both to the patient and the offspring. Currently 30% of IVF pregnancies are twins or higher-order multiple gestations [7]. Increased maternal risks due to multiple gestation include an increase in gestational diabetes and preeclampsia (see Table 17.4).

Risks to Offspring

Numerous studies have been conducted to assess the overall health of children conceived through IVF, and the majority of studies have been reassuring. A major problem with studies done thus far has been comparing a group of infertile couples to a group of normally fertile couples, as infertile couples, by definition, do not have normal reproductive function (54–58). Studies do suggest an increase in birth defects in babies born through IVF (2.6–4.3%, compared to 2–3% in the general population, see Table 17.4), although it is important to consider the limitations of existing epidemiologic studies. Interestingly, studies of spontaneous pregnancies after IVF have shown an increase in risk of birth defects, further strengthening the theory that this issue is more likely due to inherent problems with the infertile couple, and not the technology of IVF itself [69].

The vast majority of risks to the offspring are related to multiple gestation and preterm delivery, and all of the co-morbidities that are associated with prematurity. It has been shown that singletons conceived with IVF tend to be born slightly earlier than naturally conceived babies (39.1 weeks compared to 39.5 weeks), and IVF twins are not born earlier than naturally conceived twins. There may be a slight increase in low birth weight of IVF singletons compared to naturally conceived singletons [53].

Monochorionic twinning occurs in approximately 2–3% of IVF pregnancies, which is higher than the spontaneous rate of 0.4% for in vivo conceptions. This further increases the risk to the pregnancy, as complications such as twin–twin transfusion may occur (in up to 20% of monochorionic diamniotic gestations) as well as umbilical cord entanglement (in monochorionic monoamniotic gestations). This risk may be increased by assisted hatching of embryos, and caution should be employed when this procedure is not indicated [70].

A limited number of studies also suggest that ART may be associated with an increased risk of imprinting disorders, theoretically due to laboratory manipulations that occur during meiosis [71]. Because imprinting disorders are quite rare, a causal relationship with IVF is difficult to determine. Finally, data are limited in terms of motor and cognitive development of IVF offspring, but outcomes seem to be similar to those of naturally conceived children [72]. With all of these potential adverse outcomes, it is important to remember that the relative risk may be increased, but the absolute risk remains quite low. Consequently, the majority of IVF pregnancies produce healthy offspring.

Table 17.4 Potential adverse outcomes in singleton in vitro fertilization pregnancies [54]

	Absolute risk (%) ART pregnancies	OR (95% CI)[a]
Perinatal risks		
Preterm birth	11.5	2.0 (1.7–2.2)
Low birthweight (less than 2500 g)	9.5	1.8 (1.4–2.2)
Very low birthweight (less than 1500 g)	2.5	2.7 (2.3–3.1)
Small for gestational age	14.6	1.6 (1.3–2.0)
NICU admissions	17.8	1.6 (1.3–2.0)
Stillbirth	1.2	2.6 (1.8–3.6)
Neonatal mortality	0.6	2.0 (1.2–3.4)
Cerebral palsy	0.4	2.8 (1.3–5.8)
Maternal risks		
Preeclampsia	10.3	1.6 (1.2–2.0)
Placenta previa	2.4	2.9 (1.5–5.4)
Placental abruption	2.2	2.4 (1.1–5.2)
Gestational diabetes	6.8	2.0 (1.4–3.0)
Cesarean delivery	26.7	2.1 (1.7–2.6)
Genetic risks		
Epigenetic or imprinting disorders[b]	0.03	17.8 (1.8–432.9)
Major birth defects	4.3	1.5 (1.3–1.8)
Chromosomal abnormalities (post-ICSI)		
De novo sex chromosomal aneuploidy	0.6	3.0
Structural autosomal abnormalities	0.4	5.7

Used with permission from: Reddy UM, Wapner RJ, Rebar RW, Tasca RJ. Infertility, assisted reproductive technology, and adverse pregnancy outcomes: executive summary of a National Institute of Child Health and Human Development Workshop. Obstetrics & Gynecology. 2007;109(4):967–77

ART assisted reproductive technology, *OR* odds ratio, *CI* confidence interval, *NICU* neonatal intensive care unit, *ICSI* intracytoplasmic sperm injection

[a]Assisted reproductive technology singleton compared with spontaneously conceived singleton offspring

[b]Absolute risk and odds ratio reported for Beckwith Wiedemann syndrome

17.5 Controversies

17.5.1 Ethical and Religious Considerations

Infertility treatment can raise ethical or religious concerns for some patients. Patients may have concerns about the creation of human embryos and what to do with excess embryos, if available. In addition, in the situation of higher-order multiples (triplets or more), patients may be faced with the decision of whether to undergo selective reduction of one or more of the fetuses, which also has cultural and ethical implications for some. It is prudent for patients and their partners (if applicable) to discuss these issues with members of their community or their religious leaders for guidance regarding these decisions.

17.5.2 Third Party Reproduction

Some of the controversial aspects of third party reproduction have been previously discussed in this chapter, although they are some of the most debated issues in the field, and should be underscored.

17.5.3 Disposition of Embryos

As IVF involves the creation of human embryos, disposition of embryos must be clear before their creation. In particular, the wishes of a patient or couple must be clear regarding the possible situation of death of one of the intended parents, divorce, separation, failure to pay storage charges, inability to agree on disposition in the future, or prolonged lack of contact with the program. For this reason, the ASRM has a committee opinion dedicated to this subject, stating that programs should create and enforce written policies on the designation, retention, and disposal of abandoned embryos. In general, embryos may be donated (either to an infertile couple or to research) or discarded, and this should be clarified in a written document before the IVF cycle [73].

17.5.4 Genetic Information

Many controversies continue to emerge, as technological advances push the boundaries of current practice. For example, the possibility for sex selection, mitochondrial transfer, human leukocyte antigen matching, and gene editing bring new questions that must be addressed. In addition, it may be possible in the future to select embryos based on certain genetic traits that are unrelated to health or human disease. It is impossible to anticipate every possible situation where ethics will be challenged. Each of these situations is best handled with a multidisciplinary team, involving an institution's Ethics Committee if needed.

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Assisted Reproductive Technology: Laboratory Aspects

Charles L. Bormann

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18.1 Introduction

The concept of in vitro fertilization (IVF) as a treatment for infertility is straightforward: obtain eggs from the ovaries, mix them with sperm in a dish containing culture medium, and transfer the eggs back to the woman after fertilization has occurred. However, this technique took over 100 years to develop. The initial development of IVF in humans can be attributed directly to a team of two investigators, Drs. Patrick Steptoe and Robert Edwards [1]. It was in 1969 that Dr. Edwards first stated, "Human oocytes have been matured and fertilized by spermatozoa in vitro. There may be certain clinical and scientific uses for human eggs fertilized by this procedure" [2]. This understated conclusion marked the first successful attempt to fertilize human eggs in a laboratory.

In 1959, successful IVF was reported using rabbits [3]. The first human birth to result from IVF was achieved in England in 1978 [4]. John and Lesley Brown had 9 years of infertility secondary to bilateral fallopian tube obstruction. Dr. Patrick Steptoe surgically retrieved a single mature oocyte from one of Lesley's ovaries during a natural cycle. Dr. Robert G. Edwards combined John's sperm with the oocyte in the laboratory and the resulting embryo was placed into Lesley's uterus a few days later. On July 25, 1978, Louise Joy Brown was delivered by cesarean section at approximately 37 weeks gestation and weighed 5 lb., 12 oz. In 2010, 32 years later, Robert G. Edwards was awarded the Nobel Prize for Physiology or Medicine "for the development of IVF." Today, most IVF is performed after ovarian stimulation so that multiple eggs can be retrieved transvaginally with a sonographically guided needle, followed by transcervical embryo transfer.

Mammalian oocytes are maintained in meiotic arrest throughout most of follicular development; the resumption of meiosis I is induced by the preovulatory surge of LH, which is emulated during an IVF cycle by administration of hCG. Retrieval of oocytes is generally performed 34–36 h after administration of hCG in order to allow adequate time for oocyte maturation and also to avoid premature ovulation. During the oocyte retrieval, each follicle is punctured with a biopsy needle under the guidance of a transvaginal ultrasound. The fluid from within the follicle is gently aspirated by the physician into a sterile test tube containing processing medium. Heparin may be added to the processing medium to help prevent blood from clotting in the tube or search dishes. In the laboratory, aspirates are examined quickly for the presence of cumulus-oocyte complexes. Oocytes are rinsed thoroughly and placed in holding medium until time of conventional insemination or cumulous cells removal.

Clinical Case

A 27-year-old woman has recently undergone IVF for male factor infertility. She had two embryos transferred on day 5 that were considered poor quality. She did not achieve pregnancy. Although she had ten oocytes retrieved and seven had fertilized none of the remaining embryos were of sufficient quality to freeze.

18.2 Oocyte Assessment

Immature oocytes are defined as being at a stage of meiosis prior to metaphase of meiosis II (MII). This includes oocytes in prophase of meiosis I, which are identified by the presence of a germinal vesicle or nuclear envelope in the cytoplasm, without any polar body present in the perivitelline space (Fig. 18.1). If present, cumulus and corona cells are commonly very tightly condensed. As prophase I resumes, the oocyte enters into metaphase of meiosis I (MI). This intermediate stage of maturation is recognized by the disappearance of the germinal vesicle and the absence of the first polar body (Fig. 18.2). For MI oocytes, cumulus cells may be expanded, but the corona cell layer can still be compact. The extrusion of the first polar body marks the transition to a mature oocyte, which is now considered to be at MII (Fig. 18.3). Metaphase II oocytes will usually have a fully expanded cumulus cell complex. Under normal circumstances, the oocyte will remain at MII until fertilization; when meiosis II resumes, the second polar body is extruded and the male and female pronuclei form (Fig. 18.4).

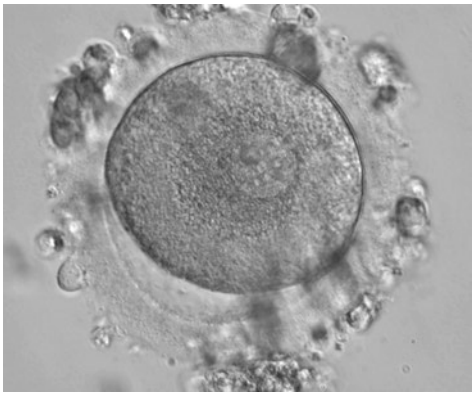

Fig. 18.1 Germinal vesicle intact representing an immature oocyte after cumulus removal (courtesy of Dr. Nina Desai, Cleveland Clinic)

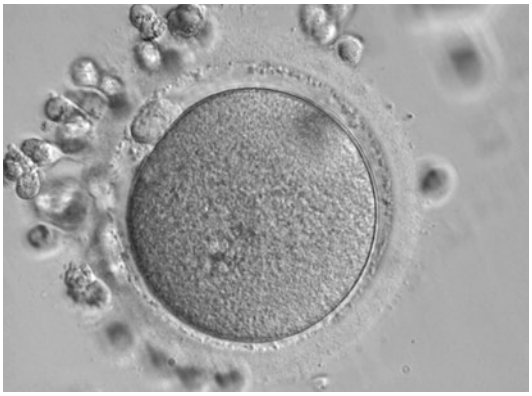

Fig. 18.3 Metaphase II (mature) oocyte. Denuded oocyte showing presence of first polar body (courtesy of Dr. Nina Desai, Cleveland Clinic)

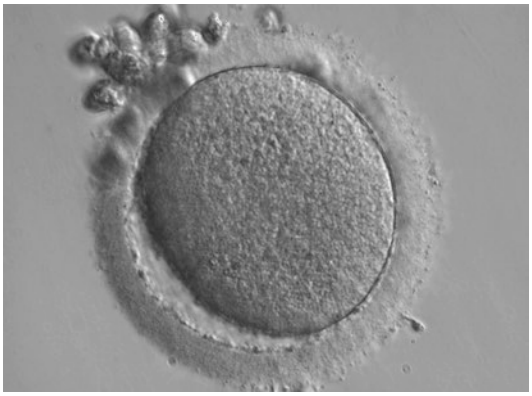

Fig. 18.2 Metaphase I (MI) human oocyte without a polar body after cumulus cell removal (courtesy of Dr. Nina Desai, Cleveland Clinic)

Fig. 18.4 Normal zygote showing presence of two polar bodies (only one seen in this view) and two pronuclei (courtesy of Dr. Nina Desai, Cleveland Clinic)

18.3 In Vitro Maturation

In vitro maturation (IVM) of oocytes is a procedure in which eggs are collected from antral follicles at a stage prior to selection and dominance. These immature oocytes are cultured under conditions that facilitate the cytoplasmic and nuclear maturation of eggs to metaphase II. This procedure is especially important for cancer patients, where the time and hormonal milieu associated with a traditional IVF cycle may adversely affect the patient's treatment and medical outcome. Likewise, patients with contraindications for ovarian stimulatory drugs such as those with polycystic ovary syndrome (PCOS) and are at higher risk for hyperstimulation with ovulation induction agents may be candidates for IVM [5].

Although the precise mechanisms that regulate the control of oocyte maturation remain obscure, it has been recognized for over 70 years that immature oocytes removed from antral follicles may undergo spontaneous maturation in culture, termed "in vitro maturation" (IVM), without the need for hormonal stimulation. With IVM, immature oocytes are typically obtained in the mid- to late-follicular phase of the menstrual cycle. To date, IVM has been most successful in young women with multiple antral

follicles that typically have a high chance of pregnancy with conventional IVF. Despite this selection bias, IVM pregnancy rates remain lower than in stimulated IVF cycles [6]. As culture conditions for IVM are optimized and pregnancy rates improve, this technology may offer a safer, less expensive, more convenient alternative to stimulated IVF.

18.4 Spermatozoa Collection, Evaluation, and Processing

The most common method of obtaining a semen sample is through masturbation and ejaculation into a sterile cup. In cases where ejaculation cannot be achieved via masturbation, whether due to religious or psychological reasons, non-toxic condoms can be used to collect the ejaculation following sexual intercourse. In cases where there is no presence of an ejaculate following orgasm, patients are asked to immediately urinate in a sterile cup and the sample is analyzed for presence of sperm. The presence of semen in the urine is clear indicator of a retrograde ejaculation. Men with this condition may be prescribed stomach-acid buffering medications in order to neutralize the pH of the urine and thus provide a more hospitable environment for the sperm during collection and processing.

In cases where men cannot achieve an erection, or ejaculation due to neurologic or psychogenic reasons, semen can still be collected via prostate massage, electrical stimulation of the prostate or applied vibration to the penis. Samples collected from men with spinal cord injuries typically have high concentrations of sperm, poor motility and contain red blood cell contamination in the ejaculate and require rigorous washing steps to isolate highly motile sperm for ICSI.

In cases of non-obstructive and obstructive azoospermia where sperm are not present in the ejaculate, spermatozoa can be collected by way of testicular dissection or percutaneous needle biopsy. This method of collection is highly invasive and is generally the last resort in obtaining sperm for ICSI. Samples obtained testicular dissection contain large amounts of red blood cells and testicular tissues; thus requiring additional steps to isolate a clean sample of spermatozoa.

18.5 Sperm Isolation for IVF and ICSI

One of the oldest and most commonly used methods of sperm isolation is the swim-up procedure. This sperm separation technique is mostly used on normozoospermic males. The swim-up method is based on the active movement of motile sperm from a pre-washed pellet of sperm into an above layer of fresh medium. The first step in swim-up involves repeat dilution and centrifugation (2–3 times) of the semen sample to separate spermatozoa from seminal plasma. Following centrifugation, the pelleted spermatozoa can be both suspended and overlaid with media or the pellet can be uninterrupted and overlaid with media. If one chooses to disrupt the pellet, extreme care must be taken when overlaying with media to prevent mixing and contamination with immotile sperm, debris, and other cell types.

The swim-up method using either the intact or disrupted sperm pellet is incubated at 37 °C for 30–60 min in a buffered media to allow spermatozoa to swim from the pellet to the culture medium. Following incubation, the upper layer of culture media is carefully aspirated without disrupting the pellet and is transferred to a clean test tube for further analysis. One advantage of this technique is that it isolates a population of sperm with greater than 90% motility and without cellular debris. The disadvantages of this technique are in the low overall recovery of motile spermatozoa due to the limited surface area of the pellet and culture media. Another disadvantage of this technique is that repeat centrifugation force viable spermatozoa to be in close contact with immotile spermatozoa, cellular debris, and leukocytes, which are known to produce very high levels of ROS and affect subsequent fertilization ability.

Density gradient centrifugation is the second most common method for isolating motile sperm for ART purposes. The majority of all density gradients used to isolate spermatozoa is discontinuous and consists of two to three layers. The most commonly used materials for density gradients are colloidal silica with covalently bound silane molecules, which have a low viscosity, are non-toxic and are approved for human use.

During centrifugation, highly motile sperm migrate faster in the direction of the sedimentation gradient and are able to penetrate this

interface faster than low-motile or non-motile spermatozoa. This unimpeded density gradient separation produces a clean fraction of highly motile spermatozoa. The pellet is washed with culture media and centrifuged at 300*g* for 10 min. This process is repeated two times to ensure complete removal of the density gradient medium prior to insemination.

There are many advantages in using a density gradient to process spermatozoa for IVF and ICSI. The entire ejaculate is used during the centrifugation process, resulting in a significantly higher yield of motile spermatozoa than can be obtained using other separation techniques. This makes this technique ideal for patients with suboptimal semen parameters (e.g., oligozoospermia and asthenozoospermia). Another advantage of this technique is that it produces a relatively clean sample of spermatozoa, free of cellular debris and leukocyte contamination. This property significantly reduces the ROS and problems associated with its contamination.

18.6 In Vitro Fertilization (IVF)

18.6.1 Conventional Insemination

Oocytes are routinely inseminated 3–6 h after oocyte retrieval is performed, depending on oocyte maturity. Individual or groups of oocytes can be incubated and inseminated either in organ culture dishes, four-well dishes, or test tubes containing equilibrated medium, with or without oil overlay. Individual oocytes can also be inseminated in 30–50-μL drops of equilibrated medium in culture dishes with oil overlay, thus reducing the number of spermatozoa necessary for the insemination. Generally, concentrations range from 50,000 to 100,000 motile spermatozoa/mL. Spermatozoa concentrations that are too high can result in increased incidence of polyspermic fertilizations (more than one spermatozoon penetrating an oocyte). Concentrations that are too low may compromise fertilization rates.

18.6.2 Intracytoplasmic Sperm Injection (ICSI)

ICSI consists of insertion of a single spermatozoon directly into the oocyte cytoplasm. This technique was first successfully applied to human oocytes in 1992 and has since revolutionized the treatment of severe male factor infertility [7]. By injecting a spermatozoon into the oocyte cytoplasm, many steps of spermatozoa processing and developmental prerequisites are bypassed without compromising fertilization rates. There is currently debate regarding appropriate indications for ICSI. Current evidence supports the following indications for the use of ICSI:

- Prior failed fertilization by conventional insemination
- Prior IVF cycle with less than 50% fertilization of MII oocytes
- Prior IVF cycle with a high rate of polyspermic fertilization
- Total motile spermatozoa concentration less than 10 million/mL
- Poor forward progressing sperm score
- Spermatozoa morphology <4% normal forms based on strict criteria

18.7 Fertilization Assessment

Fertilization assessments are performed 15–18 h after insemination for both IVF and ICSI procedures. It is necessary to examine the oocytes/zygotes within this time period to visualize the presence of pronuclei and polar bodies. Normal fertilization is characterized by the presence of two pronuclei, one male and one female, in the ooplasm and two polar bodies in the perivitelline space (Fig. 18.4). If oocytes have undergone conventional IVF, the cumulus cells must be removed to clearly see the oocyte.

Abnormal fertilization may also be represented by oligopronuclear zygotes. The term applies to zygotes that have single pronuclei. Only one pronuclei and the presence of two polar bodies may be observed in cases when the oocyte undergoes parthenogenic activation or failure of the spermatozoa head to decondense. It is possible that a second pronuclei will be developed later than the first one, therefore a repeat observation about 4 h after the first check is recommended. Failed fertilization is represented by the absence of pronuclei and presence of one or two polar bodies that may be in the process of degeneration.

18.8 Embryo Assessment

Embryos can be assessed and graded daily while they are in culture. Standard morphologic methods of grading can be applied according to observations made on embryo development until their transfer to the uterus on day 3 (72 h post fertilization) (Tables 18.1 and 18.2) or on day 5 or 6 at the blastocyst stage (Table 18.3) [8, 9]. There are numerous scoring systems proposed for embryo development. Criteria for grading include the rate of division as judged by numbers of blastomeres, size, shape, symmetry, appearance of the cytoplasm, and presence of cytoplasmic fragments.

It is important to note that evaluation and scoring by morphology alone can be subjective and may not necessarily reveal embryos with the best developmental potential. Currently, there are numerous groups working on translational research focused on noninvasive means of assessing embryos for biomarkers that are indicative of embryonic developmental competence and implantation potential. Such evaluation, in concert with morphology and genetic analysis, has enormous potential to reshape the practice of embryo selection in the future.

Table 18.1 Cleavage-stage embryo single-step grading system

Parameter measured	Score	Description of embryo grade
Cell number	#	Total number of blastomeres
Blastomere symmetry	1	Regular, even blastomere division
	2	<20% Difference between blastomeres
	3	20–50% Difference between blastomeres
	4	>50% Difference between blastomeres
Fragmentation	1	<10% Fragmentation of embryo
	2	10–20% Fragmentation of embryo
	3	20–50% Fragmentation of embryo
	4	>50% Fragmentation of embryo

Example: The grade is recorded as (cell number) C (size-fragmentation); therefore, an 8-cell embryo with even cell division and approximately 15% fragmentation by volume will be scored as an "8C,1–2"

Table 18.2 Cleavage-stage embryo two-step grading system

Embryo score	Blastomere cell number
A	Minimum of 4 cells by 40 h post-insemination Minimum of 8 cells by 64 h post-insemination
B	Minimum of 2 cells by 40 h post-insemination Minimum of 4 cells by 64 h post-insemination
C	Minimum of 2 cells by 64 h post-insemination
D	No minimums (lowest possible grade). Do not make subtractions
Subtract from the grade for irregularities as follows:	
Description of embryo	**Subtractions**
Spherical blastomeres with no fragmentation	No subtractions
Spherical blastomeres with ≤20% fragmentation	Subtract 1 grade
Slightly irregular blastomeres with ≤50% fragmentation	Subtract 2 grades
Irregular blastomeres with >50% fragmentation	Subtract 3 grades

Table 18.3 Blastocyst-stage embryo grading system

Parameter measured	Score	Description of embryo grade
Expansion status	1	Early blastocyst; blastocoel less than half the volume of the embryo, little or no expansion in overall size, zona pellucida (ZP) still thick
	2	Blastocyst; blastocoel more than half the volume of the embryos, some expansion in overall size, ZP beginning to thin
	3	Full blastocyst; blastocoel completely fills the embryo
	4	Expanded blastocyst; blastocoel volume now larger than that of the early embryo. ZP very thin
	5	Hatching blastocyst; trophectoderm has started to herniated through the ZP
	6	Hatched blastocyst; the blastocyst has evacuated the ZP
Inner cell mass	A	ICM prominent, easily discernible and consisting of many cells, cells compacted and tightly adhered together
	B	Cells less compacted so larger in size, cells loosely adhered together, some individual cells may be visible
	C	Very few cells visible, either compacted or loose, may be difficult to completely distinguish from trophectoderm
	D	Cells of the ICM appear degenerate or necrotic
	E	No ICM cells discernible in any focal plane
Trophectoderm	A	Many small identical cells forming a continuous trophectoderm layer
	B	Fewer, larger cells, may not form a completely continuous layer
	C	Sparse cells, may be very large, very flat or appear degenerate
	D	No viable trophectoderm cells discernible in any focal plane

Example: The grade is recorded as (Expansion Stage), (ICM Score, Trophectoderm Score); therefore, an expanded blastocyst with a large tightly compacted ICM and sparse elongated Trophectoderm cells will be scored as "5A,C"

18.9 Time-Lapse Imaging

Time lapse imaging (TLI) of embryos was introduced in the field as an alternative method for assessing the competency of developing embryos. This technology allows embryologists to grade embryos at specific time points without disturbing the culture environment. This method of grading opened the door to using a variety of quantifiable morphokinetic measurements to aid in embryo selection. The first scientific evidence for time-lapse selection markers was discovered by a group of researchers from Stanford in 2008 [10]. They found that three cell division time-intervals could predict successful development to blastocyst by day 2, the 4-cell stage. The cell division timing parameters were unique, because:

1. They formed a distinct timing window where blastocysts clustered very closely compared to arrested embryos.
2. They correlated with the underlying molecular health of the embryo, as gene expression analysis showed that embryos with abnormal cell division timings had defective RNA patterns.

Since the initial identification of developmental markers for blastocyst development, numerous studies have identified a multitude of parameters that have been reportedly associated with embryo implantation potential. Some of the positive predictors of implantation include: timing of compaction, timing of early blastulation, and rate of blastocoel expansion. Additionally, negative predictors of implantation have been identified using TLI. This includes the following early stage abnormal cleavage (AC) events: AC1 (where the zygote divides to more than two daughter cells), and AC2 (where one of the daughter cells divides to more than two daughter cells). In many cases, standard morphology grading alone is unable to detect AC embryos and these embryos are selected for freezing or transfer.

Despite the increasing use of TLI in this field for selecting embryos for transfer, there continues to be a large gap in high-quality evidence that supports its utility. In fact, the first randomized controlled trial designed to evaluate whether the addition of TLI parameters improves clinical outcomes compared to standard morphologic assessments did not demonstrate any significant improvements in pregnancy or implantation with these additional parameters [11].

18.10 Assisted Hatching

One of the most common unsolved problems in IVF is the fact that embryos with apparently good developmental potential do not always implant. It has been proposed that this may be due impart to defects of the zona pellucida, uterine receptivity, extensive fragmentation, modifications after freezing and thawing, or even suboptimal culture conditions. An important observation leading to the clinical introduction of assisted hatching was the finding that there were higher implantation rates from embryos that were fertilized using microsurgical techniques such as ICSI. In addition, it was observed that cleaved embryos with thinner zonae had higher implantation rates than those with thick zona pellucidas. It has also been reported that a naturally thick zona or hardening of the zona pellucida due to cryopreservation or suboptimal in vitro culture conditions may interfere with (and prevent) the natural hatching process, leading to implantation failure.

To overcome hatching failure, three different micromanipulation procedures (mechanical, chemical, and laser-induced hatching) have been used to thin or produce holes in the zona pellucida of cleavage-stage embryos. Assisted hatching techniques, designed to facilitate embryo escape from the zona, have been used in IVF centers since 1992. The initial indications for assisted hatching were patient age, zona thickness, high basal FSH value, and repeated IVF failure. Several retrospective and prospective studies assessing assisted hatching in these cases have given disparate results. Therefore, the clinical relevance of assisted hatching in cleavage-stage embryos within an assisted reproduction program is heavily debated [12].

18.11 Preimplantation Genetic Testing

Preimplantation genetic testing (PGT) includes preimplantation genetic diagnosis (PGD) performed for monogenic diseases and translocations, as well as preimplantation genetic screening (PGS) for aneuploid screening. The PGT procedure is a very early form of prenatal diagnosis for patients with a preexisting genetic risk. Technically, PGT consists of micromanipulation (biopsy), and DNA analysis of gametes and/or embryos.

18.12 Embryo Biopsy

Micromanipulation for the biopsy includes the mechanical opening of the zona pellucida and retrieval of one or two polar bodies (when performed on oocytes or zygotes), one or two blastomeres (when performed on cleavage stage embryos), and 5–10 cells (when performed on blastocysts). With the improvements in embryo culture, blastocyst conversion and high success rates with blastocyst vitrification, an increasing number of labs are performing blastocyst-stage biopsy. The human blastocyst, depending upon developmental stage, can contain more than 100 cells. As such, the biopsy of 5–10 cells from the outer layer of the trophectoderm is less likely to have a detrimental effect on the developing embryo. Additional advantages of performing biopsies at the blastocyst stage include:

1. Improved development to the blastocyst stage

2. Pre-selection of top quality embryos for biopsy
3. Improved DNA amplification with more cells biopsied
4. Lower rate of mosaicism

To aid in the biopsy of trophectoderm cells, a small opening may be made in the zona pellucida of the embryo during the cleavage stage of development. As the embryo develops and the blastocoel cavity expands, a portion of the blastocyst will herniate through the zona breach making it easily accessible for biopsy. Trophectoderm cells can be gently biopsied using a glass needle or a laser. Following biopsy, blastocysts will immediately collapse due to the opening in the zona pellucida. Blastocysts should be cryopreserved while in the collapsed state as this facilitates adequate exposure of cryoprotectants to all cells.

18.12.1 Cryopreservation

Cryopreservation of gametes and embryos maximizes success in any IVF program and prevents wastage of specimens. It is important to realize, however, that there are many ethical, religious, legal, and social implications involving embryo storage. Some countries, such as Germany, Austria, Switzerland, Denmark, and Sweden, have restricted or forbidden cryopreservation of embryos [8]. There are currently two primary categories of gamete/embryo cryopreservation strategies: slow-rate freezing and vitrification.

18.12.2 Slow-Rate Freezing

Slow-rate freeze protocols vary in permeating cryoprotectants, non-permeating cryoprotectants, and cooling and warming rates, thus making it difficult to generalize or compare cryopreservation results. The following is one general example of a cleavage-stage embryo slow-rate cryopreservation protocol.

Prior to cryopreservation, embryos that meet the program-specific freeze criteria are selected and assigned to cryopreservation. After washing embryos through processing media with 12–15 mg/mL of protein, they are exposed to the same media containing 1.5 mol/L of propylene glycol (propanediol) and then 1.5 mol/L propylene glycol plus 0.1 mol/L sucrose. Embryos are loaded into plastic straws or vials and placed in a programmable freezer, where they will be cooled at −2 °C/min from room temperature down to −4 to −6 °C. After a period of 5 min of holding the temperature, a supercooled object is pressed against the side of the container to induce "seeding." The hold is continued for a period of time, followed by continued temperature drop at a rate of −0.3 °C/min until it reaches −32 °C. At this point, the containers can be plunged directly into liquid nitrogen for storage.

18.12.3 Vitrification

Vitrification is a form of rapid cooling that utilizes very high concentrations of cryoprotectant that solidify without forming ice crystals. Ice crystals are a major cause of intracellular cryo-damage [13]. The vitrified solids contain the normal molecular and ionic distributions of the original liquid state and can be considered an extremely viscous, supercooled liquid. In this technique, oocytes or embryos are dehydrated by brief exposure to a concentrated solution of cryoprotectant before plunging the samples directly into liquid nitrogen.

Both slow-rate freezing and vitrification are being used extensively in the USA. For oocyte cryopreservation, vitrification appears to be superior to slow-rate freezing. For cleavage-stage embryos, both approaches seem to be equally successful. For freezing at the blastocyst stage, vitrification may offer more consistent results, although slow cooling is also quite efficacious [14]. With continued research, protocols for both techniques will likely be optimized.

18.12.4 Laboratory and Media Preparation

Advances in culture medium composition have significantly influenced embryo quality and pregnancy rates over the years. All media for ART can now be purchased commercially. Prior to use, they must be tested for toxins and their ability to support growth and development of embryos. Media should only be opened in a laminar flow hood, with attention to maintaining sterility during the addition of protein.

There are two basic kinds of media used for ART procedures: one used during the handling of gametes and embryos out of the CO_2 incubator, called "processing medium," and another used for culture while in CO_2 incubators, called "culture medium." Both consist of a combination of nutrients necessary to maintain early embryo metabolism and proper pH and osmolarity. A source of protein, such as albumin or synthetic serum, must be added in a percentage that can vary from 2 to 15 mg/L.

Media used for the culture of embryos are usually bicarbonate buffered and kept in an incubation chamber. It is very important that this medium is maintained at a stable pH and temperature, because embryos are extremely sensitive to variations of these two factors. Culture media, used for embryo development inside incubation chambers, should always be equilibrated inside the CO_2 environment prior to use. Overlaying of the medium with mineral oil is recommended to help avoid evaporation and increase stability of pH while the dish is temporarily out of the CO_2 environment. Oil overlay provides an effective barrier to atmospheric volatile organic compounds (VOCs), which can be embryotoxic if exposed directly to the culture medium.

18.12.5 Culture Conditions

Traditionally, laboratories have cultured embryos at an atmospheric oxygen concentration of approximately 20%. In contrast, the oxygen concentration in the fallopian tube and uterus is approximately 5% [15]. In animal models, high oxygen concentrations increase the production of reactive oxygen species. This increased oxidative stress may have deleterious effects on embryo quality [16]. Several studies suggest that culturing embryos at a lower oxygen concentration, about 5%, may improve live birth rates with IVF and ICSI [17]. Additionally, there is no evidence to date that culturing embryos under low oxygen concentrations is associated with an increased risk of any adverse outcomes, such as multiple pregnancies, miscarriages, or congenital abnormalities [17].

18.13 Monitoring Clinical Outcomes

Data analysis is a crucial part of maintaining a successful ART laboratory and practice. Routine review of identified key performance indicators (KPIs) is important to ensure proper laboratory functioning and, perhaps more importantly, to identify potential problems to permit timely correction. In fact, this is the primary reason for data analysis: to achieve early identification of factors that could negatively impact laboratory function which, in turn, allows timely insight into targets for corrective action.

The most important aspect of QA data analysis is identification of KPIs that will provide meaningful insight into laboratory functioning. While KPIs may vary among laboratories, some are routinely assessed and considered standard. These include the following:

Fertilization Rates: This is a useful indicator that provides real-time insight into variance in laboratory performance in addition, possibly, to changes in stimulation protocols. Fertilization rates from both standard IVF and ICSI should be evaluated with data stratified by the embryologists inseminating or injecting the oocytes.

Day 2 Cleavage: The rate of embryo development is a predictive indicator of embryo quality. The number of 4-cell embryos on day 2 is a common indicator of quality of the culture system.

Day 3 Embryo Development: The number of cells on day 3 gives considerable insight into performance of the lab's culture system. Embryos developing along the normal timeline should have progressed to the 7–8-cell stage. Therefore, the percentage of 2PN zygotes with >7-cell embryos provides a useful marker of overall embryo quality.

Blastocyst Formation and Embryo Freezing: Tracking total blastocyst formation, as well as quality of blastocysts as evidenced by those that meet a minimal freeze criteria helps give insight into quality of the culture system. These parameters can be measured on day 5 and/or on day 6 of culture.

Cryo-Survival: Tracking cell survival following cryopreservation/thawing of oocytes and embryos is an important marker of technical efficiency of a cryopreservation program. It is important to note that success rates may vary based on stage of tissue frozen as well as method of cryopreservation (slow-rate vs. vitrification).

Pregnancy, Implantation, and Live Birth Outcomes: The clinical outcome of an IVF cycle is perhaps the best indicator of system efficiency with implantation rates providing the most robust and timely marker of embryo quality. Assessment

of pregnancy rates per physician and embryologist performing the transfer is critical.

18.14 Future Directions

Over the past 3 decades, few areas of medicine have experienced the rapid evolution that has occurred within the field of ART. Despite this progress, success rates have plateaued in recent years, and many new challenges and opportunities for improvement lie ahead. With growing pressure to decrease multiple gestations, methods to improve embryo selection (such as PGS, transcriptomic/metabolomic profiling and time-lapse imaging) will become increasingly important. Finally, as a result of these innovations within our field, we will likely see a greater shift toward eSET in all patient populations.

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Preimplantation Genetic Diagnosis and Genetic Screening

Jason M. Franasiak and Richard T. Scott Jr

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19.1 Introduction

The development and enhancement of the assisted reproductive technologies (ART) culminating in in vitro fertilization (IVF) over the last 35 years have resulted in dramatic improvements in treatment outcomes. At the current time, IVF provides both the most successful and often the most cost-effective approach to the care of most infertile couples [1, 2].

In the short time since the first IVF birth in 1978 [3], utilization of the technology has increased dramatically. The primary goal of technology applied to embryo selection in the IVF laboratory is to distinguishing those embryos which are reproductively competent and are able to become a healthy child from those which cannot. The drive to select healthy embryos as a means to avoid the need for pregnancy termination led to the first applications of preimplantation genetic diagnosis (PGD) in 1990 [4].

The first applications for PGD came in testing monogenic disorders and sex-linked disorders. Focusing on X-chromosome linked diseases, amplification and detection of Y-chromosome specific repeat sequences allowed for selection of embryos that were female and thus not at risk of carrying the disease. This technique has evolved to detection of gene mutations on autosomes and sex chromosomes as well as translocations in either partner and allowed for selection of embryos which do not harbor the mutation for embryo transfer.

The success of PGD to predict embryos which did not have genetic disease led to attempts to apply the technology more widely to all embryos and identify those embryos with normal chromosome complements [5–13]. This practice became known as preimplantation genetic screening (PGS). This evolving effort was borne out of the historical practice of transferring multiple embryos to overcome the inability to accurately predict which embryos were competent. Even the relatively conservative embryo transfer guidelines of the American Society for Reproductive Medicine (ASRM) had previously recommended five or more in older patients. While increasing the number of embryos transferred enhanced delivery rates, the prevalence of multiple gestations, including higher order (>3) which are associated with a high morbidity as compared to singleton deliveries deliveries, are also increased at unacceptable levels [9].

The contribution of embryonic aneuploidy to the inefficiency of human reproduction is well established [14–16]. Indeed, it seemed intuitive that assessment of the ploidy status of each embryo within the developing cohort would allow selection of only euploid embryos and ought to improve IVF outcomes [17]. This premise was always valid, but early attempts at embryonic aneuploidy screening were clinical failures [18, 19]. In reality, the techniques employed for molecular analysis of a single or very small number of cells have lacked sufficient precision to be clinically meaningful. More recently, application of newer and more powerful molecular technologies has overcome some of the early limits and along with optimization of the entire process has produced meaningful improvements in clinical outcomes.

This chapter focuses on the past, present, and future of PGD/PGS applications, techniques used to obtain genetic material for analysis, and molecular strategies utilized for analysis.

Clinical Case #1: Preimplantation Genetic Diagnosis

A 32-year-old G2P1001 presents to the clinic for preimplantation counseling. She presented late to care with her first pregnancy and it was discovered after birth that the child was affected by cystic fibrosis. They desire to discuss options for preventing cystic fibrosis in their next pregnancy.

Her physician orders carrier testing and she and her partner have mutations identified in their CFTR gene. She possesses the pN1202K mutation and he the p. F508del. The treatment plan utilizing linked markers and sequencing of the gene of interest is devised in collaboration with a single gene genetics laboratory.

The patient undergoes IVF and genetic material from the embryos is obtained from the trophectoderm on day 5 for testing and

then vitrification of the embryos is performed. Testing reveals that she has two embryos of her six which are chromosomally normal and do not possess the two mutations which would result in cystic fibrosis.

After endometrial preparation with estrogen and progesterone a single embryo transfer is performed utilizing a normal blastocyst.

Clinical Case #2: Preimplantation Genetic Screening

A 36-year-old G2P1011 with male factor infertility presents in follow-up to the clinic. She had a successful birth of a child from IVF 3 years ago. The couple desires a second child but does not want twins. They have five embryos remaining which are cryopreserved. She undergoes a single embryo transfer and the resulting pregnancy is diagnoses with trisomy 21 with chorionic villus sampling. She undergoes termination of pregnancy and returns to the clinic to discuss further options. She desires a single embryo transfer but wants to limit the chance of having a chromosomally abnormal pregnancy once again.

The patient undergoes IVF with trophectoderm biopsy of the embryo on day 5 followed by embryo vitrification. The results show that two of five blastocysts are chromosomally normal and designated suitable for embryo transfer. After endometrial preparation with estrogen and progesterone a single embryo transfer is performed.

19.2 Applications for Genetic Testing of Embryos

Preimplantation genetic testing was first instituted in 1990 by Handyside et al. The report entailed two couples at risk for transmission of X-linked mental retardation and adrenoleukodystrophy [4]. Analysis of a blastomere at the six to eight cell stage with polymerase chain reaction (PCR) analysis which amplified a Y chromosome specific repeat sequence allowed for transfer to female embryos. The field's impetus at the outset was to identify only unaffected children prior to implantation and thus eliminate the need for pregnancy termination after a diagnosis was made at a later time by either chorionic villus sampling (CVS) or amniocentesis.

While PGD was initially applied to a small subset of disorders with a high likelihood, on the order of 25–50%, of being present, over the subsequent 15 years its use expanded. Testing for genetic disorders with low penetrance and late-onset became more common and the list of disorders tested consisted of over 100 conditions, although the most frequent were cystic fibrosis and hemoglobinopathies [20].

Since that time the applications of PGD have grown further to include a vast list of sex-linked and autosomal single gene disorders, HLA typing, translocations, and aneuploidy screening. Of note, there are differences in national regulations that exist. This ranges from an absolute ban on PGD in countries such as Germany and Switzerland, to countries with unrestricted utilization such as France, United Kingdom, United States, Spain, and Denmark, to countries where it is limited to specific high risk circumstances such as Finland and Portugal [21].

Finally, circumstances under which PGD is applied are also changing. In the past, it was necessary that the couple has had or poor pregnancy outcome or had strong family history of disease in order to uncover the genetic disease that was to be tested. Now, expanded carrier screening is becoming more widely utilized and in some circumstances allows for detection of a transmissible genetic anomaly before it has been phenotypically shown in couples.

19.2.1 Sex-Linked Diseases

At the outset, a readily available application of PGD came in the form of X-linked conditions. This approach was ideal for several reasons. First, in keeping with the goal of decreasing the need for pregnancy termination in couples adverse to it, it allowed for selection of couples with a 50% chance

of requiring pregnancy termination. This was most commonly done utilizing fluorescent in situ hybridization (FISH) at the outset since identification and transfer of female embryos eliminated the possibility of transmission of disease. This did, however, result in the discard of 50% of normal male embryos. As sequencing information became more readily available, it became more commonplace to test for the specific gene and thus to transfer embryos regardless of gender. This process allowed for testing of many X-linked diseases, chiefly fragile X syndrome, Duchenne muscular dystrophy, and hemophilia.

19.2.2 Single Gene Diseases

As PGD became more widespread autosomal recessive gene mutations became the most commonly tested. The most common of these included cystic fibrosis, beta thalassemia, sickle cell anemia, and spinal muscular atrophy. Autosomal dominant disorders such as myotonic dystrophy type 1, Huntington's disease and neurofibromatosis were also widely tested for utilizing PGD.

19.2.3 Translocations

Patients with balanced translocations presented a rather unique advancement for PGD. This was not a circumstance which had previously fallen under the prenatal diagnosis category as poor pregnancy outcomes related to miscarriages prior to routine testing with CVS or amniocentesis. The first case of PGD for maternal translocations which did not require use of specific DNA probes specific to each translocation was performed in 1998 with the use of FISH analysis of the first polar body [22]. Subsequent reporting with both polar bodies and blastomeres was also reported [23, 24].

19.2.4 Genetic Predisposition Disorders

As testing became more robust and sophisticated, the original goal of decreasing the need to decide whether to terminate a pregnancy with a known genetic disease expanded to include disease states that may not have been clear indications for pregnancy termination when found during prenatal testing with CVS and amniocentesis. This new group of disease included those with genetic predisposition toward disease. The use of PGD allowed for selection of embryos which did not possess the predisposition and thus eliminating the need to discuss termination for a disease with uncertain phenotypic consequences.

One of the first such case series involved PGD for patients at risk for familial adenomatous polyposis coli (FAP), von Hippel-Lindau syndrome (VHL), retinoblastoma, and Li-Fraumeni syndrome, determined by p53 tumor suppressor gene mutations [25]. This was also applied in slightly more controversial cases, for example in the case of selecting for an embryo that did not carry a gene known to be associated with Alzheimer's disease [26].

Although this area of PGD is still discussed, both the American Society for Reproductive Medicine (ASRM) ethics committee for adult onset conditions and ESHRE ethics task force concluded the use of PGD for late onset disorders was appropriate [27, 28].

19.2.5 HLA Matching

In the same vein as genetic predisposition disorders, prenatal diagnosis for human lymphocyte antigen (HLA) matching has not been part of standard care as it would then involve decisions of termination of a pregnancy that was not HLA matched but otherwise healthy. However, with IVF where a subset of embryos are chosen for embryo transfer as part of routine care, the addition of PGD to determine HLA typing was more acceptable. The first case in 2001 involved a couple who had a child with Fanconi anemia. Testing was done for both HLA typing and Fanconi anemia in hopes of producing healthy offspring that may also serve as a transplantation donor [29].

There are inherent limitations to PGD for HLA matching. There is in theory a 25% chance of finding a HLA match, but this must be taken in combination with being able to find an embryo that is both a HLA match and does not carry the disease in question. Adding aneuploidy screening on top of this results in only 12–15% of embryo being eligible for transfer [30, 31].

19.2.6 Aneuploidy Screening

Utilization of PGD for aneuploidy screening was initially borne out of a desire to improve pregnancy rates in patients with advanced reproductive age. While prenatal diagnosis for aneuploidy was utilized to decrease rates of live births of fetuses with an extra chromosome 21, 18, or 13, PGD was meant to look more widely at chromosomes to better selection and reproductive success for patients undergoing IVF.

FISH was initially employed with various combinations of chromosomes. However, this method was always limited by the inability to simultaneously screen for all 24 chromosomes [19]. FISH typically screened the seven chromosomes most frequently seen in miscarriage specimens (chromosomes 13, 16, 18, 21, 22, X and Y) analyzing only one or two blastomeres [32, 33]. Five trials examining the impact of chromosomal screening in patients with advanced maternal age [32–34], typically classified as poorer prognosis, and four trials in relatively good prognosis patients failed to show benefit when screening with FISH [35–38]. This is due in part to the limited interrogation of chromosomes and the error for a chromosome is extrapolated based on the presence or absence of a single locus.

The development of technologies for single cell whole genome amplification (WGA) allowed for analysis of all 24 chromosomes [39–41]. A wide variety of techniques have been used for WGA ranging from primer extension preamplification (PEP) PCR, degenerate oligonucleotide primed (DOP) PCR, multiple displacement amplification (MDA), end-labeling, and more recently multiple annealing and looping based amplification cycles (MALBAC). Unfortunately, there are no published studies comparing the various methods used for WGA and no clinical data regarding the predictive value of the techniques. High quality randomized trails remain to be done which include different testing platforms and actual clinical outcomes to confirm the findings. Indeed, WGA remains a complex and unresolved issue.

Platforms which utilize various types of WGA include metaphase comparative genomic hybridization (mCGH) [42, 43], array CGH (aCGH) [44, 45], single nucleotide polymorphism (SNP) arrays [5, 46, 47], oligonucleotide CGH [48] and more recently next generation sequencing (NGS) [49]. An additional method, which enables 24 chromosome evaluation without requiring whole genome amplification, is quantitative real time (qPCR) [50].

Each of these platforms varies in their reported accuracy, based upon cell line predictions; consistency between polar body and oocyte; amount of time required to complete analysis; stage of biopsy which would enable a timely, fresh embryo transfer; number of probes required, and minimum detectable imbalance [51]. Furthermore, platforms vary in the extent to which they have undergone rigorous validation of their ability to assess embryonic aneuploidy in a single cell. Most remain incompletely validated.

19.2.7 Summary

Since PGD's inception in 1990, the utilization worldwide has increased and its indications for use have expanded. At its outset monogenic disorders represented the most common indication for PGD. While single-gene cases continue to rise, PGD is most commonly utilized in the United States and Europe for aneuploidy screening according to the Society for Assisted Reproductive Technology (SART) and the European Society of Human Reproduction (ESHRE) [52, 53]. A major reason for this trend is that single gene cases most commonly need to be referred by primary care physicians who can alert their patients who have heritable diseases about this technology. However, according to available data, less than 10% of internists feel comfortable discussing PGD with their patients and less than 5% ultimately recommend it for their patients [54]. This data demonstrates the potential of PGD to impact the burden of disease in the human population has only begun to be realized.

19.3 Obtaining Genetic Material

Although technologic advances may prove otherwise, at present, there is no reliable way to diagnose genetic disease in the human embryo with precision without removing cells at some stage of development. When considering the stage of development during which the biopsy is performed, safety of the procedure and to the

predictive value of analytical result for actual clinical outcomes are important factors.

At the current time, biopsy for PGD may be accomplished at one or more of four possible developmental stages: (1) first polar body from the oocyte; (2) second polar body from the two-pronuclear embryo; (3) blastomere biopsy obtained at the cleavage stage or (4) trophectoderm biopsy at the blastocyst stage. A recent experience from the ESHRE data set suggests that ~12% of biopsies have been done at the polar body stage, ~88% have been done at the cleavage stage, and that less than 1% have been done at the blastocyst stage [55]. Of course, this varies from program to program and even country to country where regulatory restrictions may provide limits on the timing of the biopsy. Additionally, there are a number of technical and clinical considerations which guide this decision [56–58].

Defining the optimal time to obtain the critical specimen for analysis requires careful consideration of several factors: (1) Does the timing of the biopsy allow accurate identification of the genetic errors for which the embryos are being screened? Screening too early in development might miss critical errors which occur later that could impact the reproductive potential of the embryo. (2) Do the abnormalities identified in the specimen accurately and consistently predict a corresponding abnormality in the embryo? If the embryo has the potential to self-correct, then identification of imbalances in the biopsy specimen might result in assignment of an incorrect karyotype which could cause some normal embryos to be discarded. (3) Can the specimen be obtained quickly enough to allow timely embryo selection? This parameter impacts the ability to perform an embryo transfer in the same cycle as the screening. (4) Finally, does the biopsy itself compromise the embryo? The safety of the biopsy itself is sentinel.

19.3.1 Polar Body Analysis

Polar bodies (PB) represent an attractive target as they have been extruded from the oocyte and they eventually undergo apoptosis. The first PB is removed prior to conception and the second PB removed following insemination and fertilization. Initial data showed that PB biopsy did not impact embryonic development but more recent studies have called this into question [59, 60].

More significant than these concerns are the issues related to the clinical meaningfulness of the information attained from PB analysis. First, PB analysis tests only for meiotic errors in the oocyte. This is effective if single gene cases are being performed on a maternally inherited disease process. However, when it comes to aneuploidy screening, the opportunity to identify male meiotic errors or mosaic errors, which account for approximately one third of embryonic aneuploidy, is lost.

The molecular analytical challenge results from the different types of meiotic errors. Maternal meiotic errors may arise from either non-disjunction (ND) or premature separation of sister chromatids (PSSC) (Fig. 19.1). Classically, it was assumed that the substantial majority of maternal meiotic errors came from non-disjunction in meiosis I [14, 62]. In fact almost 90% of meiosis I errors arise from PSSC [63–67]. With ND, reciprocal errors in the polar bodies (meaning gain in the first PB and loss in the second PB or vice versa) indicate an imbalance and will result in an aneuploid embryo. In contrast, when PSSC has occurred then the presence of reciprocal errors indicates that the abnormality in the first meiotic division was corrected in the second meiotic division and that the embryo will be euploid for that chromosome. The molecular analytical challenge comes in distinguishing the amount of material lost or gained in a polar body. Techniques such as quantitative SNP microarray can detect a loss or gain for a particular chromosome, but cannot reliably distinguish the extent of the change. Traditional approaches such as heterozygosity analyses are less informative here as the polar bodies may contain nearly identical sister-sister homologues. Clinically, when the PB analysis returns abnormal results, the resulting embryo will be discarded. Given the high prevalence of PSSC (90% of meiosis I errors) and the 50% self-correction rate in the second meiotic division [63], this provides an exceedingly high clinical error rate and results in an unacceptably large number of euploid embryos being discarded (Fig. 19.2).

19.3.2 Blastomere Biopsy

Biopsy of the embryo itself is essential to attain the greatest opportunity to capture aneuploidy. This may be done at the cleavage stage prior to compaction or later in development after blastocyst

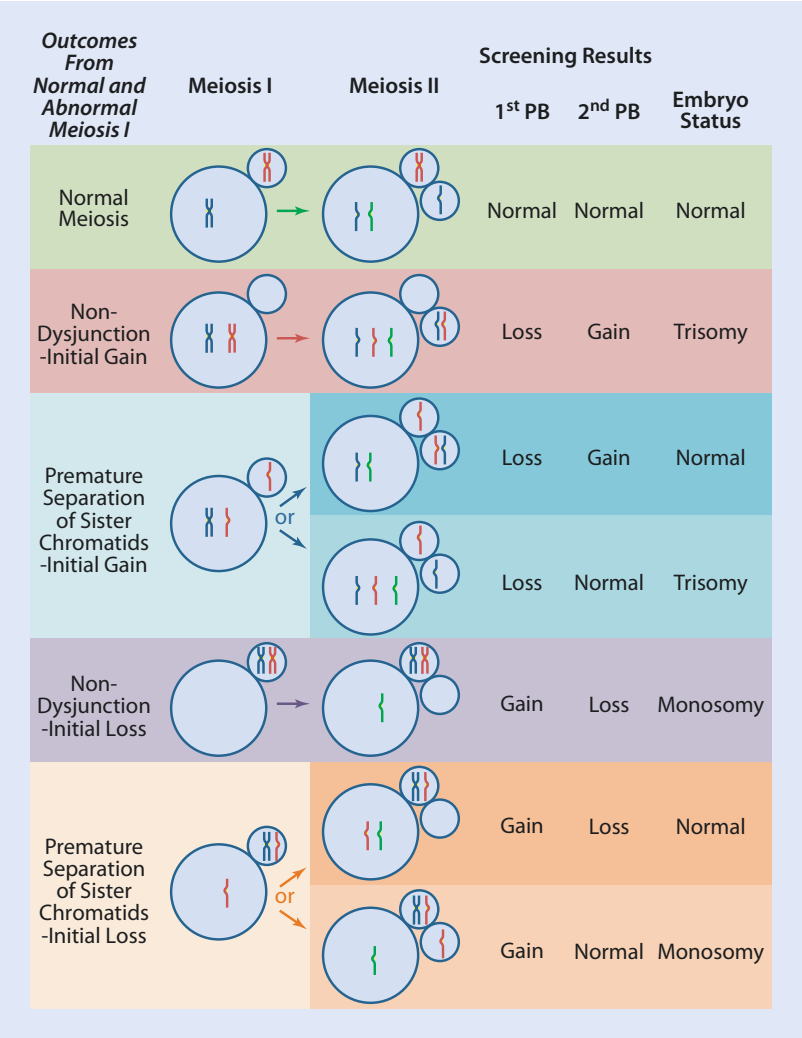

Fig. 19.1 Reciprocal errors and PSSC. Impact of the type of meiotic error and the predictive value of polar body (PB) screening results and the eventual chromosomal composition of the embryo. Note that reciprocal errors prognosticate aneuploidy after non-disjunction but euploidy after premature separation of sister chromatids (PSSC) making that result indeterminate. Adapted from [90]

formation. Removal of cells from two- and four-cell embryos is known to reduce the inner cell mass (ICM), the portion of the embryo from which the fetus is derived [68]. Common practice has been to biopsy embryos at the eight cell stage on the morning of day 3. Indeed, until recently 88% of biopsies for preimplantation genetic diagnosis (PGD) cases were performed on day 3 cleavage stage embryos, with less than 1% being performed at the later blastocyst stage [55]. Greater concerns arise when more than one cell is required, as is the case with many platforms capable of PGD.

The safety of biopsy at the eight cell stage was based upon the fact that all cells at this stage are thought to be totipotent [69] and that removal of cells did not appear to compromise ICM allocation [70]. However, both the cells and cytoplasm have become polarized at this stage and given that all cells are morphologically the same, it is not possible to determine which cells are destined to become fetal and may be more likely to be detrimental to development if biopsied. Finally, the number of cells available at the cleavage stage limits the number of cells that can safely be removed and thus limits the starting material available for amplification and analysis. Removal of even one blastomere affects outcomes [18] and two blastomeres rather than one blastomere has been shown to be detrimental to ultimate blastocyst formation [71].

19.3.3 Trophectoderm Biopsy

The final stage of development prior to embryo transfer during IVF is the blastocyst stage. At this point the blastocoelic cavity has begun to

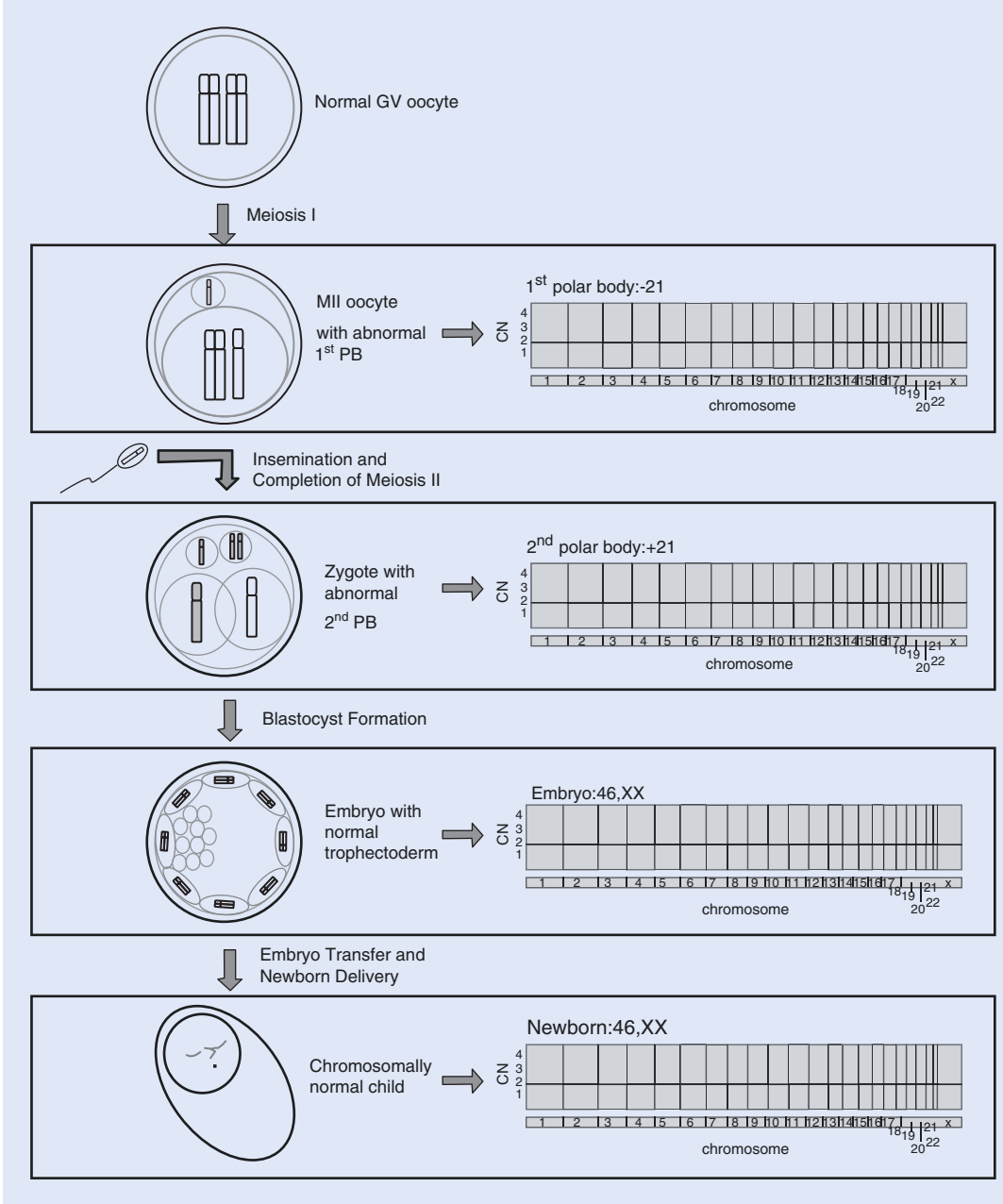

Fig. 19.2 PSSC with correction. Data from an oocyte that underwent first and second polar body analysis and subsequent embryo biopsy. The polar bodies demonstrated reciprocal errors. Given that the embryo was normal, this represents premature separation of sister chromatids with correction of the meiosis I error in meiosis II. The reproductive competence of the embryo was demonstrated by the delivery of a healthy infant. Adapted from [90]

develop and the trophectoderm cells, those destined to be amniotic membranes, and the ICM cells, those destined to become the embryo, can be easily distinguished. It is possible to remove up to five or six cells, which can be used to reliably reduce no result rates due to failed amplification when using platforms for CCS. Initially, trophectoderm biopsy was utilized as confirmatory diagnosis following a polar body or blastomere biopsy [72].

Until only recently, no class I data existed evaluating the safety of embryo biopsy at these two developmental stages: cleavage stage and blastocyst biopsy of trophectoderm. The major limitation of literature comparing the techniques was selection of controls. Given the large variety of reasons patients seek preimplantation genetic diagnosis, it can be challenging to compare two groups of patients. Recently, a blinded, paired, randomized trial was undertaken to determine the impact of embryonic biopsy [73]. This experiment was done for both cleavage stage biopsies and for trophectoderm biopsy at the blastocyst stage and showed a relative reduction of 30% in the sustained implantation rate for blastomere biopsy.

There are several possible reasons for these results. First, the trophectoderm biopsy removes proportionally fewer cells than the cleavage stage biopsy [74]. One or two blastomeres taken from an eight cell cleavage stage embryo represents 12.5–25% of the embryo, whereas four to five trophectoderm cells taken from an expanded blastocyst containing 200–220 cells represent only 2–3% of the total cells. Another possibility is that, as noted above, trophectoderm cells represent an extraembryonic component of the blastocyst whereas the specific fate of the blastomere being removed cannot be determined by morphology, and thus could represent cells with gene activation that have destined them to become the embryo [75]. The final possibility is that blastocysts simply tolerate mechanical manipulation better than cleavage stage embryos [76].

Thus, trophectoderm biopsy of four to five cells from an expanded blastocyst represents the safest way to obtain cells from a developing embryo that contains the full maternal and paternal complement of DNA that will become the conceptus. In light of the highly predictive nature of the chromosome complement in the trophectoderm for the inner cell mass, blastomere biopsy represents an unnecessary risk [77].

Embryonic mosaicism is important to note here. It is known that FISH overestimates true-cleavage stage mosaicism [78] in some cases indicating it occurs as much as 100% of the time. There is data utilizing SNP microarray which suggests it occurs 24–31% of the time [41, 78]. These mosaicism rates may account for the failures of euploid blastocyst transfers. Additionally, mosaicism likely accounts for the majority of misdiagnoses made when utilizing a validated PGD platform [79].

19.4 Strategies for Single Gene Defect PGD

There were a number of limits which have faced PGD in its infancy. They primarily relate to the fidelity of amplification and analysis of samples with extremely limited amounts of starting material. This was overcome by analyzing the DNA in multiple areas, primarily through linkage analysis. This was done by finding single nucleotide polymorphisms (SNPs) on the impacted chromosome which are different than those on the normal chromosome for each individual. Then, at the time of analysis, multiplex PCR reactions are utilized to look not only for the mutation but also for the linked markers which should be nearby. In doing this, the laboratory has several chances to detect the presence or absence of the mutation.

Unfortunately, linkage analysis is not always straightforward. The following are some of the factors that may impact the reliability of this approach:

1. It may not be possible to target the mutation directly. In such cases, additional linked markers are recommended
2. The potential for recombination exists. Recombination occurs during meiosis I and at that time DNA is exchanged between chromosomes. If the point of recombination lies between the marker and the mutation, then the marker will be moved to the normal chromosome. In this setting, the assay might perform perfectly and still prognosticate the wrong clinical result! There are ways to minimize the risk that recombination will impact the validity of the screening result [55]:
   (a) Pick markers which are as close to the mutation as possible. The closer the mutation the less likely it is that a recombination event will occur between them.
   (b) Ideally the marker would be intragenic and exhibit a high degree of heterozygosity and provide consistent high quality amplification

(c) Extragenic markers which are utilized should ideally be within 1 MB of the mutation
(d) Use a total of four markers—two upstream and two downstream of the mutation. Thus if ADO occurs but there is concurrence with the other three a result may still be determined. If only a single marker is used—specifically in those cases where the mutation is not being evaluated directly—an indeterminate will result for any discrepancy
(e) Recombination will be reflected by a discrepancy between the more proximal and distal markers. This phenomenon should be rare (~1%). If you have several embryos in a cohort with recombination in the interpreted results, it may be prudent to reevaluate the screening strategy or the fidelity of the amplification for those markers

19.4.1 Alternative Strategies for Single Gene Defect PGD

The wide spread availability of cost effective NextGen sequencing platforms may impact single gene defect PGD. A recent has demonstrated the ability to sequence through the area of the defect providing both qualitative and quantitative information [49]. This would functionally provide more markers and the depth of the sequencing appears to meaningfully enhance the precision of these assays. With the appropriate amplification strategies, it may also allow simultaneous aneuploidy screening which would empower the transfer of lower numbers of embryos while lowering loss rates and improving outcomes. There is much work to be done as the validations are not yet published.

19.5 Strategies for Aneuploidy PGS

Amplification is a critical step in the molecular analysis of these samples. There are approximately 6 pg of DNA in a single cell. Thus, an average trophectoderm biopsy of five cells would provide approximately 30 pg of DNA. Technologies capable of copy number analyses require dramatically more DNA to obtain a result: typically several hundred nanograms or more. This requires a 100,000 (or more) fold amplification of the DNA in a relatively uniform fashion.

Many techniques employ whole genome amplification. While it has the advantage of potentially amplifying the entire genome, uniformity is typically not attained and may vary as much as several thousand fold across different portions of the genome. This disadvantage may be overcome by evaluating the chromosomes at a large number of different sites and then performing statistical smoothing. Alternatively, a targeted approach focused at specific loci on each chromosome may be used. This is more uniform but still varies and requires some degree of statistical smoothing. Whole genome amplification is required for SNP microarray and CGH related technologies. In contrast, qPCR is done with targeted amplification of specific loci and next generation sequencing (NGS) may be done with either.

One metric by which to compare the available technologies is the time required to perform the test. This is of great importance in the application to real-time IVF. Often, the embryo is transferred in the same cycle as the oocyte was retrieved, a so-called fresh embryo transfer. If this is not possible, the embryo must be subjected to freezing and transferred at a later day, a so-called frozen transfer. As discussed, the biopsy is most optimally performed on the expanded blastocyst. This leaves less than 24 h within which to make a diagnosis and transfer the embryo in that fresh cycle. If diagnosis takes longer than this, embryos must be frozen, using either slow freezing methods or vitrification, and then thawed after the diagnosis is made and transferred in a separate cycle. In circumstances where results cannot be obtained in time for a fresh embryo transfer, modern vitrification techniques yield equivalent pregnancy outcomes in fresh or frozen cycles [10].

Traditional metaphase CGH requires a minimum of 72 h to yield results. There are shorter mCGH protocols, but even these take up to 24 h for the entire process to be completed and results may not available in time for a fresh transfer [59, 60, 80–82]. Thus, aCGH is an attractive

alternative, requiring only 12 h for results [83], with the added benefit of greater throughput. SNP arrays have also been used to perform CCS on blastomeres in time for a fresh transfer and also have a result rate time of 12 h [46]. Finally, qPCR results are available in as little as 4 h which enables a fresh embryo transfer after analysis of trophectoderm cells from an expanded blastocyst [84]. Thus, only qPCR analysis allows for safe biopsy of a day 5 blastocyst with results in time for a fresh transfer early on day 6.

Thus, given the ability to have results relatively quickly, DNA array technology was widely applied to CCS in the form of aCGH and SNP array. There are two main contrasting attributes of these two platforms. First is the number of DNA base probes across the 24 chromosomes. Although accuracy cannot be precisely correlated with probe number, resolution, which in CCS is the ability to distinguish between euploid and aneuploid, varies significantly. For example, BlueGnome (Illumina, USA), a widely used aCGH platform uses bacterial artificial chromosome (BAC) arrays, and possesses between 2000 and 5000 DNA probes spread across the 24 chromosomes [85]. By comparison, the Agilent (Agilent, USA) utilizes 180,000 oligonucleotide probes [48] and the Affymetrix (USA) SNP array contains 262,000–370,000 probes [41, 46, 47].

In addition to resolution differences, the way that copy number is assigned for each chromosome differs between aCGH and SNP arrays. With aCGH copy number is assigned by analyzing a ratio of red (sample) and green (control) fluorescence after differential labeling and mixing of biopsy DNA with control DNA [86]. With the Affymetrix SNP array, a single color system is used where the fluorophore hybridizes only to the biopsied DNA sample and then computational comparisons of signal intensities are made to separate control DNA hybridized arrays [87]. The former has the advantage of comparing a control sample in the same time frame under the same laboratory conditions. The latter has the advantage of comparing the sample DNA to several control samples, rather than just one, which allows for better statistical smoothing of natural inconsistencies in control samples. Furthermore, the differences in the paradigm utilized to assign copy number impact the platform's ability to gather further information from the test. For example, SNP arrays allow for a determination of parental origin of both monosomy and trisomy of parental origin from the SNP array data alone [67]. Additionally, uniparental isodisomy, although a rare event, has been validated utilizing SNP array through loss of heterozygosity analysis [5]. Finally, DNA fingerprinting from SNP array data provides clinicians with the opportunity to prevent misdiagnosis from contamination and can also be used to track which embryos implant in the event of a multiple embryo transfer for research and clinical purposes screening [88]. The array CGH platform is unable to provide these additional points of information.

19.5.1 Clinical Impact

Intuitively it seems logical that PGS would improve outcome with older patients. Aneuploidy rate is closely related to maternal age (Fig. 19.3a). Furthermore the complexity of the aneuploidy increases with age (Fig. 19.3b). The fact that a platform is accurate and reliable and has known positive predictive value does not indicate that clinical outcomes can be improved by applying the technology. This is the final step in validation: a prospective, randomized selection study. This class I data was recently obtained and demonstrated that CCS for aneuploidy utilizing qPCR, which had been cross-validated with SNP array, meaningfully enhances embryo selection and ultimately implantation and delivery rates [90]. Indeed, in this study, sustained implantation rates in the trophectoderm biopsy and CCS group was 66.4% versus 47.9% in the control group. Subsequent class one data proved the single embryo transfer of a CCS screened blastocyst had equivalent outcomes as double embryo transfer in an unscreened arm with elimination of twin pregnancies [10]. Unfortunately, not all the platforms in use for CCS have undergone rigorous validation. However, many of the shortcomings have been addressed and continued work will hopefully alleviate this concern. It is critical that this occurs lest their use results in a similar story to that of FISH.

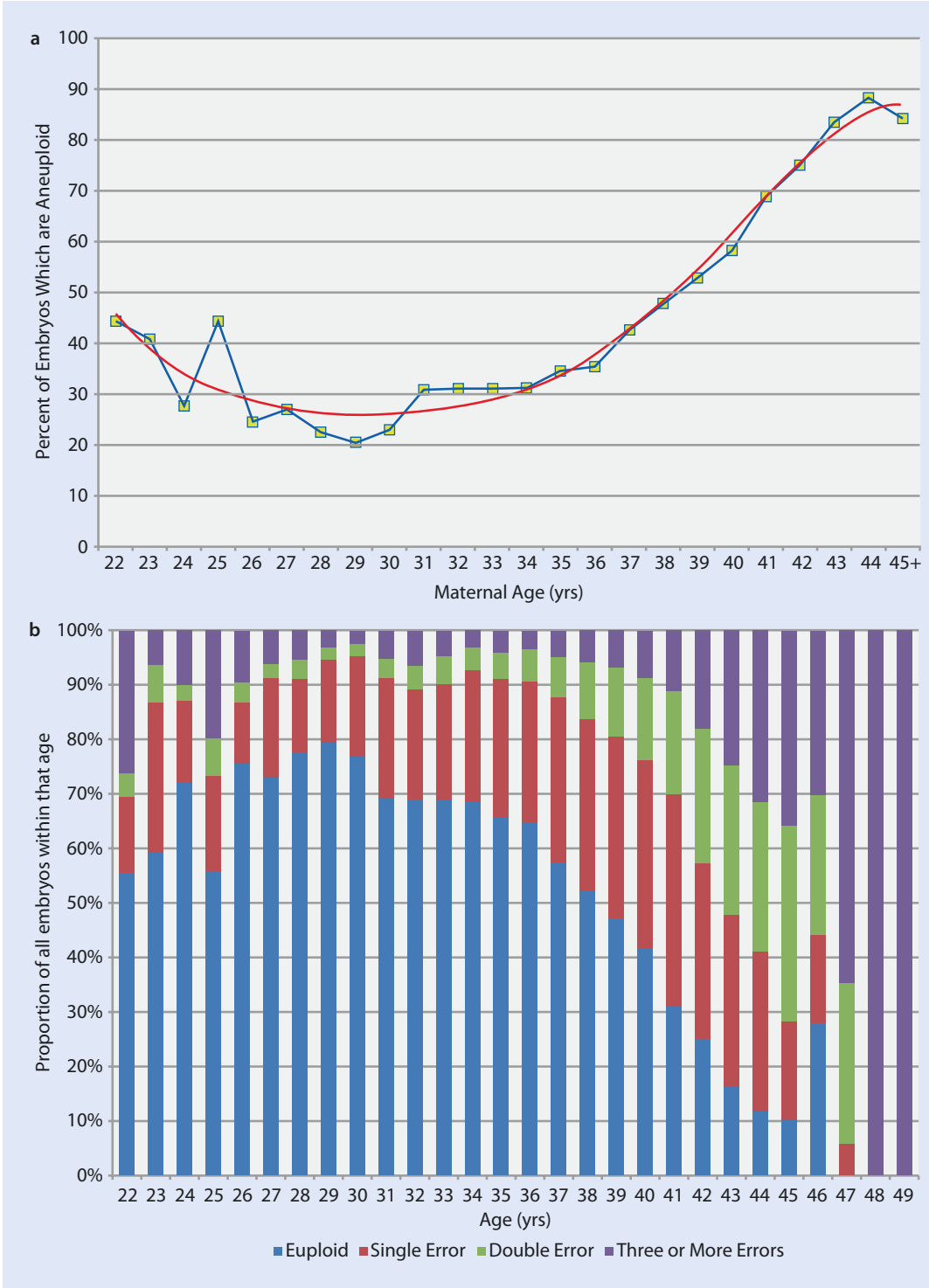

Fig. 19.3 **a** Embryonic aneuploidy rate. Aneuploidy rate relative to maternal age in 15,169 embryos undergoing aneuploidy screening for clinical IVF. Adapted from [89]. **b** Complexity of aneuploidy with age. Comparison of maternal age and the nature of the aneuploidy screening results amongst 15,169 embryos. The data are expressed as the proportion of the embryo which were euploid or as aneuploid involved a single chromosome, two chromosomes, or three or more chromosomes. Adapted from [47]

19.6 Conclusion

Preimplantation genetic diagnosis has evolved over the past several decades and existed in many forms. Its use as a mechanism for enhancing embryo selection in hopes of improving pregnancy outcomes with IVF has grown substantially in the past decade. There is also continued growth of utilization for single gene cases; however, this remains an underutilized technology.

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Hysteroscopic Management of Intrauterine Disorders: Polypectomy, Myomectomy, Endometrial Ablation, Adhesiolysis, and Removal of Uterine Septum

Michelle G. Park and Keith B. Isaacson

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20.1 Introduction

Hysteroscopy is a minimally invasive and highly accurate means of diagnosing and treating a multitude of intrauterine pathologies. During hysteroscopy, a telescope is inserted transvaginally through the cervix and into the uterine cavity. A distention media is used to expand the uterine cavity in order to visualize the endometrium and tubal ostia. Pathology can then be detected and treated with direct visualization.

As the technology has advanced, hysteroscopy has become more accessible to gynecologists both in the office and in the operating room. It has become an indispensable tool for treating fibroids, polyps, synechiae, and congenital uterine anomalies and for performing endometrial ablation procedures.

Many hysteroscopic procedures that were historically only performed in the operating theater are now performed in an office based setting often without the need for local or systemic anesthesia and often without the use of a vaginal speculum. These vaginoscopic procedures are well tolerated by the patients and allows for the most rapid return to normal activities.

Clinical Case

Mrs. F is a 29-year-old with regular cycles that are very heavy in flow who presents two spontaneous miscarriages and persistent bleeding since her third D&C following this last pregnancy 12 weeks ago. She reported no problem conceiving when trying. On her initial visit, a 3-D ultrasound was obtained which showed a uterine septum extending 2/3 down from the fundus to the cervix. The right horn demonstrated a thickened endometrial echo consistent with retained products of conception and on the left there was noted to be a type III 2 cm submucous myoma.

A 5.5 mm continuous flow hysteroscope was the instrument of choice in the office on the initial visit. Normal saline for distention was used to insert the scope vaginoscopically (without the need for a speculum or tenaculum). The hysteroscope was guided into the right horn and the retained POCs were removed in a piecemeal fashion. The septum was then cut with 5Fr scissors and the type III myoma was left in place.

This case demonstrates the value of combined U/S and hysteroscopic approach in an office setting. The patient likely had retained POCs by doing a D&C in the wrong uterine horn. Her early micarriages could be due to the uterine septum, the myoma, or other unknown causes. Since she is young, it is reasonable to encourage her to conceive spontaneously after 8 weeks post septum repair. Should another miscarriage occur, she will consider a myomectomy.

20.2 Indications

The most common indications for evaluation of the uterine cavity are abnormal uterine bleeding and infertility. Although hysterosalpingography (HSG) and modern ultrasonography are relatively sensitive in detecting intrauterine anomalies, diagnostic hysteroscopy remains the gold standard tool to visualize the cervical canal and uterine cavity. Today, there remain two schools of thought regarding initial evaluation of the uterine cavity. Many clinicians utilize HSG, transvaginal ultrasonography, and sonohysterography for initial evaluation. However, gynecologists can proceed directly to diagnostic hysteroscopy in the office or outpatient setting with little or no anesthesia for this purpose. Operative office hysteroscopy allows for a visually directed biopsy of focal lesions and treatment of intrauterine lesions, including endometrial polyps, intracavitary leiomyomas, uterine septa, and uterine synechiae.

20.3 Basic Hysteroscopic Hardware

20.3.1 Types of Hysteroscopes

Until recently, all hysteroscopes incorporated an optical system that includes mechanisms to deliver light into the uterine cavity and return the reflective image to an eyepiece or a camera. The

most commonly used system is the rigid hysteroscope with an objective lens at the tip offset by 0, 12, 25, or 30° from the horizontal plane. Other systems include the fiber-optic diagnostic single-channel flexible hysteroscopes and digital flexible dual-channel hysteroscopes. All hysteroscopes include at least one channel for the inflow of distention media. Continuous flow hysteroscopes incorporate a second channel for the return of distention media and the placement of operative instruments. Since CCD and CMOS camera chips have been miniaturized and less expensive, new hysteroscopes have incorporated the "chip on a stick" and LED light design. This eliminates the need for a light source, and allows the image to be projected directly through a USB port for visualization and image capture.

Resectoscopes are all continuous flow systems that allow the attachment of operative instruments, including monopolar and bipolar electrodes in a variety of shapes. These are 7- to10-mm systems and are more typically utilized in the operating room setting. All resectoscopes incorporate a rod lens system for light delivery and image return.

Since the publication of the last edition of this text, intrauterine tissue shavers have become available in small sizes (6.5 mm O.D.) appropriate for hysteroscopic polypectomies and larger more robust designs for hysteroscopic myomectomies. One of the shavers combines bipolar and mechanical energy but most are purely mechanical. In general this technology is far more expensive than resectoscopic RF systems but this may change with time. The other drawback for myomas is their inability to easily remove the intramural portion of submucous myomas.

20.3.2 Distention Media

The uterine cavity is a potential space that must be distended with either gas or fluid media in order to visualize the endometrium and intrauterine pathology in three dimensions. Each distention media has its own advantages and disadvantages.

20.3.3 Low-Viscosity Fluids

Low-viscosity fluids are the most common distention media used today because they are suitable for both diagnostic and operative hysteroscopy, are relatively inexpensive, and are relatively low risk to use. The two groups of low-viscosity fluids are isotonic electrolyte-containing fluids and nonelectrolyte media that may be hypotonic or isotonic.

Isotonic electrolyte-containing fluids can be used for all operative procedures except those that require monopolar electrosurgery. Two commonly used fluids are 0.9% sodium chloride and acetated lactated Ringer's solution. Nonelectrolyte media is required for monopolar electrosurgery (i.e., resectoscopic) procedures.

When electrolyte-containing solutions are used (e.g., saline), the procedure should be discontinued when a fluid deficit of 2500 mL is reached. The patient may require a diuretic if this fluid deficit is higher.

Hypotonic nonelectrolyte-containing fluids are required when the monopolar resectoscope is used, and several types are available. The most common fluids used are 5% mannitol, 3% sorbitol, and 1.5% glycine. The theoretical advantage of 5% mannitol is that it is isotonic, which, in theory, may reduce the risk of cerebral edema with excessive absorption [1]. Excessive absorption of all electrolyte-free media can lead to hyponatremia and its potentially life-threatening complications. Sodium levels fall 10 mEq/mL for every 1 L of electrolyte-free media absorbed.

When a fluid deficit of 1000 cm^3 of nonelectrolyte solution is identified, electrolytes should be drawn and the case should be terminated. Consideration should be given to administer diuretics with close monitoring of electrolytes following surgery. Injection of 3–4 mL of dilute vasopressin (10 units in 200 mL saline) into the cervix prior to distention of the cavity can decrease both intraoperative bleeding and intravasation for at least 20–30 min [2].

20.3.4 Carbon Dioxide

Carbon dioxide is less commonly used today and is only appropriate for diagnostic procedures.

20.3.5 Dextran 70

One of the first fluid-distention media used was high-viscosity hypertonic dextran 70. A 32% solution of dextran 70 in 10% dextrose in water is a

nonelectrolytic, nonconductive fluid with syrup-like consistency that can be used for both diagnostic and operative hysteroscopy. It is rarely used today as hysteroscopic distention media because of higher rates of allergic reactions and hypertonic-associated problems, including pulmonary edema [3].

20.3.6 Diagnostic Hysteroscopy

Diagnostic hysteroscopy is used to examine the intrauterine cavity. Patients should be on suppressive hormones or have the procedure timed to occur during the proliferative phase of their menstrual cycle in order to improve visualization. Diagnostic hysteroscopy can be performed in the office setting or the operating room, depending on the level of discomfort the patient experiences during the procedure.

Usually diagnostic hysteroscopy can be performed in the office setting without the need of any analgesia or anesthesia. Narrow, rigid, and flexible hysteroscopes generally have diameters <4 mm that can be inserted with minimal discomfort to the patient. If the patient experiences discomfort, oral analgesics may be required, and if cervical dilation is necessary, a paracervical block may also be placed.

It is important to insert the hysteroscope slowly and under direct visualization of the canal so that a false channel is not created and uterine perforation can be avoided. In order to allow for controlled insertion of the hysteroscope, some patients may require cervical softening prior to hysteroscopy. Misoprostol is most commonly used for cervical softening. Vaginal misoprostol (200–400 mcg) can be given 8–12 h before surgery, or oral misoprostol (400 mcg) can be given 12 and 24 h before surgery. This should only be used if cervical stenosis is documented since routine use can lead to a cervix that is too soft and cannot maintain distention. If a patient is menopausal, the misoprostol is only effective after vaginal estrogen has been administered.

Once the uterine cavity is entered, visualization of landmarks is critical. A panoramic inspection of the endocervix, lower uterine segment, endometrial cavity, and tubal ostia should be performed as the hysteroscope is inserted. A careful, thorough survey is necessary so that pathology is not missed. Myomas of the endocervix and lower uterine segment can easily be missed with too rapid insertion of the hysteroscope.

20.3.7 Operative Hysteroscopy

For operative procedures, larger hysteroscopes and resectoscopes are used that require cervical dilation prior to insertion and are therefore used primarily in an operating room under sedation or with general or regional anesthesia.

20.3.8 Complications

The overall complication rate associated with hysteroscopy is reported to be 2.7% [4]. The operative complications of hysteroscopy include uterine perforation, excessive hemorrhage, air embolus, pulmonary edema, pelvic organ injury secondary to thermal damage if electrode surgical systems are used, excessive fluid absorption, major vessel injury, intrauterine scar formation, and infection [5]. The different procedures described below carry all these risks to varying degrees, depending on the pathology being treated and the equipment being used. These complications are more or less likely depending on the procedure being performed. For example, perforation is more likely during adhesiolysis of severe Asherman's syndrome, and fluid overload is more likely in procedures that require incisions deep into the myometrium, exposing the uterine venous sinuses, as in hysteroscopic myomectomy.

20.3.9 Office Hysteroscopy

Office hysteroscopy is the gold standard tool for evaluation of intrauterine pathology. While it has been available for over 20 years, it has recently become more commonplace due to improved technology, improved reimbursements, and patients' desire to avoid general anesthesia and the operating room setting. Also, there have been advances in office-based hysteroscopic procedures such as tubal occlusion for sterilization that increased awareness of this valuable tool for providers and patients. Patients are able to undergo the procedure with minimal discomfort, most often with no or minimal oral and local analgesia.

When performing any procedure in the office setting, the clinician must be especially cognizant of possible complications of hysteroscopy given the limited resources available to manage any complications that may be encountered.

Office hysteroscopy is an effective and well-tolerated alternative to day-surgery hysteroscopy. One study randomized 40 women requiring polypectomy into either office hysteroscopy or day-surgery hysteroscopy. The results demonstrated that the office hysteroscopy subjects experienced minimal pain during the procedure and had faster recovery times and lower postoperative analgesia requirements than the subjects who underwent day-surgery hysteroscopy. Ninety-five percent of women who underwent office hysteroscopy stated they would repeat outpatient hysteroscopy if their polyps recurred. Eighty-two percent of women who underwent day-surgery hysteroscopy stated that they would like to try office hysteroscopy if their polyps recurred [6]. Several studies such as these have demonstrated the safety and significantly lower cost of office hysteroscopy when compared to day surgery [6, 7]. Additionally, it seems that patients are well able to tolerate these hysteroscopic procedures in the office and they have the added benefit of avoiding general anesthesia. Given all of these benefits, clinicians should give serious thought to developing facilities for office hysteroscopy if they are lacking and promoting office hysteroscopy to their patients when appropriate.

20.3.10 Equipment

The two categories of hysteroscopes used in the office setting are rigid and flexible. Generally, the hysteroscopes used for office hysteroscopy range from 3 to 5 mm in diameter. Larger operative hysteroscopes that are 8–10 mm in diameter are generally reserved for the operating room because they require significant cervical dilation, which can be very uncomfortable for the patient. When diagnostic hysteroscopy is performed, the hysteroscopes are introduced without the operative sheaths so as to decrease the diameter of the hysteroscope and minimize trauma to the cervical canal. Rigid hysteroscopes may be used, which are 2.7–5 mm in outer diameter. Zero-, 15-, or 30-degree hysteroscopic lens are appropriate for diagnostic hysteroscopy. If the cervical canal is tortuous or the uterus is especially anteflexed or retroflexed, flexible hysteroscopes are generally superior to rigid hysteroscopes. They have the added advantage of having a tip that can deflect from 0° to 110° and curve with the natural course of the endometrial canal.

If operative procedures are planned in the office, operative sheaths must be used, which increase the diameter of the hysteroscope system to approximately 4–5 mm. These outer sheaths are needed to accommodate the instruments and provide an outflow of fluid, making the hysteroscope a continuous flow system with both an inflow and outflow channel. The inflow channel is essential for appropriate distention of the uterine cavity, and the outflow channel is used to flush out any blood and debris that may accumulate, thereby improving visibility.

The standard operative tools available in the office setting include cold scissors, biopsy cups, and graspers. Recent technologic advances have also made bipolar electrodes available for use in the office. These 5-Fr electrodes are small enough to pass through any standard operative port of the 5-mm office hysteroscope. Additionally, because they are bipolar electrodes, physiologic electrolyte-containing distention media can be used without dissipating the energy generated at the end of the electrode.

20.3.11 Technique

The traditional approach to office hysteroscopy involves the use of a speculum to visualize the cervix and a tenaculum to grasp the cervix and provide traction to straighten the cervical canal. Although this approach may be necessary for some patients when a difficult entry is encountered, in a vast majority of patients, the clinician may dispense with the speculum and tenaculum by traversing the vagina via vaginoscopy. Bettocchi et al. have shown that hysteroscopy can be performed very successfully with this technique, while decreasing the amount of pain experienced by the patient [8].

With this technique, the hysteroscope is placed just inside the vaginal canal and the distention media is instilled. The vaginal walls distend, allowing the clinician to advance the hysteroscope through the vaginal canal under direct visualization and without causing trauma to the walls. The

hysteroscope is directed along the posterior wall of the vagina until the posterior fornix is reached. It is then pulled back slightly and angled upward until the cervical os is visualized. The hysteroscope can then be introduced into the cervical os, which then distends the endocervical canal. With the inflow distention media on low flow, the hysteroscope is guided through the canal under direct visualization so that the clinician may follow the natural course of the canal with minimal trauma to the surrounding tissue. Unless cervical stenosis is encountered, this procedure can be performed with minimal discomfort to the patient. In many cases, no analgesia or anesthesia is required.

20.3.12 Pain Management

With appropriate pain management, office hysteroscopy is successful in 90–95% of cases [9, 10]. Many patients are able to tolerate hysteroscopy without any analgesics or anesthetics, especially when the vaginoscopic technique is used. However, if the patient experiences significant discomfort, the clinician should be armed with tools to manage the patient's pain so that the hysteroscopy can be completed successfully.

20.3.13 NSAIDs and Anxiolytics

Patients can be pretreated with nonsteroidal anti-inflammatory drugs (NSAID) so that prophylactic pain management can be initiated before the procedure has even begun. Patients should be told to take NSAIDs 1–2 h prior to their procedure. The patient may take this orally before arriving at the office, or it may be administered at the office in the form of IM ketorolac once they arrive. Anxiolytics may also be administered to calm the patient; Lorazepam is usually the drug of choice.

20.3.14 Topical Analgesia

Lignocaine spray of 30, 50, or 100 mg to the cervix and cervical canal was shown to decrease pain at the time of insertion of the hysteroscopy, and it was also shown to decrease vasovagal reactions [11]; 25 mg of lignocaine cream with 25 mg of prilocaine, administered to the cervix, was shown to have the same benefits. There have not been many studies in support of this method of analgesia. However, given the current data, topical analgesia is a reasonable option to reduce patient discomfort.

20.3.15 Paracervical Block

If cervical dilation is required, a paracervical block may be performed to minimize patient discomfort. In most studies, paracervical block was performed by placing 10 mL of mepivacaine or lidocaine via a 22-gauge spinal needle at 3, 5, 7, and 9 o'clock positions paracervically. The studies are conflicted regarding the efficacy of the paracervical block. Some studies showed no significant difference in pain scores between groups, while an equal number of other studies showed a statistically significant decrease in pain overall [12]. In one study, patients were randomized into injections of lidocaine or saline. There was improvement in pain at insertion of the hysteroscope in the group with lidocaine, but patients commented that the injections were as painful as the hysteroscopic procedure. Transcervical and intracervical blocks have not been shown to be effective [12]. It is imperative that the surgeon placing the paracervical block be aware of the maximum dose of the local anesthetic based on the patient's weight as well as signs of allergy. Protocols should be in place to prevent, recognize, and treat these events should they occur.

20.3.16 Conscious Sedation

Conscious sedation is characterized by decreasing the patient's consciousness while allowing them to maintain their own airway. They are usually still able to respond to physical and verbal stimuli. Because the effects of the narcotics may be unpredictable, the patient's vital signs must be continuously monitored. Typical agents used for conscious sedation are fentanyl, propofol, and midazolam. Antiemetics may also be infused to counteract the gastrointestinal upset caused by the narcotics.

There are several requirements, outlined by the American Congress of Obstetricians and Gynecologists, which an office must fulfill in order to safely perform conscious sedation in the office setting [13]. The patient's oxygenation must be monitored continuously; this is most commonly done using pulse oximetry. If

deeper sedation is administered, the patient's ventilatory function should also be monitored by direct observation, auscultation, or capnography. The patient's circulatory function should be monitored with a continuously displayed electrocardiogram, blood pressure and heart rate measurements every 5 min, and pulse plethysmography. Anesthesiologists or nurse anesthetists must be present to monitor the patient during conscious sedation. Gynecologists may administer conscious sedation after undergoing certification. However, they must keep in mind that an additional health-care professional must be present whose sole responsibility is monitoring and attending to the patient. The clinician must also be certified in ACLS, PALS, or BLS and must be sufficiently free after the procedure is performed to monitor the patient until stable for discharge.

Respiratory suppression is a significant concern when administering conscious sedation. The office must have all the equipment necessary to manage complications from respiratory suppression. An oxygen source must be present, as well as suction, resuscitation equipment including a defibrillator, and emergency medications. This equipment must be maintained and tested according to manufacturer's specifications. If any of these requirements are lacking, conscious sedation is not recommended.

20.3.17 Patient Selection

Several factors must be considered when determining if a patient is a reasonable candidate for office hysteroscopy. First and foremost, a thorough history must be taken of the patient. The patient should be screened for significant comorbid conditions compromising her ability to tolerate the stresses of hysteroscopy. If a history of severe anxiety for example, the patient should be considered for day-surgery hysteroscopy [14].

In terms of the pathology that can safely be treated in the office setting, clinicians should consider what the patient will be able to tolerate and be wary of what complications can potentially be encountered. Appropriate procedures include diagnostic hysteroscopy, endometrial biopsies, lysis of adhesions, small polyps, tubal occlusion, and global endometrial ablation. These procedures are appropriate because they are relatively short, so that the patients will be able to tolerate them, and they have very low risk of complications.

Any procedure requiring lengthy operative time (>15 min) is not suggested for an office-based procedure. The operating room is more appropriate in order for the patient to remain comfortable. Myomas or polyps larger than the internal os of the cervix (>1 cm) as well as extensive lysis of adhesion and large septum resections are more appropriately and safely done in the operating room.

20.4 Complications

20.4.1 Fluid Intravasation

Complications may be encountered regardless of the type of distention media used. A complication that may be seen, regardless of the type of distention media, is fluid overload, which occurs in approximately 0.2% of cases [15]. This is seen when there is significant intravasation of the distention media. To prevent this from occurring, intrauterine pressure should be maintained at the lowest possible pressure while still maintaining adequate intracavitary visibility. Ancillary staff should be available to aid the clinician with the procedure and monitor the fluid deficit closely. If there is evidence of rapid intravasation of distention media, the procedure should be terminated. Clinicians should be especially cautious when performing lysis of adhesions, myomectomies, resection of septums, or any other procedures that may open vascular channels and potentiate intravasation.

For isotonic electrolyte-containing solutions, the maximum deficit in an office setting should not exceed 1000–1500 mL. If these deficits are surpassed, the patient should be monitored and the procedure terminated. There is no place to use electrolyte-free media in an office-based setting.

However, in most cases, procedures done in the office should be relatively quick, and fluid deficits should not be approaching maximum levels. Hysteroscopic procedures should be discontinued at lower fluid deficits than the thresholds described above, given that there is limited acute care available in the outpatient setting [14]. Therefore, if a long operative time is anticipated for a procedure, it may be better to schedule it for the operating room where the patient can be monitored closely as the fluid deficit increase.

20.4.2 Perforation

The most frequent reported complication of hysteroscopy is uterine perforation, which occurs at a rate of 14.2/1000 procedures according to a survey conducted by the American Association of Gynecologic Laparoscopists in 1993 [15]. These perforations commonly occur during dilation of the cervix, as well as during the hysteroscopic portion of the procedure. Perforations of the lower uterine segment and fundus of the uterus may be seen. In most cases, no treatment is required. However, if there are signs of intra-abdominal bleeding, such as when there is a lateral perforation through major vessels, the patient should be transferred immediately for laparoscopic exploration with possible repair of the defect. Management of perforation with an activated electrosurgical device also warrants emergent surgical evaluation. These thermal injuries usually occur when clinicians are activating the electrodes while moving them away rather than towards themselves. Additionally, certain procedures such as lysis of adhesions and resection of septums have much higher rates of perforation than other hysteroscopic procedures [16, 17]. Therefore, clinicians should be especially cautious when performing these procedures in the office.

20.4.3 Vasovagal Reaction

A vasovagal reaction is not uncommon in office hysteroscopy. One study reported a rate of 0.72% in patients undergoing hysteroscopy without analgesia [18]. Smaller hysteroscopes and improved pain control were shown to decrease the rates of vasovagal reaction [19]. A vasovagal episode is generally preceded by a feeling of lightheadedness, nausea, diaphoresis, bradycardia, and/or pallor. If these symptoms are encountered, the procedure should be immediately terminated. Patient should be placed in Trendelenburg position or with her legs raised. Her vital signs should be monitored closely, and an intravenous fluid bolus may be necessary. In most cases, patients recuperate quickly with these interventions. If a patient has known history of vagal reactions, one can pretreat with 0.4 mg of atropine IM prior to the procedure.

20.4.4 Bleeding

Most bleeding encountered during hysteroscopy is self-limiting. However, persistent bleeding may be encountered. This can be seen when the cervical canal is lacerated during dilation, after uterine perforation, or when vessels in the myometrium are transected, such as when clinicians are performing myomectomies or septum resections. If persistent bleeding is seen, electrocautery is most often ineffective. The primary treatment should be a Foley catheter placed into the cavity to tamponade the bleeding [20]. The Foley catheter is placed into the cavity and inflated with 15–30 mL of water until the bleeding stops [21]. If persistent bleeding is encountered despite these measures, more aggressive exploration in the operating room may be warranted.

20.4.5 Hysteroscopic Polypectomy

Symptomatic polyps are generally characterized by abnormal bleeding, postcoital staining, chronic vaginal discharge, or dysmenorrhea. Abnormal bleeding symptoms include intermenstrual spotting or heavier menstrual flow. There is good evidence that polyps can decrease fertility and that their removal will improve the chances of pregnancy [22].

It is obvious that symptomatic endometrial polyps should be removed. However, it is also important to remove asymptomatic polyps, particularly in postmenopausal women [23]. Although the vast majority of polyps are benign, endometrial cancer and hyperplasia will be found in approximately 2% of endometrial polyps and are associated with coexisting malignancies elsewhere in the endometrium. In one study of over 1400 polyps, endometrial cancer was found in 27 polyps (1.8%) [23]. All but one of these women were postmenopausal, and only 26% were asymptomatic.

20.4.6 Technique

Polyps can generally be removed with hysteroscopic scissors and pulled through the cervical canal intact. Larger polyps, with thick stalks, require resection with morcellation, most often with a resectoscope so that the tissue can be removed piecemeal. More recently, mechanical hysteroscopic morcellators have been increasingly

used to manage both polyps and fibroids. A variety of morcellators are available, and each utilizes an electromechanical drive system to power a cutting blade inserted into a metallic cylinder. A vacuum is created that suctions the polyp into the cylinder, and the tissue is collected via the outflow tract.

Of the various methods described above for removing polyps, none have been shown to be more safe or effective than another, though there is a significant increase in the cost of the procedure if disposable devices are used. The choice of equipment should be based on the surgeon's preference when cost, safety, and efficacy are taken into account.

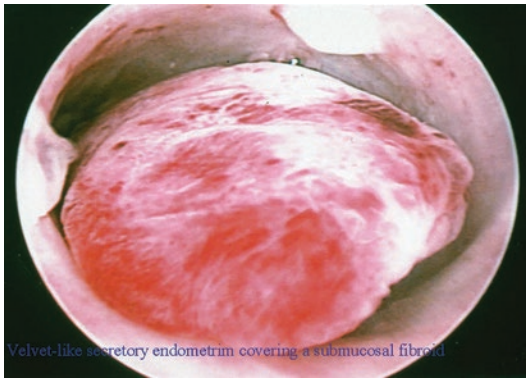

Fig. 20.1 Hysteroscopic view of a type 0 myoma. It is pedunculated, and the total myoma lies within 100% of the endometrial cavity (reproduced with permission from Bradley LD. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

20.4.7 Hysteroscopic Myomectomy

Patients with symptomatic myomas generally present with abnormal uterine bleeding (i.e., menorrhagia), infertility, pelvic pain, or pressure. When determining the optimal surgical approach for their removal, it is important to determine the fibroid location in relation to the endometrial cavity. Fibroids can be described using the European Society of Hysteroscopy classification system, which groups fibroids into types based on the degree to which the myomas intrude into the endometrium and myometrium (Table 20.1).

20.4.8 Classification

Type 0 myomas are pedunculated, with the myoma lying completely within the endometrial cavity (Fig. 20.1). Type I myomas are described as "sessile" with <50% intramural extension (Fig. 20.2). Type II myomas are submucosal

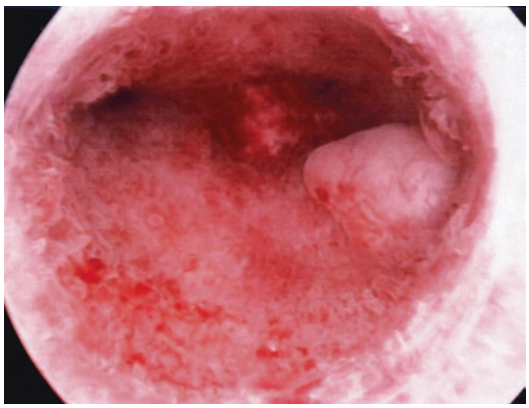

Fig. 20.2 Hysteroscopic view of a type I myoma that involves less than 50% of the myometrium (reproduced with permission from Bradley LD. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

Table 20.1 Hysteroscopic and sonohysterographic classification system for myomas encroaching upon the endometrial cavity

Hysteroscopic type [24]	Sonohysterographic class [25]	Description
Type 0	Class 1	Pedunculated myomas, where 100% of the myoma lies within the endometrial cavity with no intramural extension
Type I	Class 2	Sessile myomas, with <50% intramural extension
Type II	Class 3	Submucous myomas, with >50% intramural extension

in location, with >50% intramural extension. These include transmural myomas, which extend from the submucosal to serosal edge. When viewed hysteroscopically, type II myomas form a bulge that can be seen in the endometrial cavity.

This system was originally designed to classify myomas exclusively on hysteroscopic appearance. However, this approach has significant limitations. During hysteroscopy, myomas can be compressed and recede into the myometrium as a result of the pressure of the distention media, thereby preventing full visualization of the myoma. For this reason, preoperative evaluation with ultrasonography is required to accurately determine how many myomas are present and how deeply the myomas penetrate the myometrium. Magnetic resonance imaging (MRI) can also be used for this purpose.

20.4.9 Surgical Approach According to Stage

For a successful surgical outcome, it is important to preoperatively identify the size, number, location, and intramural extension of uterine myomas. These characteristics predict the surgeon's ability to completely resect the fibroids during one surgical procedure. Most often, when there is a large type II myoma, the procedure has to be terminated prior to completion due to excessive fluid absorption [26].

The degree of surgical difficulty and, thus, the risk to the patient are related to the depth of penetration and size of the myomas. Pedunculated hysteroscopic type 0 (class 1) myomas up to 3 cm in diameter can usually be easily removed hysteroscopically. Larger hysteroscopic type 0 myomas (>3 cm) and hysteroscopic type I (class 2) myomas can also be approached hysteroscopically. However, the risk of fluid intravasation increases as a result of increased surgical time and the opening of myometrial venous channels during resection. Additionally, there is poorer visibility with larger myomas given the more limited space within the uterus, inability to distend the cavity well, and the large amount of myoma "chips" that accumulate within the cavity. Often, incomplete removal of larger myomas requires two or more separate operative procedures. Only the most experienced hysteroscopist would attempt a hysteroscopic resection of an intracavitary myoma 5 cm or larger.

Likewise, hysteroscopic resection of type II (class 3) myomas should only be approached by high volume hysteroscopists. They are more commonly approached abdominally by laparoscopy or laparotomy. Hysteroscopic removal of type II myomas is also associated with a greater risk of fluid intravasation and uterine perforation and commonly requires two or more procedures for complete removal.

When patients have multiple intracavitary fibroids throughout the endometrial cavity, they would benefit from a "two-staged" operative hysteroscopic myomectomy, in which myomas are removed from only one uterine wall at a time. This is to decrease the risk of apposition of the uterine walls and the development of postoperative intrauterine synechiae.

20.4.10 Technique

There are various techniques for removing pedunculated and submucosal myomas, including avulsion, scissors, wire-loop resection with bipolar or monopolar equipment, morcellation, and laser vaporization. However, hysteroscopic wire-loop resection still remains the most popular method of removing myomas and will be the technique discussed in this section.

When monopolar energy is used for wire-loop resection, the current setting should be 60–80 W cutting current and requires an appropriately sized grounding pad. Higher settings may be necessary with very fibrous, dense, or calcified myomas. When the bipolar generator is used, it automatically adjusts the power to default settings.

Once the submucosal myoma is identified, the wire-loop electrode is advanced in clear view and retracted towards the surgeon behind the myoma. As the wire loop is drawn towards the surgeon, small, crescent-shaped "chips" or fragments of myoma are created. The whorled fibrous appearance of the myoma is clearly different from the fascicles of soft underlying myometrium. The fibrous tissue should be methodically resected until the border of the underlying myometrium is reached. However, increased bleeding from the myometrial bed and fluid intravasation may be encountered if the myometrium is breached. The resecting loop should stay within the pseudocapsule of the myoma

and not cut this myometrium. If the hysteroscopist stays within the pseudocapsule, the likelihood of a uterine perforation will nearly be zero.

Myoma chips can remain free-floating until they interfere with visualization and are then removed with polyp forceps, Corson graspers, suction curette, or with the loop itself under direct visualization. All free-floating tissue fragments should be removed and sent for histologic examination. Removing all free-floating tissue also prevents delayed vaginal extrusion of this tissue material, malodorous discharge, adhesions, and infection.

Intermittently throughout the procedure, the intrauterine pressure should be lowered to 30 mmHg or the least amount of pressure that is possible while still maintaining visualization. This rapid reduction in intrauterine pressure will aid in enucleation of the myoma via a decompression mechanism that releases the encapsulated myoma from its myometrial bed. The myoma may appear to increase in size. In fact, more myoma protrudes into the endometrial cavity allowing a more complete resection without having to resect myometrium. False-negative views can occur during hysteroscopy because of the high intrauterine pressures. The "disappearing phenomena" refers to the flattening of endometrial polyps or fibroids, resulting in a falsely negative hysteroscopic study. This disappearing phenomenon can be avoided by decreasing the intrauterine pressure at the end of the procedure and reinspecting the endometrial cavity. As a general rule, the distention pressure within the uterine cavity should be the lowest pressure that gives the surgeon adequate flow and visualization. This will allow the myoma to protrude within the cavity and minimize unwanted fluid absorption.

20.4.11 Intraoperative Ultrasonography

Intraoperative ultrasonography guidance during operative hysteroscopy is useful for resection of myomas that are hard to define. Ultrasound guidance allows constant visualization of the uterine walls, as well as the hysteroscopic instruments. Therefore, the hysteroscopist may know when they are in danger of perforating the uterine wall. This added imaging allows for resection beyond the limit conventionally defined by hysteroscopy [27].

20.4.12 Fertility Preservation

If the patient desires fertility, overzealous resection of the myometrium must be avoided. Asherman's syndrome may occur when large portions of overlying endometrial tissue are resected with a sessile myoma. Patients who desire fertility and have multiple intracavitary myomas, especially those with myomas on opposite walls, may require resection in two separate occasions to minimize chances of intrauterine synechiae developing postoperatively.

20.4.13 Complications

Complications of hysteroscopic myomectomies include uterine perforation, bleeding, infection, and fluid intravasation. Uterine perforation most often occurs with cervical dilation with a blunt dilator. These patients can be observed in the recovery room and sent home when stable.

Major bleeding after a hysteroscopic myomectomy is rare. When excessive bleeding is encountered, it is generally secondary to myometrial bleeding. When the bleeding is excessive, it can be controlled with a 25-cm^3 catheter balloon left in place for 4–12 h.

20.4.14 Endometrial Ablation

Endometrial ablation was developed as a minor surgical procedure to treat women with intractable heavy menses unresponsive to medical management, who no longer desire fertility. Each year approximately 200,000 women undergo an endometrial ablation. Compared to hysterectomy, endometrial ablation offers the advantages of avoiding the morbidity and prolonged recovery associated with major surgery when patients fail medical management. However, the disadvantages include recurrence of bleeding over time. Up to 35% of women who receive an endometrial ablation will receive a hysterectomy within 5 years of the procedure. Endometrial ablation should only be offered to women who are willing to accept eumenorrhea, hypomenorrhea, or cyclical bleeding rather than amenorrhea as a final clinical result. Only 40% of women having an ablation will be amenorrheic.

20.4.15 Endometrial Ablation Techniques

There are several methods for endometrial ablation. The three "first-generation" hysteroscopic techniques include electrosurgical laser ablation, endomyometrial resection, and electrosurgical rollerball ablation. Second-generation techniques, also referred to as "global" methods, differ in that they do not require the use of the resectoscope to perform the ablation. Hysteroscopy is an integral part of only one of these systems.

20.4.16 First-Generation Hysteroscopic Endometrial Ablation

Mimicking the physiologic effect of Asherman's syndrome, the ultimate goals of endometrial ablation were to create severe endometrial scarification and secondary amenorrhea.

Endomyometrial resection utilizing a resectoscope was first reported by DeCherney and Polan in 1983 [28]. This technique utilizes unipolar electrocautery and is performed with hypotonic nonelectrolyte-containing distention media. This technique was the forerunner of hysteroscopic *rollerball endometrial ablation*, which has become the "gold standard" to which all emerging endometrial ablation technology is compared. Each of these devices destroys the basalis layer of the endometrium and is designed to result in hypomenorrhea or amenorrhea.

20.4.17 Technique: First-Generation Hysteroscopic Endometrial Ablation

The general concept of hysteroscopic endometrial ablation involves thorough destruction of the basalis layer of the cornua and lower uterine segment. For this reason, the patient should ideally be scheduled during the early proliferative phase. Otherwise, hormonal suppression of the endometrium is required to thin the endometrium and increase the chances of success of the ablation. Hormonal suppression also increases visualization by ridding the cavity of excess blood and tissue. Hormonal options for endometrial suppression include the use of depot leuprolide acetate, danacrine, oral contraceptive pills, or progesterone-only pills 4–8 weeks prior to surgery. Surgical preparation with suction or sharp curettage immediately prior to ablation has also been used with reported success.

20.4.18 Vasopressin

Vasopressin is used to decrease the risk of fluid absorption, fluid overload, and intraoperative bleeding [29]. A dilute solution of vasopressin (10 units in 50 mL saline) is injected as 5-mL aliquots into the stroma of the cervix at 12, 3, 6, and 9 o'clock positions. This causes intense arterial and myometrial wall contractions for 20–45 min. Vasopressin is not approved by the FDA for this purpose and should not be used in patients who are hypertensive.

20.4.19 Technique: Rollerball and Endomyometrial Resection

Several varieties and shapes of electrodes are available to perform hysteroscopic endometrial ablation, including ball, barrel, ellipsoid, and large-caliber loops. Most surgeons perform rollerball endometrial desiccation with a 3-mm rollerball probe, with the goal of systematically destroying the entire endometrium to the lower uterine segment and cornual region. The technique of endomyometrial resection is also a popular method for endometrial ablation and is performed with a 90-degree wire-loop electrode.

Following a systematic surgical plan ensures optimal clinical outcomes. Excellent visualization of the entire uterine cavity and endocervix is imperative. All intrauterine landmarks are clearly delineated hysteroscopically before initiating the procedure. Once a panoramic view of the endometrium is accomplished, the surgeon should determine if there is any previously unrecognized pathology. If a subtle lesion is discovered, then a directed biopsy with a wire-loop electrode is performed and the specimen labeled and submitted separately.

Once the surgeon visualizes all of the landmarks, the lower uterine segment is cauterized circumferentially to mark the endpoint and lowest level of endometrial ablation therapy. Ablation of the endocervix is avoided to minimize the risk of cervical stenosis. Cervical stenosis can result in

cyclic pain, dysmenorrhea, and, in severe cases, hematometra.

After the lower uterine segment is identified and coagulated circumferentially, the cornua and fundal region are treated initially. With the rollerball, the electrode is advanced to the fundus and then directed at the cornua utilizing a "touch technique" to desiccate the cornua. It must be remembered that the thinnest region of the uterus is at the cornua. Extra care must be taken to avoid forward pressure, which could cause perforation. The most challenging part is the fundus, since the rollerball cannot truly be rolled against the fundus. The fundus should be addressed as the first step. The posterior wall should be resected next, followed by the lateral walls and anterior walls. Traditional technique utilizes direct tissue contact, such that one-half of the rollerball is buried in the endomyometrial juncture. The rollerball should only be activated when the electrode is moving toward the surgeon to avoid perforation. Perforation with the rollerball has the added risk of inflicting burns to the pelvic viscera beyond the uterine wall. Intermittently, the rollerball may need to be cleaned and debris evacuated to provide optimal visualization.

The endomyometrial resection with a wire loop follows the same principles. This loop is generally 3–4 mm deep. The loop should be buried into the endometrium just below the superficial level of the myometrium. The cut is performed using 60–80 W of cutting current. The loop is then advanced under direct view from the fundus to the lower uterine segment. Thus, the endomyometrial junction is shaved off, creating "crescent-shaped" tissue fragments.

At the conclusion of the endometrial ablation procedure, the intrauterine pressure is reduced in order to identify bleeding areas which may be treated with coagulation current.

20.4.20 Outcomes

The majority of patients who undergo endometrial ablation are satisfied with their clinical outcome, and at least 90% will notice symptomatic improvement. However, 5–10% of patients may ultimately be required to undergo additional interventions, such as repeat ablation or hysterectomy [30].

Hysteroscopic endometrial ablation is an outpatient procedure associated with a rapid return to work, minimal complications, and high patient satisfaction. Approximately 20–60% of patients undergoing endometrial ablation with rollerball techniques will achieve amenorrhea, 65–70% will become hypomenorrhic, and 5–10% will fail. Approximately 35% of patients treated by endometrial ablation will require a subsequent operation [30]. Women receiving appropriate preoperative counseling may find this attractive in treating menstrual disorders.

20.4.21 Second-Generation Endometrial Ablation Devices

Second-generation or "global" endometrial ablation refers to destruction of the entire endometrium with devices that require little or no hysteroscopic skills. Currently, FDA-approved second-generation devices available in the USA utilize intrauterine balloons, hot saline irrigation, cryosurgery, bipolar radio frequency, and microwave energy.

Second-generation technology offers the advantage of shorter procedure times while retaining the acceptable outcome rates similar to traditional rollerball ablation. However, these second-generation devices have limited or no ability to treat intracavitary pathology. For this reason, it is important for clinicians who routinely use these ablation devices to be skilled in operative hysteroscopy so that they will be equipped to treat any intracavitary pathology that they may encounter prior to performing the ablation procedures.

20.4.22 Complications of Endometrial Ablation

When energy using heat is used, the most concerning complication is perforation of the uterus with concurrent thermal damage to the surrounding viscera. Perforations of this kind require immediate laparoscopy to determine whether or not thermal injury to the pelvic organs has occurred. Thermal injury to the bowel that is not repaired may result in breakdown of the intestinal wall with spillage of bowel contents into the abdomen. When this occurs, it generally results in massive pelvic infections that may progress to disseminated intravascular coagulopathy. Other complications include skin burns with circulating hot saline, direct coupling vaginal burns from monopolar energy, and unwanted bladder or bowel thermal injuries from cryotherapy.

20.4.23 Hysteroscopic Resection of Adhesions

Most commonly, intrauterine adhesions form in the postpartum or postabortion period. Unfortunately, there is usually no way to avoid this complication during these critical periods, such as when a patient presents with postpartum hemorrhage and requires intrauterine procedures (i.e., D + C) for hemostasis. Early detection of intrauterine synechiae is a key preventative feature following intrauterine surgery, curettage, or spontaneous abortion. This is because early detection allows for identification of adhesions while they are still filmy, thin, and easily resected with prompt adhesiolysis [24, 31–34].

The incidence of Asherman's syndrome in a select group of women, especially after curettage for missed or incomplete abortion, is reported in the range of 17%, but rates as high as 30% are reported in the literature, the majority of which are mild in severity [35–38]. Furthermore, in at-risk women, such as those who undergo curettage postpartum, the rate is speculated to be even higher [5, 39].

Table 20.2 Classification system for intrauterine adhesions[a]

Grade	Finding
Minimal	Less than 1/4 of the uterine cavity is involved
	Thin, filmy adhesions
	Fundus and ostia are clear of adhesions
Moderate	1/4–3/4 of the uterine cavity is involved
	No agglutination of uterine walls; only adhesions are present
	Upper uterine cavity and ostial areas are only partially occluded
Severe	More than 3/4 of the uterine cavity is involved
	Agglutination of uterine walls or thick adhesion bands
	Upper uterine cavity and ostial areas are totally occluded

[a]Adapted from [25]

20.4.24 Pathophysiology

Any intervention that destroys the endometrium may generate adhesions of the myometrium in the opposing uterine walls. The key predictive factor to intrauterine adhesions is the gravid uterus. The gestational changes noted with a gravid uterus soften the uterine wall, resulting in greater denudation of the basalis layer during surgical interventions. The basalis layer is crucial because it is the regenerative layer of the endometrium [40].

20.5 Classification of Intrauterine Adhesions

20.5.1 March Classification

Intrauterine adhesions can be characterized based on the extent of uterine involvement [25]. Minimal adhesions are defined as adhesions that involve less than one-fourth of the uterine cavity and are thin and filmy. The fundal and ostia areas are minimally involved or devoid of any adhesions. Moderate adhesions involve one-fourth to three-fourths of the uterine cavity but no agglutination of the uterine wall is seen. The tubal ostia and fundus are only partially occluded. Severe adhesions involve greater than three-fourths of the uterine cavity with agglutination of the uterine walls or thick bands with occlusion of the tubal ostia and upper uterine cavity. The March classification system is simple and easy to apply, but it is not prognostic [25] (Table 20.2).

20.5.2 American Society for Reproductive Medicine Classification

According to the 1988 ASRM (formerly the American Fertility Society) classification system, synechiae are classified in three stages, with stage III being complete obliteration of the uterine cavity (see Table 20.2) [41]. The ASRM classification system provides both an indirect and direct grading of intrauterine adhesions with HSG and hysteroscopy, respectively. The location of the adhesions is presumed to be prognostic for

Table 20.3 American Fertility Society intrauterine adhesions classification system[a]

Extent of cavity involved	<1/3	1/3–2/3	>2/3
	1	2	4
Type of adhesions	Filmy	Filmy and dense	Dense
	1	2	4
Menstrual pattern	Normal	Hypomenorrhea	Amenorrhea
	0	2	4
Prognostic classification		HSG[b] score	Hysteroscopy score
Stage I (mild)	1–4	–	–
Stage II (moderate)	5–8	–	–
Stage III (severe)	9–12	–	–

[a] Reproduced from American Fertility Society. The American Fertility Society classifications of adnexal adhesions, distal tubal occlusion, tubal occlusion secondary to tubal ligation, tubal pregnancies, Mullerian anomalies, and intrauterine adhesions (Fertil Steril 1988; 49:944–55, with permission from Elsevier the American Society for Reproductive Medicine)
[b]All adhesions should be considered dense

reproductive outcome given that most implantation occurs in the top fundal portion of the uterine cavity and cornual adhesions may cause tubal obstruction. In addition, unlike the March classification system, the significance of endometrial sclerosis or atrophy is included in the ASRM system by ascertaining the menstrual pattern (Table 20.3).

20.5.3 Clinical Manifestations

The most common presentation of intrauterine adhesions is menstrual disturbance and/or reproductive disturbance (infertility and recurrent pregnancy loss). If conception occurs, it may be complicated by preterm labor or abnormal placentation such as placenta previa or placenta accreta. Menstrual disturbances are most often categorized by amenorrhea or hypo- or oligomenorrhea but can also be seen in eumenorrheic women.

The most common single presentation is infertility, representing 43% of reported cases. The second most common is amenorrhea, which is seen in 37% of cases [42]. The rate of abnormal placentation, although elevated in women with intrauterine adhesions, is the least common presentation reported in women with intrauterine adhesions.

20.6 Diagnosis

20.6.1 Sonohysterogram

A sonohysterogram is performed with transvaginal sonography (TVS) and can enhance the detection of intrauterine adhesions. Saline serves as a homogenous, echo-free contrast medium enabling better visualization of the uterine cavity than transvaginal ultrasonography alone. Alborzi et al. [43] published the largest series to evaluate the diagnostic accuracy of sonohysterogram compared to laparoscopy and hysteroscopy, which are the gold standards for diagnosis. The prospective study reviewed 86 women with infertility. In this study, sonohysterogram had a high diagnostic accuracy for the detection of Asherman's syndrome. Sonohysterogram accuracy in diagnosis was greater than that for hysterosalpingogram, with a sensitivity of 76.8%, a specificity of 100%, a PPV of 100%, and a NPV of 97.7%.

Transvaginal ultrasound should be performed in the late follicular or early luteal phase of the cycle, as the endometrium is thick enough to appear more echogenic than the myometrium and not too thick to obscure the midline echo. The classic appearance of the three-layer endometrium enables better imaging of uterine defects

than the postmenstrual endometrium, which is thin, <3 mm. The typical appearance of uterine synechiae is focal, hyper-echoic, irregular, cord-like structures seen within the echo-free space between the basalis layers, which interrupt the continuity of the endometrial cavity. These structures can vary in size (2–6 mm) and/or in location within the cavity [44].

20.6.2 Hysteroscopy

Diagnostic hysteroscopy is the gold standard for the diagnosis of intrauterine adhesions, with demonstrated superiority to sonohysterogram and hysterosalpingogram, specifically in false-positive rates. Both radiologic techniques have high false-positive rates. Hysteroscopy has the added advantage of being able to assess intrauterine adhesions and classify them by location, shape, size, and nature.

20.7 Surgical Treatment

20.7.1 Hysteroscopic Surgery

Hysteroscopy has become not only an accurate tool for the diagnosis of adhesions but also the main method for their treatment. Hysteroscopic lysis of adhesions is indicated when the extent of adhesions is moderate to severe or access to tubal ostia is blocked. The significance of mild adhesions is still controversial, yet surgical treatment may be considered if all other causes of infertility or recurrent pregnancy loss have been excluded and/or successfully corrected and the patient still experiences persistent reproductive failure.

The basic technique involves resection of the intrauterine adhesions either by sharp and/or blunt dissection. Successful hysteroscopic resection can be accomplished by the use of sharp dissection using semirigid scissors, electrosurgery, and/or fiber-optic laser. Electrocautery is used by some clinicians. However, the disadvantage of its use in this context is possible thermal damage to the endometrium.

Adhesiolysis begins inferiorly and is carried out cephalad until a panoramic view of the endometrial cavity can be obtained and the tubal ostia are seen. The initiation of the adhesiolysis is from the internal os. The maintenance of adequate distention is key to the successful resection of intrauterine adhesions; distention provides traction to the scar tissue so that they may be more effectively resected with hysteroscopic scissors. In cases of severe disease, transabdominal ultrasound guidance with a full bladder is very helpful in preventing the creation of a false passage or uterine perforation.

20.7.2 Postoperative Adjunctive Therapy

Despite advances in the development of techniques for adhesiolysis, the two basic problems associated with poor outcome with these procedures still exist: the inability to treat extensive or severe adhesions and the lack of methods to prevent recurrence of the adhesions postoperatively. The use of Foley catheters, antibiotics, and high-dose estrogen postoperatively to prevent recurrence of adhesions is still widely debated, and no consensus exists [40]. A typical regimen is conjugated estrogen, 0.625–1.25 mg twice daily, or estradiol, 2 mg twice daily for 25 days, followed by 12 days of progesterone (10 mg is prescribed).

Another approach is to place a postoperative intrauterine stent. Either a pediatric catheter inflated with 15–20 cm^3 or a balloon uterine stent specifically designed for this purpose (Cook OB/GYN, Spencer, Indiana) may be inserted for 7–10 days to prevent the juxtaposition of the uterine walls.

A final approach is to perform office hysteroscopy within the first 7–14 days following extensive myomectomy to evaluate the endometrium for synechiae. If detected early, the adhesions are filmy and easily lysed with the distal tip of the hysteroscope. In some circumstances, hysteroscopic visualization every 7–10 days may be required until regeneration of the endometrium is confirmed and filmy adhesions treated. When performed too late, dense fibrous adhesions may be encountered, requiring repeat operative hysteroscopic adhesiolysis.

20.7.3 Complications

Complications after hysteroscopic adhesiolysis include all the standard risks seen with any operative hysteroscopy. The perforation risk is highest

during hysteroscopic adhesiolysis [45]. The risk of postoperative infection after hysteroscopy in general is 1.42%, but the risk of early onset endometritis is highest after lysis of synechiae compared to other hysteroscopic procedures including uterine septa [45].

20.7.4 Outcome

The success of surgery can be assessed by repeat hysteroscopy or imaging or simply by the presence of withdrawal bleeding, suggestive of adequate regeneration of the endometrium. Successful pregnancy outcome is also a parameter or measure of success in women trying to conceive and seems to be correlated with the severity of the intrauterine adhesions.

A number of series have been published reporting the outcome of hysteroscopic treatment of intrauterine adhesions. However, randomized clinical trials are lacking. A report of 40 consecutive women with recurrent pregnancy loss (24 women) or infertility (16 women) resulting from intrauterine adhesions showed excellent surgical results with mild or moderate disease [46]. Of the 40 women, 10 had mild adhesions, 20 had moderate adhesions, and 10 had severe adhesions, according to March classification system. Hysteroscopic adhesiolysis was performed with hysteroscopic scissors or monopolar electro surgery. Prophylactic antibiotics were used, and, postoperatively, a pediatric Foley was placed and estrogen was administered. All women with recurrent pregnancy loss conceived after adhesiolysis; 71% were term or preterm with a viable pregnancy. Among the women with infertility, 62% conceived, resulting in a 37.5% live birth rate. Adhesion re-formation was absent or rare in women with mild or moderate adhesions, reported as 0–10%. However, adhesion reformation was seen in 60% of women with severe intrauterine adhesions, and none of the patients with severe adhesions conceived. Only one perforation was reported in a patient with severe adhesions.

Valle and Sciarra [16] reviewed 81 infertile women and reported a term pregnancy rate of 81%, 66%, and 15%, respectively, in women with mild, moderate, and severe disease. Among these women with recurrent pregnancy loss, the term pregnancy rate was 94%, 89%, and 65% in women who had mild, moderate, and severe adhesions, respectively. The literature is unified and quite clear that for women with severe intrauterine adhesions, the reproductive outcome remains poor even after hysteroscopic adhesiolysis [16, 46]. The recurrence rate of severe adhesions was 48.9% and decreased to 35% after repeat adhesiolysis [16].

The overall live delivery rate following adhesiolysis in women was 43.5% during a mean follow-up period of 39.2 month (± 4.5 months). The live delivery rate based on the stage of adhesions was 33.3%, 44.4%, and 46.7% for stages I, II, and III, respectively. The live birth rate among women who tried naturally was 61.9% vs. 28% after in vitro fertilization. Similar pregnancy rates were noted in women who conceived naturally whether the resectoscope or the coaxial bipolar system was used. The mean time to conception in these women was 12.2 months, and all pregnancies were achieved within 2 years post-adhesiolysis.

Increase in pregnancy complication rate was noted. Preterm rate was 50%, and hysterectomy for abnormal placentation (placenta accreta) was seen in 2 of the 20 patients (10%). In addition, Zikopoulos et al. [47] reviewed the literature of existing studies examining delivery rates in women undergoing hysteroscopic adhesiolysis. A large array of techniques were used in these studies. He identified seven published studies in the last decade. A total of 126 women were reported with an overall delivery rate of 38.1% (48/126) among all the studies analyzed.

Pabuccu et al. [46] reported the highest success rate among women with recurrent abortion, with a delivery rate of 70.8% vs. women with infertility with a 37.5% delivery rate. The overall delivery rate was similar to that reported by Siegler and Valle [48] in 1988. They reviewed a series of studies that encompassed 775 subjects, of which 302 (38.9%) achieved a term delivery.

The mainstay of diagnosis and treatment of intrauterine adhesions remains hysteroscopy. However, one cannot stress enough the need to exert due diligence and avoid forced or extensive interventions on the post-gravid uterus to minimize the development of intrauterine adhesions. Mild and moderate adhesions are associated with improved reproductive outcome post adhesiolysis, but severe intrauterine adhesions carry a very poor prognosis.

20.8 Uterine Septa

20.8.1 Etiology

Uterine septa are created when there is a failure of resorption of the midline septum between the Müllerian ducts. The etiology of a septate uterus remains to be elucidated. Sporadic case reports on family pedigrees suggest familial aggregation exists, but no clear genetic cause has been linked to the development of a septate uterus [28, 49]. In general, 92% of women with congenital uterine anomalies have a normal karyotype, 46 XX, and approximately 8% of women have an abnormal karyotype [50]. In rare cases, early in utero exposure to radiation, infection, such as rubella, and teratogens (diethylstilbestrol, thalidomide) has been implicated as the causal factor of the uterine anomaly.

20.8.2 Classification

A number of classification systems have been reported for Müllerian anomalies. However, the classification system proposed by the ASRM in 1988 is most commonly used to describe or define Müllerian defects. The classification system organizes uterine anomalies into six major uterine anatomic types or categories. In this classification system, a septate uterus is a class V anomaly. It is among the vertical fusion defects described by the ASRM classification system. Va is a complete septate uterus, and Vb is a partial septate uterus.

A septate uterus is characterized by a smooth external fundal contour with two uterine cavities. The extent of the septum or the degree of septation can vary from a small midline septum to total failure of resorption, resulting in a complete septate uterus with a longitudinal vaginal septum.

20.8.3 Incidence

The reported incidence of Müllerian or uterine anomalies is between 0.5 and 6% of reproductive-age women and highest among women with poor reproductive outcome. The overall incidence of Müllerian defects reported in a series by Acien [51] was 5% among women with normal reproductive history, 3% among infertile women, and 5–10% among women with first-trimester recurrent miscarriage and greater than 25% in women with late first- or early second-trimester loss or preterm delivery. The most frequent to least frequent anomaly is bicornuate uterus, arcuate uterus, incomplete uterine septum, uterus didelphys, complete uterine septum, and a unicornuate uterus [51]. In this combined series of women, bicornuate uteri and complete or partial septums represented 74% of the uterine anomalies.

In women with recurrent pregnancy loss, the relative frequency of a septate vs. bicornuate uterus is less clear. This is often attributed to old surgical data that often did not definitely differentiate a septate from a bicornuate uterus. In one of the largest studies of patients with recurrent pregnancy loss that were evaluated with either laparoscopy or sonohysterogram, the septate uterus was more prevalent in women with recurrent pregnancy loss than the controls [52]. A septate uterus is the most likely anomaly in patients with recurrent pregnancy loss.

20.8.4 Pathophysiology of Pregnancy Complications

The key presentation in women with a septate uterus is difficulty in maintaining a pregnancy and not a decreased ability to conceive (infertility). Additionally, a septate uterus is thought to impair normal reproductive performance by increasing the risk or incidence of early and late abortion, preterm delivery, and the rate of obstetrical complications [53].

The pathogenesis of pregnancy complications in women with a septate uterus has not been completely elucidated. The most widely accepted causal theory includes the inadequate vascularization of the fibroelastic septum and altered relations between the myometrial and endometrial vessels, thus exerting negative effects on fetal placentation.

The septum is primarily composed of avascular, fibromuscular tissue. Hence, it has been proposed that the endometrium lining the septum responds poorly to estrogen, resulting in irregular differentiation and estrogenic maturation [54]. Implantation on this poorly vascularized, fibrous septum leads to abnormal implantation, defective embryonic development, and subsequent abortion [55–57].

20.8.5 Diagnosis

Uterine imaging techniques used as a method of detection of uterine anomalies include HSG; TVS, with or without three- and four-dimensional technology; saline infusion sonohysterosonography (SHG); and MRI.

20.8.6 Hysterosalpingogram

HSG is a useful screening test and should be the first step in the evaluation of the uterine cavity. HSG is a simple, safe, relatively noninvasive radiological procedure performed under fluoroscopic guidance that enables visualization of the uterine cavity, but it is limited in differentiating between a septate and bicornuate uterus. Consequently, the limitations of HSG require additional evaluation.

20.8.7 Ultrasonography

Two-dimensional ultrasound and SHG can also be used to diagnose women suspected of Müllerian anomalies. The diagnostic accuracy of two-dimensional TVS and SHG compared to HSG is shown in Table 20.4 [58]. However, the presence of a uterine septum is best diagnosed with a three-dimensional ultrasound scan. One study examined 61 patients with a history of recurrent miscarriage or infertility. Subjects underwent a hysterosalpingogram, two-dimensional TVS, and three-dimensional TVS. The study demonstrated that three-dimensional ultrasonography was superior in the detection of arcuate uteri and major congenital anomalies. It facilitated visualization of the uterine cavity and myometrium, allowing easier diagnosis of septate uteri [59].

20.8.8 Magnetic Resonance Imaging

MRI can accurately predict the presence of uterine anomalies and has become the imaging method of choice to confirm inconclusive results from other methods. The clear advantages of an MRI include the ability to distinguish between myometrial and endometrium tissue, image the uterus in several planes, and define uterine contour [47, 60–62]. Because MRI can delineate the uterine contour, a septate uterus can be distinguished from a bicornuate uterus, unlike with other radiographic imaging modalities. Furthermore, the uterine septum can be further characterized by the absence of myometrial tissue and vascularization in the septum. Instead, a uterine septum is seen to have a fibrous consistency throughout its entire length. In a review of 23 cases of Müllerian anomalies, the correct diagnosis was made in 96% of cases with MRI, compared to 85% for TVS [63].

Another advantage of MRI is its ability to detect the associated anomalies in other organ systems typically seen with Müllerian anomalies such as renal or the urinary tract anomalies. The major disadvantage includes the lack of portability and higher costs compared with other imaging modalities.

After initial imaging has been performed with either HSG, TVS, or SHG, an MRI may be used to determine the contour of the uterine fundus to

Table 20.4 Diagnostic accuracy of HSG, TVS, and SHG for uterine malformations[a,b]

Examination	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
HSG	75.0 (21.9–98.7)	95.1 (85.4–98.7)	50.0 (13.9–86.1)	98.3 (89.7–99.9)
TVS	0.0 (0.0–69.0)	95.2 (85.6–98.7)	0.0 (0.0–69.0)	95.2 (85.6–98.7)
SHG	75.0 (21.9–98.7)	93.4 (83.3–97.9)	42.9 (11.8–79.8)	98.3 (89.5–99.9)

HSG hysterosalpingogram, *SHG* sonohysterography, *TVS* transvaginal sonography, *PPV* positive predictive value, *NPV* negative predictive value

[a]Reprinted from Fertility and Sterility, 73/2, Soares SR, dos Reis MMBB, Camargos AF, Diagnostic accuracy of sonohysterography, transvaginal sonography, and hysterosalpingography in patients with uterine diseases, 406–11, Copyright 2000, with permission from Elsevier

[b]The numbers in parentheses are the limits of the 95% confidence interval

distinguish between a septate uterus and bicornuate uterus. There must be a less than 1 cm indentation at the fundus to qualify as a septate uterus. If there is any ambiguity in terms of the diagnosis, the gold standard for the accurate and proper classification for the diagnosis of a uterine anomaly is laparoscopy and hysteroscopy.

20.9 Surgical Treatment

20.9.1 Indication

The most common and accepted indication for surgical resection of a uterine septum is recurrent pregnancy loss in either the first or early second trimester. The goal of surgical repair of a uterine septum is restoration of a normal uterine cavity. However, normal surgical restoration of the uterine cavity does not necessarily imply good reproductive prognosis, as uterine vascularization may also be impaired.

Most studies would support the observation that primary infertility in the presence of a septate uterus is not an indication for hysteroscopic metroplasty. This procedure should only be considered after a comprehensive infertility evaluation. If no other etiologies are discovered, and the patient's infertility persists, this procedure may be undertaken. On the other hand, the simplicity and low morbidity of the hysteroscopic metroplasty has led many experts to recommend immediate removal prior to more comprehensive evaluation, especially in women with advanced age, given that uterine septums increase the rate of miscarriage.

Hysteroscopic metroplasty is the method of choice, with benefits that include lower morbidity, faster recovery, and lower risk of infection, hemorrhage, and adhesions than with metroplasty via laparotomy or laparoscopy. Additionally, by avoiding large incisions into the myometrium, hysteroscopic metroplasty does not result in a recommendation for cesarean delivery in future pregnancies.

20.9.2 Hysteroscopic Technique

Operative hysteroscopy is usually performed under general anesthesia using either an operative hysteroscope or resectoscope. The basic technique usually involves simple incision of the septum rather than removal or resection. However some large, broad-based septums are sometimes excised partially.

Micro-scissors are the method of choice for surgical resection of the septum. However, one limitation of the scissors is increased difficulty in dissecting and cutting broad-based septums. In these instances, electrocautery with the wire loop can be used. Generally, electrocautery is avoided secondary to theoretical concern that the use of electrocautery will cause thermal damage on the endometrium and myometrium, with potential risk of uterine rupture with a subsequent pregnancy.

Hysteroscopic metroplasty is performed ideally in the early follicular phase of the menstrual cycle when the endometrium is thin, hence, not requiring any preparation of the endometrium. Classical teaching has described the use of a laparoscope to visualize the uterine fundus while it is transilluminated with the hysteroscope. This allows for assessment of myometrial thickness, which is important to optimally resect the septum while avoiding uterine perforation. However, this is not necessary in most cases. More recent data suggest that a transabdominal intraoperative ultrasound can be used both safely and adequately to prevent perforation.

Resection or horizontal incision of the septum is carried out from the lower margin of the septum and continued cephalad toward the tubal ostia, always staying in the midline and horizontal plane of the septum. Incision into the myometrium should be minimized. One can safely assume that the base of the septum has been reached if increased bleeding is noted.

The surgical technique used for a complete uterine septum includes placement of a plastic uterine dilator, balloon hysterosalpingogram catheter, or Foley balloon through the contralateral cervix to indent the septum wall. It also functions to prevent the loss of distention medium through the second cervical opening. The hysteroscope is then inserted into the opposite cervix, and resection is initiated over the indented septum wall, enabling a safe resection. It is important to identify the point above the cervix at which the resection can be initiated. Once the passage is created, resection is then completed as described above, while sparing the cervical tissue. Limited studies exist, but the recommendation is to spare

the cervical portion and preserve the septum below the internal os in order to minimize the risk of cervical incompetence in subsequent pregnancies [4, 64, 65].

The endpoint of resection can be characterized by a number of parameters. Visualization of pink, vascular myometrium, distinct from the white, avascular tissue of the septum, is important. It is also important to examine the relationship of the resection to the tubal ostia and the proximity of the resection to the uterine serosa, which can be assessed via the laparoscope or ultrasound. A successful resection of the septum is defined when the tubal ostia are seen together with no separation in between, an enlargement of the uterine cavity is demonstrated, and an improvement in the uterine shape is accomplished.

20.9.3 Abdominal Metroplasty

Abdominal metroplasty is at times performed if the uterine septum cannot be resected hysteroscopically. This entails either a wedge resection at the fundus that removes the septum, as in the Jones procedures, or opening the uterus in the midline and removing the septum, as in the Tompkins metroplasty technique. Abdominal metroplasty is rarely performed and should only be undertaken by skilled surgeons.

20.9.4 Postoperative Care

Postoperative estrogens or intrauterine contraceptive devices have been used. However, randomized studies in women undergoing hysteroscopic resection of their uterine septums have noted no difference in the postoperative intrauterine adhesion rates, when followed up with HSG or hysteroscopy, despite the use of either agent [66, 67]. Studies have also not shown any added value with use of prophylactic antibiotics.

20.9.5 Complications

The complications associated with the hysteroscopic metroplasty can be viewed in two major categories: those intrinsic to operative hysteroscopy and those related to the technique and instruments used for septum resection.

The major concern with the use of the electrosurgical systems is uterine rupture at the actual site of the septum with a later pregnancy secondary to weakening of the uterine wall from thermal damage. However, vaginal delivery is still recommended unless extensive damage has occurred through thermal injury or a fundal perforation has occurred. The rate of uterine perforation is lower with resection of a uterine septum than it is for intrauterine adhesiolysis. It is reported to be less than 1%.

20.9.6 Outcome

Many studies exist that report both presurgical and postsurgical outcomes in women with hysteroscopic metroplasty. However, to date there are no published randomized clinical trials that compare pregnancy outcomes in treated vs. untreated groups of symptomatic women. Hence, surgical outcomes after treatment of septate uteruses are based on retrospective studies evaluating the reproductive outcome of women, often using patients as their own control. The overall reported rate of successful pregnancy after hysteroscopic metroplasty is 85–90% [48, 68–70].

Hickok et al. wrote a small retrospective series of 40 women with uterine septums. Preoperatively, they observed a miscarriage rate of 77.4%, delivery rate of 22.6%, and uncomplicated delivery rate of 6.5%. After hysteroscopic metroplasty, the miscarriage rate was seen to be 18.2%, delivery rate was 81.8%, and uncomplicated delivery rate was 77.3% [71]. Kupesic reviewed the reproductive outcome from 13 studies in women with untreated septate uterus and reported on 1304 pregnancies. They observed a miscarriage rate of 81.9% and a preterm delivery rate of 9.6% [72]. But the authors caution that the group of women reviewed may represent a biased group of women; women with a septate uterus and normal reproductive outcome may have been excluded. Kupesic also reported a review of the existing literature with regard to the reproductive outcome before and after hysteroscopic metroplasty for the septate uterus. In 388 patients, 1059 pregnancies were achieved before metroplasty and 362 pregnancies after surgery. The miscarriage rate and preterm and term delivery rates before and after were 87.8%, 9.0%, and 3.2% vs. 14.6%, 5.2%, and 80.1%, respectively [72]. These and other studies demonstrated an

improvement in fertility after metroplasty. The chance of pregnancy was not affected by maternal age, number of previous pregnancy losses, and method of septal resection (micro-scissors, resectoscope, or laser) nor the type of septum present, partial or complete [58, 73].

However, other studies demonstrate that there is no improvement in outcome in women with recurrent pregnancy loss after metroplasty. Kirk et al., in a series of 146 women, showed no increase in the number of living children after metroplasty [74]. However, there was also no negative effect of hysteroscopic metroplasty on fertility potential in women with recurrent pregnancy loss.

The possible adverse effect of the presence of a septate uterus on the outcome of assisted reproductive technology is still debated. The existing studies do not demonstrate any impairment on ovarian response to stimulation nor implantation rates in the presence of Müllerian anomalies to include a septate uterus. However, the studies do report a higher rate of abortion and preterm delivery if the septum is uncorrected [72, 75]. Although the hysteroscopic metroplasty is not intended to enhance fertility, it may be indicated for the improvement of their pregnancy outcome, especially after multiple treatment failed assisted reproductive cycles.

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Gynecologic Laparoscopy

Mohamed A. Bedaiwy, Howard T. Sharp, Tommaso Falcone, and William W. Hurd

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21.1 Introduction

During the last four decades, the discipline of gynecologic surgery has seen significant advancement in the use of minimally invasive surgery. The laparoscopic technique itself was revolutionized by the continuous fine tuning of the traditional instruments and the addition of new ones. The last decade has witnessed the introduction of robotic assistance and the laparoendoscopic single-site surgery (LESS) as new enhancements to the field. In addition, laparoscopy has become one of the most common surgical procedures performed in the USA and worldwide. It became the gold standard for many gynecologic procedures, such as removal of ectopic pregnancies and the treatment of endometriosis.

A recent study looked at a Cohort of 264,758 women who underwent hysterectomy for benign gynecologic disorders at 441 hospitals across the USA from 2007 to 2010. Use of robotically assisted hysterectomy increased from 0.5% in 2007 to 9.5% of all hysterectomies in 2010. During the same time period, laparoscopic hysterectomy rates increased from 24.3 to 30.5% [1].

For other procedures, including laparoscopically assisted hysterectomy and treatment of gynecologic cancers, the relative risks and benefits of the laparoscopic approach are still being determined.

This chapter will give an overview of the history and modern use of laparoscopy. Laparoscopic complications and specific laparoscopic techniques are considered in subsequent chapters.

■■ Clinical Case

A 42-year-old G3 P3 presents to her gynecologist's office with long standing history of vaginal bleeding, frequency of micturition, and constipation over the past 3 years. Her gynecologic history is significant for a diagnosis of mutifibroid uterus. She has had bilateral tubal ligation. Her Pap smears have been normal. Her vital signs are stable and her review of systems is noncontributory. Her pelvic exam reveals normal appearing vagina and cervix. Her bimanual examination is difficult because of her obesity, but does reveal 16 week size uterus. Pelvic ultrasound shows a mutifibroid uterus with the dominant fibroid being intramural and measured 7 cm in the maximum diameter with a 12 mm endometrial stripe. Laboratory evaluation includes a negative blood hCG, white blood cell count, hemoglobin of 9.8 g/dL, and platelet count of 350,000 per mcL. After reviewing different treatment options, she elected to go for total laparoscopic hysterectomy. On the day of surgery, peritoneal access was obtained via Palmer's point. Upon inserting the right lower quadrant trocar, the trocar tip wen in the right common iliac vein. Immediate laparotomy was performed and vascular surgery was called for help.

21.1.1 History

Hippocrates described the first example of an endoscope, an early rectal speculum, in Greece between 460 BC and 375 BC. The ruins of Pompeii, Italy (70 AD), provided the next example, a three-bladed vaginal speculum, similar to a modern-day speculum. Next, Philipp Bozzini in Germany (1773–1809) developed a light conductor that he called "Lichtleiter," which directed light into the patient's body and then reflected the image back to the eye of the surgeon. John D. Fisher (1798–1850) described an endoscope to inspect the vagina, and he later modified it to examine the bladder and urethra. In 1853, Antoine Jean Desormeaux pioneered the first functional endoscope, which was mainly used for urologic cases. This instrument had mirrors and a lens with a lamp flame as the light source, which burned a mixture of alcohol and turpentine.

The first experimental laparoscopy ("celioscopy") was performed in Berlin in 1901, by Dr. Georg Kelling, who placed a cystoscope into the abdomen of dogs to evaluate the ability of insufflated air to stop gastrointestinal hemorrhage [2]. Dr. Hans Christian Jacobaeus of Sweden published the first description of "laparothoracoscopy" in 1910 as a technique to evaluate patients with peritoneal tuberculosis. However, laparoscopy made little headway into clinical practice until after World War I. It took until the 1960s for laparoscopy to be accepted in the USA and Europe as a safe and valuable surgical procedure.

For many years, gynecologic laparoscopy was performed almost exclusively for diagnostic purposes and for sterilizations. By the 1970s, the role

of laparoscopy had expanded to include lysis of adhesions and treatment of endometriosis [3]. The technology and equipment advanced over the next four decades such that laparoscopy is now used for a wide variety of procedures ranging from treatment of ectopic pregnancies and ovarian cysts to hysterectomy, incontinence procedures, and management of gynecological malignancies.

21.2 General Techniques for Laparoscopy

21.2.1 Primary Trocar Placement

For many years, the standard techniques used for creating a pneumoperitoneum and placing a laparoscopic port into the abdomen were either a closed technique or an open approach. In the last decades, multiple alternative approaches and locations have been reported. The five most common approaches are as follows:

- Standard closed technique (Veress needle insufflation followed by primary trocar insertion)
- Direct trocar insertion (no insufflation prior to trocar insertion)
- Open laparoscopy
- Left upper quadrant (LUQ) insertion technique.

Both reusable and disposable instruments are commonly used. The ultimate safety of many of the newer techniques and instruments has yet to be determined.

21.2.2 Standard Closed Technique: Veress Needle and Primary Trocar Insertion

The standard closed technique was used almost exclusively for decades and continues to be widely used today. Both the Veress needle and primary trocar are blindly placed through a periumbilical incision into the peritoneal cavity. Using this approach with reusable instruments, the combined risk of injuring retroperitoneal vessels, bladder, or bowel has been found to be less than 1 in 1000 cases [4]. This approach has become the "gold standard" against which all other techniques are judged.

For the standard technique, the patient is placed in a horizontal position, and the abdominal wall is elevated by manually grasping the skin and subcutaneous tissue. This is done to maximize the distance between the umbilicus and the retroperitoneal vessels. Alternatively, a penetrating towel clips placed at the base of the umbilicus could be used to elevate the anterior abdominal wall.

In a woman of ideal weight (body mass index [BMI] <25 kg/m^2) or only slightly overweight (BMI 25–30 kg/m^2), the lower anterior abdominal wall is grasped and elevated, and the Veress needle is inserted toward the hollow of the sacrum at a 45° angle (Fig. 21.1) [5]. In the

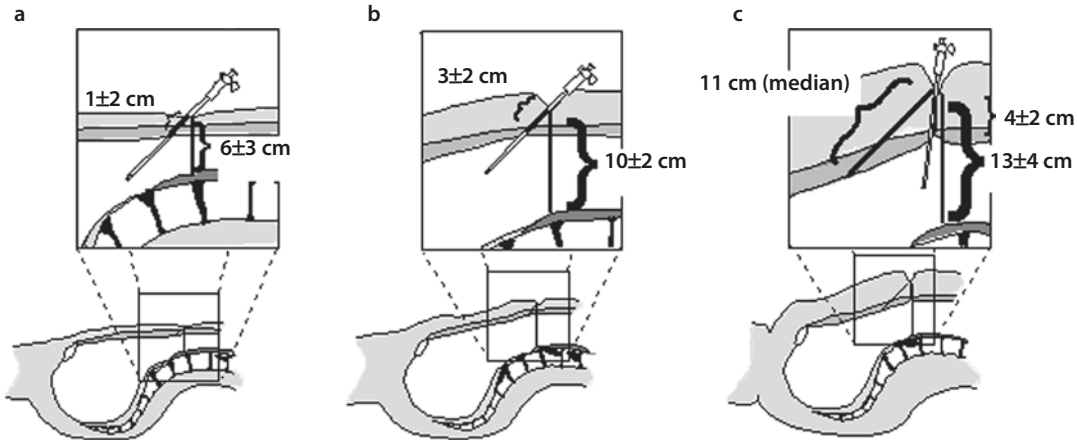

Fig. 21.1 Changes in the anterior abdominal wall anatomy with weight. Diagram of representative sagittal views derived from magnetic resonance and computed tomographic imaging for patients in three groups: (**a**) Ideal weight (body mass index [BMI] <25 kg/m^2). (**b**) Overweight (BMI 25–30 kg/m^2). (**c**) Obese (BMI >30 kg/m^2). An 11.5-cm Veress needle is superimposed on each view for comparison (reproduced with permission from Hurd WW, Duke J, Falcone T. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

thinnest patients in this group, the retroperitoneal vessels are much closer to the abdominal wall and the margin for error is reduced, with as little as 4 cm between the skin and these vessels. In the obese patient (BMI >30 kg/m^2; weight usually >200 lb) a more vertical approach, approximately 70–80°, is required to enter the peritoneal cavity because of the increased thickness of the abdominal wall. It is important to avoid subcutaneous tunneling of the Veress needle and/or the trocars prior to puncturing the fascia of the anterior abdominal wall.

Verification that the Veress needle tip is in the peritoneal cavity is done by a number of methods, including the "hanging drop test," injection and aspiration of fluid through the Veress needle, and close observation of intra-abdominal pressure during carbon dioxide insufflation. After a pneumoperitoneum has been created, the Veress needle is removed and the primary port trocar (most commonly 5 or 10 mm in diameter) is placed at an angle identical to that used for the Veress needle.

21.2.3 Direct Trocar Insertion

Direct trocar insertion is a technique whereby the primary trocar is inserted without having previously inserted the Veress needle and insufflating the abdomen with carbon dioxide [5]. This could be achieved blindly or via the optical-trocar-assisted technique. The direct primary trocar is inserted at an angle similar to that described above for the closed technique. The peritoneal cavity is then insufflated with carbon dioxide through the umbilical port.

The optical trocar insertion allows visualization of the layers that are being penetrated during entry via a laparoscope in the cannula. It is assumed that this approach could reduce the risk of injury since the technique is no longer blind. However, vascular and visceral injuries are reported with this approach. On the other side, seeing the injury as it happens will allow prompt recognition and repair, nullifying the consequences of delayed diagnosis and management.

This technique decreases the risk of extraperitoneal insufflation by allowing the surgeon to confirm intraperitoneal placement of the primary trocar before insufflation. Although small randomized studies have not demonstrated an increased risk of injuries, some series suggest that this technique might increase the risk of bowel injury [5, 6]. A larger randomized study demonstrated no major complications on comparing the two approaches. However, minor complications including preperitoneal insufflation, failed entry or more than three attempts necessary to enter the peritoneal cavity with the trocar were significantly more frequent in the Veress needle technique group [7]. In a recent meta-analysis comparing the Veress needle to direct trocar insertion, pooled analysis showed a borderline significant reduction for major complications based on five events in 2 RCTs (n = 978) and a reduction in minor complications in favor of direct trocar insertion [8].

21.2.4 Open Laparoscopy

Open laparoscopy, first described by Dr. Harrith Hasson in 1971, refers to creating a small incision in the abdomen and placing the port through the incision without using a sharp trocar [6, 9]. The skin and anterior rectus fascia are incised with a scalpel, and the peritoneal cavity is bluntly entered with a Kelly or Crile forceps. A laparoscopic port with a blunt-tipped trocar is then placed into the peritoneal cavity. For the "Hasson" technique, fascial sutures are used to assist subsequent closure and help maintain a pneumoperitoneum [6]. This method almost eliminates the risk of retroperitoneal vessel injury and is preferred by many laparoscopists for this reason. Although open laparoscopy does not entirely avoid the risk of bowel injury, many laparoscopists use this approach in an effort to decrease this risk in patients with previous abdominal surgery suspected of having adhesions.

Randomized controlled trials comparing the Hasson and Veress techniques showed no significant reduction in major complications, but the Hasson technique showed significantly less minor complications and failed entries. CO_2 leakage was far more common when using the Hasson technique [8]. In addition, a recent meta-analysis concluded that there are less minor complications and failed attempts when using the Hasson or direct entry technique when compared to the Veress method, but there is limited evidence regarding major complications [8].

21.2.5 Left Upper Quadrant Technique

This approach was developed for use in patients with previous abdominal surgery with suspected or known periumbilical bowel adhesions, during pregnancy, and with large pelvic masses. It is performed by using a LUQ site to place both the Veress needle and primary laparoscopy port into the abdomen. This point, sometimes referred to as Palmer's point, is in the mid-clavicular line beneath the lower rib margin (Fig. 21.2).

It is important to know the anatomy of the LUQ before using this technique. The most important organs that are closest to this site are the stomach and left lobe of the liver [10]. Although a small series has shown the risk of complications to be small, the relative risk of complications with this technique remains to be demonstrated by a large study [11].

Often times, a supraumbilical entry site is selected over the umbilicus for a variety of indications with large masses [12]. A recent study evaluated distances to vital retroperitoneal vasculature that were encountered with 45- and 90-degree angle entry from the umbilicus and 2 commonly described supraumbilical entry points at 3 and 5 cm cephalad from the umbilicus. According to the theoretic modeling, supraumbilical primary port placement can be implemented safely irrespective of the angle of entry as all the distances are greater than at the level of the umbilicus [13].

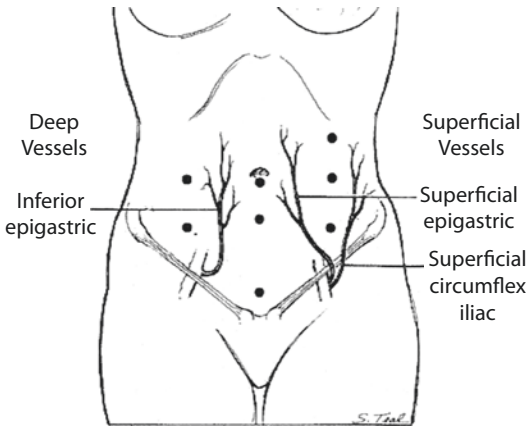

Fig. 21.2 Ideal port sites in relation to the deep and superficial vessels of the anterior abdominal wall (reproduced with permission from Hurd WW, Duke J, Falcone T. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

It is important to understand that all the studies cited above on the angle of insertion are theoretical models based on imaging. In fact due to the alterations of the abdominal wall such as lifting in order to obtain primary access will change the distances and relative anatomy.

21.2.6 Placement of Secondary Ports

Secondary ports are required to perform most gynecologic laparoscopy procedures today. After identifying the epigastric vessels by transillumination and visualizing them intra-abdominally through the laparoscope, 1–4 secondary ports are placed, depending on the procedure [14]. A midline port is often placed 3–4 cm above the pubic symphysis. Lateral ports are placed approximately 8 cm from the midline and 5 cm above the pubic symphysis to avoid the inferior epigastric vessels (see Fig. 21.2) [15]. This lateral site corresponds to McBurney's point in the right lower quadrant and is approximately one-third of the distance from the anterior iliac crest to the pubic symphysis (Fig. 21.3). Additional lateral ports for the principal surgeon are required for most operative laparoscopy cases. The site chosen is typically at the level of the umbilicus lateral to the rectus muscle. This site offers the surgeon a comfortable use of both hands and allows access to most areas of the pelvic or abdominal cavity.

Secondary ports are placed with sharp trocars under direct laparoscopic visualization to avoid injuring intraperitoneal structures. These trocars

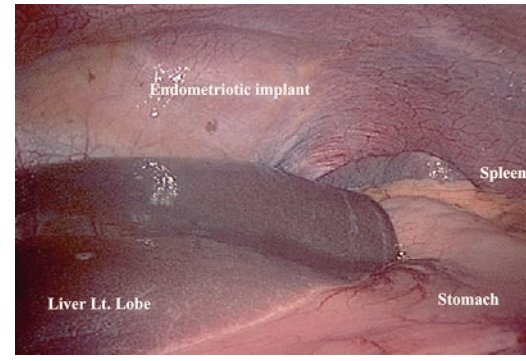

Fig. 21.3 Anatomy of the left upper abdomen (reproduced with permission from Hurd WW, Duke J, Falcone T. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

should be placed directly into the peritoneal cavity without tunneling. After removal, the intra-abdominal gas pressure is reduced to observe for signs of hemorrhage indicative of abdominal wall vessel injury. If the port diameter is ≥10 mm, the fascia and peritoneum should be closed with a full-thickness suture to reduce the risk of subsequent herniation. When comparing bladed to radially expanding trocars, three studies ($n = 408$) showed less minor complications and a trend toward pain reduction when using a radially expanding trocars [8]. Radially expanding trocars reduce minor vascular complications when compared to bladed trocars [8].

21.2.7 Removal of Ports and Port-Site Closure

At the conclusion of the procedure, port removal should be performed in a way to minimize patient risk. Secondary ports should be removed under direct visualization to detect any bleeding that might have been masked by the port or the intra-abdominal pressure. All carbon dioxide used for pneumoperitoneum should be allowed to escape prior to removal of the umbilical port to minimize postoperative shoulder pain and avoid pushing bowel into the incision sites as residual gas escapes.

21.2.8 Multifunctional Laparoscopic Instruments

Traditionally, power instruments were used during laparoscopy because suture ligation, the most common hemostatic method used during laparotomy, is difficult to perform laparoscopically. *Electrocoagulation* was perhaps the first power instrument used during laparoscopy. This instrument is heated by passing electrical current through the tip of a grasping instrument, which is then used to coagulate tissue.

In the last four decades, other methodologies have been developed, most notably electrosurgery. *Unipolar electrosurgery* passes current through the patient to cut or coagulate tissue. *Bipolar electrosurgery* was developed in an effort to minimize the risk of inadvertent injury to adjacent tissue, particularly the bowel. Bipolar electrosurgery offers an increased margin of safety because the electrical current is confined to the tip of the instrument, but the cutting ability is reduced. *Lasers* offer a precise, rapid, and accurate method of thermally destroying the tissue; however, hemostatic effects are less and lasers are costly. The *ultrasonic scalpel* is an ultrasonically activated instrument that moves longitudinally at a rate of 55,000 vibrations per second and is able to cut tissue and coagulate small vessels without heat or electrical energy. Tips available for this instrument include grasper/scissors, a hook blade, and a ball tip.

Over the past decade, significant improvements in the design and functionality of these instruments were achieved. The most important refinement was the additional cutting following coagulation. This technology uses the combination of pressure and energy to create the seal by melting the collagen and elastin in the vessel walls and reforming it into a permanent seal. Subsequently, the tissue is then divided using an internal blade. The technology reduces thermal spread to 2 mm. Controlled coagulation and cutting are achieved by a wide variety of commercially available instruments including LigaSure, LigaSure Advance, Gyrus, Harmonic Scalpel, and EnSeal.

21.3 Laparoscopic Procedures

21.3.1 Diagnostic Laparoscopy

Laparoscopy has been used effectively as a valuable diagnostic tool for a wide variety of abdominal and pelvic pathologies. It has been used for the assessment of acute or chronic pain, suspected ectopic pregnancy, endometriosis, adnexal torsion, or other extragenital pelvic pathologies. In most cases, the laparoscope is placed through an infraumbilical port, and a probe is placed through a second suprapubic port to manipulate the pelvic organs, if only a diagnostic laparoscopy is performed. However, for operative laparoscopy other than the simplest procedures, the suprapubic port is not useful and is quite uncomfortable. If operative laparoscopy is performed, the accessory trocars should be placed in the right and left lower quadrants. For advanced laparoscopy, an accessory trocar at the level of the umbilicus lateral to the rectus muscle will allow the principal surgeon to operate comfortably and have access to the pelvis. If tubal patency is a concern, a dilute dye can be injected transcervically, a procedure termed chromopertubation.

Before initiating any surgery, the peritoneal cavity should be thoroughly inspected using a systematic approach. With the surgeon controlling the movement of the laparoscope, each quadrant of the abdomen and then the pelvis should be carefully inspected. Care should be taken to inspect the appendix, omentum, peritoneal surfaces, stomach, surface of the bowel, diaphragms, and liver (Figs. 21.4 and 21.5) [16]. The spleen is usually difficult to see except in thin women (see Fig. 21.3). If any suspicious lesions are observed, fluid should be obtained for cytology (pelvic washings) prior to biopsying the lesion for frozen section.

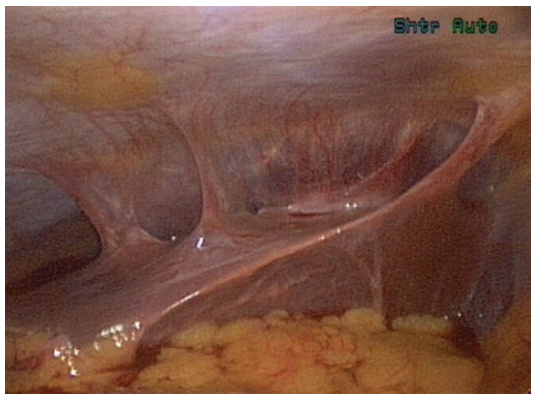

Fig. 21.4 Sub-diaphragmatic adhesions of Fitz-Hugh-Curtis syndrome. These two physicians, Dr. Curtis in 1930 and Dr. Fitz-Hugh in 1934, described the relationship with gonococcal infection (reproduced with permission from Hurd WW, Duke J, Falcone T. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

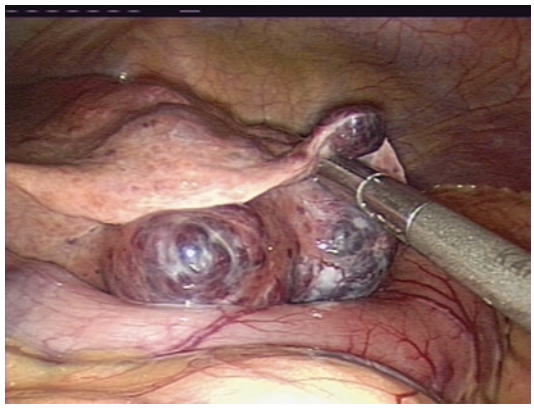

Fig. 21.5 Liver hemangioma (reproduced with permission from Hurd WW, Duke J, Falcone T. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

Laparoscopic pelvic assessment is often performed in a non-standardized fashion depending on the surgeon's discretion. Reporting positive or negative findings is random and lesions in atypical locations such as anterior and posterior cul-de-sac, deep inguinal rings, and ovarian fossa may be missed, and patient care would be less than optimal. We proposed a method for systematic pelvic assessment based on anatomical landmarks [17].

In this system, the pelvis was topographically divided into two midline zones (zones I and II) and two paired (right and left) lateral zones (zones III and IV). Zone I is the area between the two round ligaments from their origin at the uterine cornua to their insertion in the deep inguinal rings. Zone II is the area between the two uterosacral ligaments from their origin from the back of the uterus to their insertions in the sacrum posteriorly. Zone III is the area between the uterosacral ligament inferiorly and the entire length of the fallopian tube and the infundibulopelvic ligament superiorly. Zone III contains the tubes and the ovaries. Zone IV is the triangular area lateral to the fallopian tube and the infundibulopelvic ligament and medial to the external iliac vessels up to the round ligament. This system was validated in a retrospective study and prospective evaluation is ongoing [17].

21.3.2 Tubal Sterilization

Tubal sterilization is one of the most commonly used methods of birth control. Laparoscopy is one of the most common techniques used for permanent sterilization in the world. Original laparoscopic techniques used electrocautery or electrosurgery to coagulate the midportion of the tubes. Other techniques, including clips and silastic bands, have gained popularity. The pregnancy rates vary by age of the patient, ranging from 1 to 3% after 10 years [18, 19]. Given the recent discoveries indicating that the Fallopian tube is the site of origin of ovarian cancer, the uptake of salpingectomy increased significantly as a method of sterilization in different parts of the world [20].

21.3.3 Lysis of Adhesion and Tubal Reconstructive Surgery

Adhesions are frequently encountered pelvic pathology. They are usually the result of previous pelvic infections secondary to PID or a ruptured appendix, endometriosis, or previous surgery. These adhesions may contribute to infertility or chronic pelvic pain. Lysis of adhesions is performed bluntly or by sharp dissection using scissors or a power source. Extreme caution should be used if adhesions <1 cm from ureter or bowel are lysed using unipolar electrosurgery because of the unpredictable nature of current arcing. The other power techniques, such as the ultrasonic scalpel, may be a better choice for adhesiolysis near bowel for surgeons that do not have experience with unipolar cautery.

Tubal reconstructive surgery is still performed even in the era of in vitro fertilization (IVF) and is almost exclusively performed laparoscopically. Fertility-enhancing procedures include adhesiolysis, fimbrioplasty, and terminal neosalpingostomy. Prior to and during these procedures, *chromopertubation* is carried out to document proximal tubal patency by injecting dilute indigo carmine dye through the cervix using a cannula. Laparoscopic surgery is performed using the principles of microsurgery to avoid tissue damage, including delicate handling of tissues and minimal use of electrosurgery for hemostasis.

Laproscopic fimbrioplasty or neosalpingostomy has been shown to be effective in young women with hydrosalpinges with no other infertility factors, however, the evidence is fair. On the other side, there is a good evidence to recommend laparoscopic salpingectomy or proximal tubal occlusion in case of r surgically irreparable hydrosalpinges to improve IVF outcome. In addition, there is enough evidence to support the value of microsurgical anastomosis for tubal ligation reversal even in women above the age of 40 years old [21].

Patients with mild tubal disease and preservation of fimbria have excellent pregnancy rates after laparoscopic surgery. Although these patients remain at risk for subsequent ectopic pregnancy, the risk of multiple gestations associated with IVF is avoided for patients who subsequently achieve a viable intrauterine pregnancy.

Unfortunately, adhesions often reform after lysis. Multiple techniques have been used in an effort to decrease reformation. Gentle tissue handling and good hemostasis also appear to be important. Barrier methods have been shown in clinical trials to decrease adhesions but have yet to be proven to improve pain relief or future fertility.

21.3.4 Fulguration of Endometriosis

Laparoscopy is the primary surgical approach used to treat endometriosis. Endometriosis lesions may be resected or ablated, using scissors or any of the power instruments. These treatment approaches have been shown in randomized controlled trials to improve fertility and decrease pelvic pain.

21.3.5 Ectopic Pregnancy Treatment

Laparoscopy has become the surgical approach of choice for most ectopic pregnancies [22]. The embryo and gestational sac are removed either through a longitudinal incision (linear salpingotomy) or by removing the tube (salpingectomy). Both were compared in a recent RCT. The cumulative ongoing pregnancy rate was similar after salpingotomy (60.7%) compared to 56.2% after salpingectomy. However, persistent trophoblast occurred more frequently following salpingotomy compared to salpingectomy. Recurrent ectopic pregnancy rate was 8% following salpingotomy and 5% following salpingectomy [23]. Even a ruptured tubal pregnancy can be treated laparoscopically, as long as the patient is hemodynamically stable.

21.3.6 Ovarian Cystectomy and Oophorectomy

Ovarian pathological conditions, including cysts, commonly result in gynecologic complaint such as pain. The underlying pathology ranges from physiologic and self-limiting functional cysts to ovarian torsion and other benign conditions, to ovarian malignancy. Ovarian cysts are usually

characterized ultrasonographically and treated when necessary by laparoscopy or laparotomy, depending upon the size of the cyst and the level of suspicion for malignancy [24]. The most important concept in adnexal surgery is to avoid spilling the cyst content whenever possible.

21.3.7 Myomectomy

Many women with symptomatic fibroid uterus prefer a myomectomy over hysterectomy to preserve fertility or the uterus [25]. In some cases, myomectomy can be performed laparoscopically. The challenges in the case of intramural myomas are related to hemostasis, effective closure of the resulting myometrial defect, and removal of the specimen from the abdomen. Vasopressin can be injected into the uterus to help maintain hemostasis. The excised fibroid can be removed by morcellation or colpotomy. Power morcellators are available to expedite the process. Barrier techniques may be used to decrease subsequent adhesion formation. Some early case series have reported increased risk of subsequent uterine rupture during pregnancy after laparoscopic myomectomy compared to those performed by laparotomy [26]. However, several randomized clinical trials have shown no increased risk in expert hands [26]. A totally laparoscopic approach should be attempted only by gynecologists skilled in laparoscopic suturing. Recently, the uptake of the laparoscopic approach for laparoscopic myomectomy and laparoscopic hysterectomy was challenged by the FDA recommendation against power morcellation [27].

21.3.8 Laparoscopic Management of Pelvic Pain

Many women have severe dysmenorrhea that is unresolved despite medical management but wish to maintain future childbearing potential. In these patients, two laparoscopic approaches have been attempted with some success. Laparoscopic uterosacral nerve ablation (LUNA) is performed by stretching and dividing each uterosacral ligament using electrosurgery or laser alone or in combination with scissors. Care must be taken to avoid injuring the ureters. This procedure has been shown to have some temporary success, but a Cochrane review has questioned the validity of this procedure [28].

Laparoscopic presacral neurectomy (LPSN) is a second approach for central pain. This technically challenging procedure is performed by careful retroperitoneal dissection between the common iliac artery on the right and the inferior mesenteric artery where it crosses over both left common iliac artery and vein on the left. The superior hypogastric plexus, which includes the presacral nerves, is dissected from the left common iliac vein and periosteum of sacral promontory and a 2–3-cm segment is resected. Surgical risks include vascular complications, and long-term risks, such as constipation, are more common than with LUNA. Although both LUNA and LPSN appear to give some patients at least temporary relief from central pain, many clinicians believe that there is insufficient evidence to recommend the use of nerve interruption in the management of dysmenorrhea, regardless of the cause [28].

21.3.9 Hysterectomy

Laparoscopy hysterectomy, first described by Dr. Harry Reich in 1992, is commonly performed today [29]. The three basic laparoscopic approaches for hysterectomy are laparoscopic-assisted vaginal hysterectomy (LAVH), laparoscopic hysterectomy, and laparoscopic supracervical hysterectomy (LSH). Although the basic techniques for each of these approaches are fairly standardized, controversy exists over the risks, benefits, and most appropriate indication of each.

21.3.10 Laparoscopic-Assisted Vaginal Hysterectomy

LAVH is the most commonly employed and technically straightforward of the three. Using 3–4 ports, the peritoneal cavity is surveyed and lysis of adhesions is performed if necessary. Then the infundibulopelvic or utero-ovarian ligaments are occluded and divided, depending on whether the ovaries will be removed. The round ligament is divided, the utero-vesicle peritoneum is incised, and the bladder dissected from the anterior uterus. This step results in an increased risk of bladder injury compared to either abdominal or

vaginal hysterectomy. At this point, the uterine arteries laparoscopically are sometimes occluded and divided, although this is associated with an increased risk of ureter injury compared to either abdominal or vaginal hysterectomy. Finally, the posterior cul-de-sac is incised.

The surgeon proceeds vaginally for the remainder of the case, dissecting the vesicovaginal septum anteriorly to enter the anterior cul-de-sac, ligating the uterine vessels if not previously done, removing the uterus and ovaries if appropriate, and closing the vaginal cuff.

21.3.11 Laparoscopic Hysterectomy

Laparoscopic hysterectomy (LH), the common second approach, is performed initially like the LAVH, except that the entire hysterectomy is performed laparoscopically. This approach is often used when there is little or no uterine descent, which makes the vaginal approach unfeasible.

After the infundibulopelvic (or utero-ovarian) and round ligaments are occluded and divided, the bladder is dissected away from the anterior uterus. The ureters are identified, and the uterine vessels and uterosacral ligaments are occluded and divided. The posterior cul-de-sac is incised, the vagina is circumferentially separated from the cervix, and the specimen is removed vaginally. The cuff is closed laparoscopically or vaginally.

21.3.12 Supracervical Hysterectomy

The LSH is a third common laparoscopic approach to hysterectomy for benign indications [30]. The technique begins in a manner identical to LAVH and LH. However, prior to reaching the level of the uterine arteries, the fundus is transected at the uterocervical junction. In order to minimize residual cyclic vaginal bleeding and decrease the risk of developing cervical dysplasia or cancer, the glandular tissue endocervix is cored out or cauterized. The uterine specimen is removed through a 12-mm port abdominal using a power morcellator. The recent debate about tissue extraction following the laparoscopic approach for myomectomy and hysterectomy is yet to be settled [27].

This approach eliminates both the vaginal and abdominal incision, thus decreasing the risk of infection. The risk of ureteral injury is also decreased, since the procedure stops above the level of the uterine artery. However, a risk of subsequently developing cervical dysplasia or cancer remains due to the presence of the cervical stump. For this reason, routine Pap smears are required, and some patient will require additional surgery related to cervical abnormalities. Furthermore, at least two randomized clinical trials have failed to show superior results in bladder function or sexual function [31, 32]. These studies did show a higher reoperation rate for bleeding and prolapse.

Although small trials have tried to assess the value of laparoscopic hysterectomy, a large multicenter, randomized trial that compared laparoscopic with abdominal hysterectomy and laparoscopic with vaginal hysterectomy has provided insight into the role of this procedure [33]. The study confirmed that the laparoscopic approach offers no advantage over the vaginal approach. It also confirmed that the laparoscopic approach is associated with less postoperative pain, shorter hospital stay, and faster convalescence compared with the abdominal approach. It demonstrated that the laparoscopic approach was associated with a slightly higher risk of urinary tract injury. The shorter length of hospitalization with laparoscopic hysterectomy offsets some of the additional costs incurred by longer operating room times and the expense of disposable instruments [34].

21.3.13 Power Morcellation Following Laparoscopic Surgery

Morcellation is used to allow removal of large specimens that cannot be retrieved otherwise. It enabled the laparoscopic option to treat patients with large uteri or uterine myoma. One of the major limitations of this technology is the possible spread of undiagnosed cancer. This concern led the FDA to issue a warning against the use of such technology [27]. That led to many gynaecologists to refrain and many institutions to recommend against the use of the minimally invasive for women where tissue morcellation is required. In the USA, there was a significant decrease in the proportion of minimally invasive hysterectomies and myomectomies performed during the 8 months after the FDA warning statement on the use of power morcellation [35].

Overall, uterine sarcomas are difficult to diagnose preoperatively. The risk of an unexpected uterine sarcoma following surgery for a presumed benign indication is approximately 1 in 350, and the rate of leiomyosarcoma is 1 in 500 [36]. If undiagnosed sarcoma is morcellated that will indeed worsen the prognosis and negatively affect the overall survival. It is imperative that preoperative endometrial biopsy and cervical assessment to avoid morcellation of potentially detectable malignant and premalignant conditions is strongly recommended [37]. Morcellation is contraindicated for patients with hereditary cancer syndromes, and in women with established or suspected cancer where a gynaecologic oncology consultation is mandatory. Irrespective of the current local hospital policy about power morcellation, each patient should be counselled about the possible risks associated with the use of morcellation, including the risks associated with underlying malignancy. Modified morcellation techniques including the use of bags for containment are currently being tested for safety and efficacy. Despite the fact that the FDA has approved the first tissue containment system for use with certain laparoscopic power morcellators to isolate uterine tissue that isn't suspected to be cancerous, there is no clear evidence to support that their use would nullify or prevent the dissemination of undiagnosed uterine sarcomas [38].

21.3.14 Oncologic Procedures

Laparoscopy originally was used in gynecologic oncology for second-look procedures after surgical and chemical treatment of the malignancy. More recently, laparoscopy has been used for the initial staging of gynecologic cancer, including hysterectomy, peritoneal washes with biopsy, partial omentectomy, and pelvic and periaortic lymphadenectomy. Techniques have also been developed for laparoscopically assisted radical vaginal hysterectomy.

The laparoscopic approach to gynecologic cancer remains controversial. There is some concern that laparoscopy might increase the risk of intraperitoneal spread of ovarian cancer. Until the risk, benefits, and the effect on long-term prognosis have been shown to be equal to laparotomy, the laparoscopic approach will remain under close scrutiny.

21.3.15 Robotically Assisted Laparoscopic Surgery

Robotic technology has attempted to address the limitations of conventional laparoscopic surgery. The use of a remotely controlled robot has the potential to facilitate these procedures by allowing the surgeon to be seated comfortably while providing the surgeon a three-dimensional view with improved dexterity and access.

The most commonly used robotic system is the "da Vinci system" (Intuitive Surgical, Mountain View, CA, USA). The FDA approved it for use in abdominal surgeries in 2000. There are three main components: the surgeon console, the surgical cart, and the vision cart. The surgeon sits at a console separate from the surgical field. Movement of handles at the console results in movement of surgical instruments at the operative field. In this system, the surgeon looks into a console that has a dual lens system within the 12-mm laparoscope. The system provides true binocular 3D vision that is similar to looking into a microscope that enables the surgeon to see fine structures up to a tenfold magnification. Movement of the laparoscope is accomplished through the movement of the handles at the console.

The most impressive part of the system is the intra-abdominal articulation of the microinstruments 2 cm from the tip. This articulation serves the same function as a human wrist, mimicking the movements of a hand. This articulating wrist has 7 degrees of freedom of the instruments, providing an opportunity for better suturing, dissection, and reconstructing tissue by allowing the surgeon access to deep pelvic structures. The movement of the instrument tip is intuitive and requires minimal training.

The cart contains the instrument arms and camera arm. The vision cart allows all members of the surgical team to visualize the procedure. Not only does this system provide visual advantages for more precise surgery, improved dexterity, surgeon comfort with less hand fatigue, and improved instrument articulation but also it eliminates unintentional hand tremors.

There are some limitations with the use of robotic technology. One is the initial system cost, maintenance costs, and expense of disposable instruments. Another is the lack of tactile

feedback during the procedure, requiring the use of visual cues to properly carry out surgical tasks. For appropriate docking of the robot, it is imperative that a dedicated staff specifically trained on the device is available during all procedures.

Another limitation of the robotic system is its bulky size. Increased surgical operation time is a main limitation of the robotic system. This is attributed to the time required for robot preparation and docking as well as console time. Sait reported an operative time of 92.4 min for a laparoscopic hysterectomy, compared to 119.4 min for a hysterectomy with robotic assistance. However, they also showed a significant learning curve, shortening the length of operation times with increasing robotic experience [39]. Similar results were independently corroborated in a randomized controlled trial [40].

Cost is an important limitation that should be considered. Robotic surgical systems are very costly, adding approximately $3500 per procedure and approximately $2.5 billion nationally per year. This is a huge expense considering little evidence of improved outcomes over standard laparoscopy. Added to these costs, Medicare and most US private insurers do not pay additional fees for use of robotics. To overcome this, hospitals most likely will increase charges for procedures or diagnoses for which robots are used [41, 42]. The reality is that robotics overall is more costly than laparoscopy, but if it allows more surgeons to perform MIS, then maybe in the end it will end up being less costly. Wherever and whenever feasible, robotic-assisted laparoscopic surgery should not replace conventional laparoscopic or vaginal procedures for women undergoing benign gynecologic diseases. This was supported by the findings of a 2012 Cochrane Review [43]. The advantages and the disadvantages of the robotic systems are summarized in Table 21.1.

Table 21.1 Advantages and disadvantages of the da Vinci robotic system

Advantages	Disadvantages
3D visualization	Initial system cost
Improved ergonomics	No tactile feedback
Improved dexterity	Lack of research on efficiency
7 Degrees of freedom	Insufficient cases to train residents
Elimination of fulcrum effect	Large size of systems
Motion scaling	
Improved suture capabilities and knot tying	

21.3.16 Robotic Gynecologic Surgery

Robotic systems have the potential to convert surgical procedures that we presently perform by laparotomy to laparoscopy and are currently utilized in the fields of reproductive endocrinology and fertility, gynecologic oncology, and female pelvic medicine/reconstructive surgery (Table 21.2). It has been used in robotically assisted tubal anastomosis.

21.3.17 Robotically Assisted Tubal Reanastomosis

For a variety of reasons, sterilization reversal is an alternative to IVF, particularly for patients younger than the age of 35. The immediate and the long-term postoperative outcomes were compared with laparoscopic tubal anastomosis without robotic assistance [44]. The operative times were longer with the use of the robot. The tubal patency rates and clinical pregnancy rates were not significantly different. The major difficulty with laparoscopic tubal anastomosis, with or without robotic assistance, is the limited needle angles to the tubes due to operating through fixed ports. It has been reported that robotic technology is successful in facilitating laparoscopic tubal anastomosis using the da Vinci system. All of the tubal anastomoses were performed with the use of three or four robotic arms, three or four robotic instruments, and one assistant trocar. While the use of robotics prolonged surgical and anesthesia times as well as increased cost, there was no significant difference in pregnancy outcomes compared to a laparotomy technique. Additionally, patients were able to return to normal activities faster than after a laparotomy [45, 46].

Table 21.2 Current uses of robotics in reproductive surgery, gynecologic oncology, and reconstructive pelvic surgery

Reproductive surgery
Simple hysterectomy
Myomectomy
USO, BSO
Tubal reanastomosis
Resection of endometriosis
Ovariopexy
Gynecologic oncology
Radical hysterectomy
Pelvic and para-aortic lymphadenectomy
Appendectomy
LAVH
USO, BSO
Sentinel lymph node biopsy
Omentectomy
LARVH
Ovarian cystectomy
Radical parametrectomy
Radical vaginal trachelectomy
Radical cystectomy
Reconstructive pelvic surgery
Bladder repair
Hysterectomy
Vesicovaginal fistula repair
Sacrocolpopexy

21.3.18 Robotically Assisted Myomectomy

Myomectomy remains the best choice of treatment of symptomatic fibroids in patients desiring to preserve their fertility, even with the new modalities such as uterine artery embolization [26, 47]. Open myomectomy used to be the treatment modality until the emergence on minimally invasive technique. Laparoscopy yielded better cosmesis and shorter postoperative pain and hospital stay. However, this procedure was very challenging. A limitation included needing to precisely dissect the fibroid without unnecessary breaching of the endometrial cavity. Since laparoscopic suturing is a difficult skill to master, it is complicated to suture the fibroid beds in layers with precise approximation of edges, which is needed to prevent uterine rupture during labor. These challenges limited the enthusiasm and acceptance of this technique.

Many studies demonstrated the feasibility of robotically assisted myomectomy [26, 48]. Most recently, the operative and immediate postoperative surgical outcomes of robotically assisted laparoscopic myomectomy, standard laparoscopic myomectomy, and open myomectomy were compared. Blood loss, operative time, and hospital stay were lower for the robot-assisted group. These results showed an association of robotic-assisted myomectomy with decreased blood loss and length of hospital stay compared with traditional laparoscopy and to open myomectomy [49].

21.3.19 Robotically Assisted Resection of Endometriosis

Nezhat et al. compared robotic treatment of stage I or II endometriosis to conventional laparoscopy in a retrospective cohort controlled study in 2010. Forty patients were treated for endometriosis by robot-assisted laparoscopy, and 38 patients were treated by standard laparoscopy. There were no significant differences between these groups in blood loss, hospitalization, or complications, but the mean operative time with the robot was 191 min (135–295) compared with 159 min (85–320) during standard laparoscopy. Since both treatments have excellent outcomes and the robotic technique required a longer operative time, it was concluded that the robot has no added value for the treatment of early stage endometriosis [50].

Most recently, we reported on the safety and feasibility of robotic surgical treatment of advanced pelvic endometriosis. Fifty women underwent a robotic procedure for advanced endometriosis. Twenty-one (42%) had stage III and 29 (58%) had stage IV endometriosis. The median total operative time was 209 (range: 97–368) min, including patient positioning, robot docking, performing surgery, and closure of the port sites. Median actual operative time was 154

(range: 67–325) min, and both total OR time and actual operative time were comparable between the two groups. There was no difference between the two groups regarding estimated blood loss and uterine weight. Pathological evaluation confirmed the endometriosis diagnosis in all patients [51]. In a more recent series, operating time was identified as the only risk factor for the length of the hospital stay and the postoperative complications in patients with stage 3 and stage 4 disease [52].

21.3.20 Clinical Applications in Gynecologic Oncology

The traditional approach to gynecologic oncology surgeries involves a total hysterectomy, bilateral salpingo-oophorectomy, and dissection of both pelvic and para-aortic lymph nodes. These surgeries and others are now being conducted with the use of robotic surgical systems in select patients.

The specific advantages of robotic-assisted endoscopic procedures in gynecologic oncology arise from the da Vinci's enlarged operative field without the need for large fascial incisions. This allows for more easily identifiable pelvic anatomy while patients experience decreased postoperative morbidity and faster recovery to permit rapid initiation of adjuvant radiotherapy or chemotherapy. The safety profile of the da Vinci utilized for gynecologic oncology applications appears reassuring, with less blood loss and a low complication rate in managing ovarian, endometrial, and cervical cancers, respectively [53–55].

In a recent survey of the Society of Gynecologic Oncology (SGO) members to evaluate the current patterns of use of minimally invasive surgical procedures, including traditional, robotic-assisted, and single-port laparoscopy, and to compare the results to prior 2004 and 2007 surveys, there a significant increase in the uptake of the MIS approach. Overall, three indications for laparoscopy have expanded beyond endometrial cancer staging to include surgical management of early stage cervical and ovarian cancers, but the use of single-port laparoscopy remains limited. There was an increase in the overall use and indications for robotic surgery. This significant rise included radical hysterectomy or trachelectomy and pelvic lymphadenectomy for cervical cancer and total hysterectomy and staging for endometrial cancer. These procedures were found to be significantly more appropriate for the robotic platform in comparison to conventional laparoscopy [56].

21.3.21 Clinical Applications in Female Pelvic Medicine and Reconstructive Surgery

In the literature, robotics have been utilized in the repair of both vesicovaginal fistulas and in the treatment of post-hysterectomy vaginal vault prolapse with sacrocolpopexy [57]. It has been shown that the involvement of obstetrics and gynecology and urology residents has no effect on the surgical outcome of robotic-assisted sacrocolpopexy (RASCP) [58]. The question remains that although the use of robotics combines the outcomes of an open procedure, the benefits of minimally invasive surgery and easy adoptability, does it outweigh the increased cost and time [59]? Like in other disciplines, it has been shown that the robotic approach is longer and more costly than the conventional approach in urogynecologic disorders [60].

21.3.22 Single-Port Laparoscopy

The concept of natural orifice surgery has been recently revisited. Advancements in surgical instruments, optics, and ports have allowed the development of single-port laparoscopy or LESS. LESS can be used for salpingostomy or salpingectomy to treat tubal ectopic pregnancy [61].

Recent studies indicate that the procedure has low rate of complications and similar surgical outcomes compared to conventional laparoscopy. LESS has also been found to be associated with a reduction of gas leakage. The use of LESS has the advantages of reduced postoperative pain, earlier return to daily activities, reduced incidence of port-site hernias and hemorrhage, and improved cosmesis and patient satisfaction. However, data on long-term effectiveness are lacking [62].

LESS is now being used to treat benign and malignant adnexal disease and for hysterectomy. For adnexal disease, LESS can be used to remove ovarian cysts, for salpingo-oophorectomy, to remove endometriosis, and to remove malignant masses. Single-port access total hysterectomy is more commonly used now, with

various advancements in place to overcome the limited free movement and technical difficulty. Combining LESS with the da Vinci robot system allows further benefits, including better cosmesis, reduced morbidity from injury during trocar placement, a reduced incidence of postoperative wound infections and hernia formation, and improved dexterity [62].

In a review of 6 RCTs and 15 observational studies including a total of 2085 patients (899 single-incision laparoscopies and 1186 conventional laparoscopies), the surgical outcomes were evaluated. In the pooled analysis, there was no difference in the risk of complications between single-incision laparoscopy and conventional laparoscopy in gynecologic surgery. However, some studies suggest that single-incision laparoscopy may have longer operative time for adnexal surgery, but not for hysterectomy [63]. It remains uncertain if such a new technology is cost-effective with comparable long-term surgical outcomes.

21.3.23 Laparoscopic Complications

Overall, laparoscopy has a relatively favorable complication profile compared to the same procedure performed via laparotomy. In addition to the procedure-related complications, laparoscopy is associated with uncommon but significant complications related to trocar insertion. These injuries involve primarily blood vessels, bowel, and bladder. Given its mostly blind nature, insertion of the Veress needle and primary trocar for initial entry by trocar insertion remains the most hazardous part of laparoscopy, accounting for 40% of all laparoscopic complications and the majority of the fatalities. Despite decades of research and development to find safer methods for initial laparoscopic entry, major vessel injuries have been reported using virtually all types of trocar insertion methods [64]. The following is a brief discussion of avoidance and manage of these complications.

21.3.24 Retroperitoneal Vessel Injury

Techniques used to place primary and secondary laparoscopic ports into the peritoneal cavity are often accompanied by a small but unavoidable risk of injury to blood vessels located in the anterior abdominal wall and the major blood vessels located in the retroperitoneal space. Injury of major abdominal blood vessels is a rare but treatable life-threatening complication of laparoscopy, which occurs in approximately 3 per 10,000 laparoscopies [65]. These injuries most commonly occur during insertion of the Veress needle or primary trocar.

21.3.25 Prevention

The majority of retroperitoneal vessel injuries during laparoscopy occur during blind placement of the Veress needle or primary trocar through a periumbilical incision [66]. To minimize this risk, surgeons need to be aware of anatomic considerations so that they can determine the most appropriate direction and angle of insertion for each patient, as discussed above. The different approaches for primary prevention of vessel injuries are discussed in the following sections.

21.3.26 Awareness of the Patient's Position

For greatest safety, the surgeon should make sure they are aware of the patient's position in relation to horizontal prior to laparoscopic instrument placement. Most laparoscopic surgery is performed in the Trendelenburg position to keep bowel away from the operative field in the pelvis. If the patient is placed in Trendelenburg position with the feet elevated 30° relative to the head prior to instrument insertion, instruments inserted at 45° from horizontal will actually be placed at 75° from the horizontal plane of the patient's spine, which is likely to increase the risk of major vessel injury, particularly in slender patients [67].

21.3.27 High-Pressure Entry

Another technique used in conjunction with closed laparoscopy in an effort to decrease the risk of major vessel injury is "high-pressure entry." Rather than inserting the primary umbilical trocar after obtaining intra-abdominal pressure of 18–20 mmHg, many surgeons increase the pressure to 25–30 mmHg. The rationale is to make the anterior abdominal wall stiffer such that the downward pressure exerted by trocar insertion does not decrease the distance of the umbilicus to

the retroperitoneal vessels [68]. Although no controlled studies large enough to demonstrate an advantage have been published, large series including more than 8000 cases suggest that the risk of major vessel injury using this technique is approximately 1 in 10,000 cases (0.01%), compared to a risk of 4 in 10,000 cases (0.04%) reported using standard pressures [69].

21.3.28 Verify Veress Needle Location

Use of the Veress needle used to insufflate the peritoneal cavity is associated with a small risk of intravascular insufflation and venous gas embolism, reported to occur in approximately 1 in 100,000 laparoscopic procedures [70].

Several methods have been used to demonstrate the intraperitoneal location of the Veress needle tip. First, the Veress needle should be placed with the valve open, so that entering a high-pressure arterial blood vessel will immediately result in extrusion of blood through the needle. Second, after needle placement, a syringe should be used to aspirate the Veress needle, to verify that a low-pressure venous blood vessel has not been entered. This is often followed by the "hanging drop test," wherein a drop of saline is placed at the open end of the Veress needle hub. When the abdominal wall is elevated, the drop often disappears into the shaft if the tip is located in the relatively low-pressure peritoneal cavity but will usually not disappear if the tip is preperitoneal or embedded in some other structure.

The "Waggle test" is another maneuver used by some to verify that the needle has not entered the retroperitoneal space. After the needle is placed in the proper position, the hub is moved from side to side using gentle lateral pressure. Lack of lateral mobility suggests that the tip is anchored in the immovable retroperitoneal space, and the needle should be slowly withdrawn until lateral movement is possible. This technique is difficult to interpret in obese patients because the abdominal wall itself can limit lateral movement of the Veress needle, even if it is placed through the base of the umbilicus at the proper angle.

It is recommended that at least one of these methods be used when placing a Veress needle into the abdomen [71]. However, none of these methods absolutely verify intraperitoneal placement of the needle tip. Once insufflation is begun, the strongest predictor of intraperitoneal placement appears to be an initial filling pressure of <10 mmHg.

21.3.29 Other Laparoscopic Entry Methods

Multiple insertion methods and instruments have been developed in an effort to decrease the risk of trocar complications. Although each method has theoretical advantages compared to the traditional closed techniques, none has completely eliminated the risk of major vessel injury.

21.3.30 Open Laparoscopy

Open laparoscopy is a widely used alternative technique for placement of the primary laparoscopic port. The Hasson technique is fundamentally a minilaparotomy incision followed by placement of the primary port directly into the peritoneal cavity [72]. Open laparoscopy almost completely prevents the risk of major vessel injury, decreasing the rate to 0.01%, compared to a rate of 0.04% associated with closed techniques using a Veress needle [69].

21.3.31 Direct Trocar Insertion

Direct trocar insertion is a laparoscopic entry technique wherein the primary trocar is placed without prior insufflation, with or without elevation of the anterior abdominal wall manually or with towel clips. This approach is slightly faster than standard closed laparoscopy and avoids the risks of Veress needle placement. Unfortunately, this technique might increase this risk of major vessel injury. Large series (>10,000 cases) report a major vessel injury risk of 0.06–0.09% compared to 0.04% using a standard closed technique [71]. This risk of major vessel injury might be one reason why direct trocar insertion is one of the least frequently used techniques by gynecologists.

21.3.32 Left Upper Quadrant

LUQ insertion of the Veress needle and primary trocar through a site in the LUQ is recommended by some surgeons to decrease the risk of

complications associated with bowel adhesions in women with prior abdominal surgeries. The LUQ insertion site (Palmer's point) is located 3 cm below the middle of the left costal margin, and instruments are routinely inserted perpendicular to the patients' skin.

Major vessel injuries have not been reported using this technique. Anatomic studies indicate that the abdominal wall is uniformly thin in this location and the distance from the skin to the retroperitoneal structures is >11 cm in most patients [17]. However, because this distance can be <7 cm in many slender patients, it is recommended that, in slender patients, instruments placed through Palmer's point be directed 45° caudally relative to the patient's spine [73].

21.3.33 Alternative Primary Trocar Design

Alternative primary trocars have been developed, including shielded disposable trocars, optical trocars, and radially expanding trocars [71]. Unfortunately, their use does not prevent major blood vessel injuries. Currently, there is no evidence of benefit of one technique or instrument over another in terms of preventing major vascular injury. When comparing bladed to radially expanding trocars, studies showed less minor complications and a trend toward pain reduction when using a radially expanding trocars [8].

21.3.34 Treatment

Major vessel injuries are a rare but unavoidable laparoscopic complication associated with the closed entry techniques. Every laparoscopic surgeon that uses a closed technique should develop a plan of action for major vessel injury. The surgeon should also become familiar with the availability of laparotomy instruments, blood products, vascular clamps, and surgical consultants. This is especially important when these procedures are performed in a free-standing outpatient surgical facility.

When a major vascular injury is suspected, the following steps should be taken without delay. The nursing personnel should prepare for emergency laparotomy, and anesthesia personnel should consider placing additional intravenous lines and calling for blood products and additional assistance. The surgeon should immediately perform a laparotomy via a midline incision, and blood loss should be minimized using direct pressure over the injury site. When the injury occurs in a medical center, a trauma surgeon or vascular surgeon should be called in to identify and repair the vascular injuries.

The treatment approach is different when a major vessel injury occurs in a facility where vascular surgery personnel and equipment are not available. In these instances, a laparoscopic surgeon without experience in vascular surgery should not attempt to open the retroperitoneal area to repair the vessel [71]. This approach can further injure the vessels, and resultant lack of circulation to the lower extremities can have catastrophic results. Rather, the abdomen should be packed tightly with dry laparotomy pads, and the abdomen quickly closed with either running full-thickness sutures or towel clips [74]. The patient should then be transported by the most expedient method to the nearest fully equipped trauma center.

21.3.35 Abdominal Wall Vessels Injury

Anterior abdominal wall vessels at risk for injury can be divided into two groups: superficial and deep [15]. The superficial vessels consist of the superficial epigastric and circumflex iliac arteries, which are located in the subcutaneous tissue. The deep vessel at risk is the deep inferior epigastric artery, which is located beneath the rectus abdominus muscles immediately above the peritoneum.

Damage to the superficial vessels is often asymptomatic at the time of surgery, whereas damage to a deep vessel usually leads to immediate and rapid blood loss. If unrecognized, damage to either type of vessels can result in postoperative hemorrhage or hematoma.

21.3.36 Prevention

The primary method for avoiding injury to any of these vessels is to visualize the vessels via transillumination and direct laparoscopic visualization prior to lateral trocar insertion. Transillumination of the anterior abdominal wall with the laparoscopic light source is an effective way to visualize the superficial vessels in almost 90% of patients

[14]. The inferior epigastric vessels cannot be seen by transillumination since they lie beneath the rectus abdominus muscle and fascia but can be directly visualized laparoscopically immediately beneath the peritoneum in the majority of patients where they lie between the insertion of the round ligament at the inguinal canal and the medial umbilical fold. Since both the deep and superficial vessels are located an average 5.5 cm from the midline, risk of vessel injury can be minimized by placing secondary trocars 8 cm lateral to the midline and 8 cm above the pubic symphysis [15].

21.3.37 Treatment

When a superficial vessel is found to be bleeding after the port is removed, the most effective approach is to grasp the vessel with a Crile "hemostat" forceps, followed by cautery or ligation. In cases where the injured vessel cannot be grasped, a pressure dressing is often sufficient.

When an inferior epigastric vessel is injured, the result is immediate and brisk bleeding from the port site into the peritoneal cavity. Anesthesiology personnel should be alerted because additional intravenous lines and blood products might be required if the patient becomes hemodynamically unstable. If another port is available, an attempt should be made to occlude the injured vessel with a laparoscopic bipolar electrosurgery instrument above and below the injury. If another port has not yet been placed or electrosurgery is not effective, the bleeding can be temporarily slowed by placing a Foley catheter through the port site into the peritoneal cavity. After the bulb is inflated with saline, the catheter is retracted to hold the bulb tightly against the peritoneal surface and a Kelly forceps used to cross-clamp the catheter on the skin side to maintain traction.

If bipolar electrosurgery is unsuccessful, precisely positioned sutures can be placed above and below the injury using port-site closure instruments. These sutures should be tied deep to the skin above the fascia.

If hemostasis cannot otherwise be achieved, the incision should be widened and the injured vessels individually ligated. The port-site incision should be enlarged transversely to at least 4–6 cm, the fascia of the anterior rectus sheath incised, and the lateral edge of the rectus abdominus muscle retracted medially. The bleeding vessels can be grasped with hemostatic forceps and selectively ligated above and below the injury.

Delayed bleeding can occur when the abdominal pressure decreases after removal of the carbon dioxide, especially if the method used to occlude an injured vessel becomes loose as the patient awakes from anesthesia and is moved [75]. Signs of hemodynamic instability in the recovery room necessitate a return to surgery because uncontrolled bleeding from a lacerated inferior epigastric artery can be life-threatening.

21.3.38 Gastrointestinal Injury

Despite the continued development of both laparoscopic instruments and techniques, gastrointestinal injury continues to be a common, yet potentially avoidable complication of laparoscopy. In the last four decades, the risk of this complication appears to have increased from approximately 3 per 10,000 procedures to as high as 13 per 10,000 procedures [65, 76]. Most bowel injuries occur during placement of the Veress needle or primary trocar and usually when bowel is adherent to the anterior abdominal wall from previous surgery [77]. Other gastrointestinal injuries result from operative procedures including adhesiolysis, tissue dissection, devascularization injury, and thermal injury.

It is essential to minimize morbidity related to gastrointestinal injuries both by prevention and early recognition. Despite an increasing awareness of these risks, gastrointestinal injuries continue to be the most lethal type of injuries associated with laparoscopy, with a mortality rate reported as high as 3.6% [76].

21.3.39 Preventive Measures

No method has yet to be discovered that completely prevents gastrointestinal injuries during laparoscopic port placement [78]. However, it is well established that patients with previous abdominal surgery are at increased risk of gastrointestinal injury during laparoscopy since adhesions to the anterior abdominal wall occur in approximately 25% of these patients. For this reason, certain measures have been used in an effort to decrease the risk of gastrointestinal injuries in these patients.

Two commonly used techniques for high-risk patients are open laparoscopy, as first described by Hasson, and a LUQ closed technique utilizing Palmer's point [78, 79]. Unfortunately, neither of these techniques has been shown in prospective comparison studies to decrease the risk of intestinal injury relative to the open technique [78, 80, 81].

Another alternative approach is the use of an optical-access trocar. These devices are designed to increase safety by visualizing each layer of the abdominal wall during port placement. Unfortunately, these devices have not been shown to decrease the risk of gastrointestinal injuries [82].

21.4 Recognition and Treatment

21.4.1 Veress Needle Injuries

The spring-loaded tip of the 14-gauge Veress needle does not prevent perforation of adherent bowel or bowel with limited excursion related to physiologic attachments, such as the transverse colon [83]. Most bowel perforations caused by the Veress needle do not need to be repaired as long as the puncture is not actively bleeding or associate with a tear {Loffer, 1975 #68). Even in the case of colonic puncture, nonoperative management with copious irrigation appears to be sufficient [84].

21.4.2 Stomach Injuries

Injury to the stomach during laparoscopy is relatively uncommon and was reported to occur in less than 3 in 10,000 cases in the earlier days of laparoscopy [85]. Risk factors include a history of upper abdominal surgery and difficult induction of anesthesia, as a gas distended stomach can be below the level of the umbilicus. Routine decompression of the stomach with a nasogastric tube prior to Veress needle or trocar placement has virtually eliminated this risk, even when a LUQ approach is used.

Trocar injury to the stomach requires surgical repair, either via laparotomy or laparoscopy [86]. The defect should be repaired in layers with a delayed absorbable suture by a surgeon experienced in gastric surgery. The abdominal cavity should be irrigated, being careful to remove all food particles as well as gastric juices. Nasogastric suction is maintained postoperatively until normal bowel peristalsis resumes.

21.4.3 Small Intestine Injuries

Intraoperative injuries to the small intestine often go unrecognized during surgery. Injury should be suspected whenever multiple anterior abdominal wall adhesions are present. When the primary trocar and sleeve penetrate completely through both walls of bowel adherent near the umbilicus, the injury will not be visible. Whenever the routine 360° survey of the abdominal cavity reveals bowel adherent near the point of insertion, a 5-mm laparoscope should be placed through a lower quadrant port to view the umbilical port site and search for injury. An injury to nonadherent bowel with the Veress needle or a trocar during initial port placement or during lysis of adhesions may fall out of view into the abdomen. If such an injury is suspected, the bowel should be run with laparoscopic bowel graspers or manually using a laparotomy incision until an injury is satisfactorily excluded.

Postoperatively, unrecognized trocar injuries to the small intestine usually present with symptoms of nausea, vomiting, anorexia, abdominal pain, peritoneal signs, and possibly fever on the second to fourth postoperative day. Although the bacterial load of the small intestine is low, the contents are not sterile, and sepsis is a common result of undiagnosed injuries.

A full-thickness injury to the small intestine of 5 mm or greater should be repaired in two layers, sewing perpendicular to the long axis of the intestine to avoid stricture formation. This can be accomplished with an initial interrupted layer of 3–0 delayed absorbable suture to approximate the mucosa and muscularis. A serosal layer of 3–0 delayed absorbable suture is commonly placed in an interrupted fashion. This is usually performed by laparotomy or by minilaparotomy at the umbilical site, where the injured bowel loop is pulled through to the skin surface and repaired. Laparoscopic repair has also been reported by surgeons with advanced gastrointestinal surgical skills [87]. If the laceration to the small bowel exceeds one-half of the diameter of the bowel lumen, segmental resection is recommended.

21.4.4 Large Intestine Injuries

Trocar injuries to the large intestines are reported to occur with frequency of approximately 1 per 1000 cases [88]. Due to the high concentration of coliform bacteria in the large intestine, unrecognized injuries can result in serious intra-abdominal infections that can quickly become life-threatening.

Whenever a large intestine injury is suspected, the area should be carefully inspected using atraumatic bowel graspers. If adhesions or anatomy make laparoscopic inspection difficult, laparotomy is reasonable. An occult injury to the rectosigmoid colon may be detected using the "flat tire test," in which the posterior cul-de-sac is filled with normal saline and air is injected into the rectum using a proctosigmoidoscope or a catheter-tipped bulb syringe [89]. Visible bubbles indicate a large intestine injury.

The management of large intestine injuries depends upon size, site, and time between injury and diagnosis. In general, once the diagnosis of colonic injury is made, broad-spectrum antibiotics should be administered and consultation should be sought with a surgeon experienced with these types of injury. In the case of a small tear with minimal spillage of bowel contents, the defect is closed in two layers with copious irrigation. When a larger injury has occurred or the injury involves the mesentery, a diverting colostomy is sometimes necessary. In the case of delayed (postoperative) diagnosis, tissue inflammation usually makes a diverting colostomy necessary.

21.4.5 Port-Site Hernia

For the first two decades of laparoscopy, ports were placed almost exclusively in the midline, where the anterior and posterior rectus fascia fuses. These midline ports usually consisted of a 10-mm port at the umbilicus and a 5-mm suprapubic port. Port-site hernias at these locations are rare, and those reported are usually limited omental herniation through the umbilical site.

The use of lateral ports for more complex operative laparoscopy has resulted in a dramatic increase in the risk of port-site herniation. In one retrospective review, port-site hernias occurred in 5 of 3500 (0.17%) procedures, with all hernias occurring where ports with diameters ≥10 mm were placed lateral to the midline [90]. Since the rectus fascia splits laterally to form both anterior and posterior sheaths below the arcuate line, bowel herniation can occur between these two fascial layers in what has been called a "Spigelian hernia."

21.4.6 Prevention

To minimize the risk of port-site herniation, both the anterior and posterior fascial sheaths should be closed after removal of all ports 8 mm and larger. This closure is usually performed with the aid of one of a number of commercially available devices or needles that incorporate the peritoneum as well as both fascial layers. Unfortunately, port-site herniation is not completely prevented by careful fascial closure [91].

21.4.7 Recognition and Treatment

Trocar-site hernias usually present as a palpable mass beneath a lateral trocar-site skin incision that manifests during a Valsalva maneuver. Ultrasonography can distinguish herniated bowel from a hematoma. A persistent mass associated with pain indicates an incarcerated hernia and represents a surgical emergency.

Herniated bowel can often be reduced laparoscopically, followed by careful inspection of the affected segments. Although simple repair of the peritoneal and fascial defects is all that is required in most healthy patients, in some cases synthetic mesh may be needed.

21.4.8 Bladder Injuries

Injury to the bladder related to laparoscopic port placement is relatively uncommon and usually related to insertion of the primary trocar in the presence of a distended bladder or insertion of a suprapubic midline trocar in a patient whose bladder dome had extended cephalad secondary to previous surgery [92].

21.4.9 Prevention

The risk of trocar injuries to the bladder can be decreased by draining the bladder with a catheter prior to primary trocar placement. In patients

with prior lower abdominal surgery, it seems prudent to place the suprapubic trocar above any previous transverse skin incisions. In all patients, an attempt should be made to visualize the superior bladder margin laparoscopically prior to suprapubic trocar placement [14]. In cases where the superior margin of the bladder cannot be seen, the bladder can be filled with 300 mL to better define its margin. An alternative approach is to use a lateral port site rather than a midline suprapubic site, although the decreased risk of bladder injury may be offset by an increased risk of vessel injury.

21.4.10 Recognition

Laparoscopic bladder injuries are often difficult to recognize intraoperatively. Visible leakage of urine at the time of injury is unlikely in patients with a Foley catheter in place. A common sign of bladder injury is significant bleeding from a suprapubic port site placed in the relatively avascular midline. Frank hematuria suggests a full-thickness injury. An uncommon, but pathognomonic, sign of bladder injury during laparoscopy is insufflation of the Foley catheter bag with carbon dioxide [93].

If bladder injury is suspected during laparoscopy, an indigo carmine solution can be instilled retrograde through a urethral catheter to detect small leaks. Cystoscopy or, less commonly, intentional cystotomy may be used to inspect the bladder mucosa in questionable cases, or to determine the extent of a known injury and to insure that there is no ureteral involvement.

Postoperative recognition of a bladder injury can likewise be difficult. Whenever a patient returns within days of laparoscopy with significant abdominal findings, the possibility of an occult bladder injury should be considered [92]. Bladder injury should be included in the differential diagnosis in the presence of painful urination and microscopic hematuria. Elevation of blood urea nitrogen (BUN) and a serum creatinine suggests intra-abdominal spill of urine with transperitoneal reabsorption. Drainage from a suprapubic incision can be evaluated further by instillation of a dilute indigo carmine solution into the bladder.

21.4.11 Treatment

When a bladder injury is diagnosed in the postoperative period, a retrograde cystogram should be performed to determine the extent of the injury. If surgery is indicated because of peritoneal signs of uncertain etiology, cystoscopy prior to laparotomy may be extremely helpful in determining surgical approach.

Small, uncomplicated, and isolated injuries of superior portion of the bladder can be treated with catheter drainage alone [94]. A retrograde cystogram should be performed after 10 days of continuous drainage and will document spontaneous healing in 85% of patients with small injuries. Primary surgical repair is required for larger injuries and those that involve the dependent portions of the bladder, including the trigone, especially if there is a risk of concomitant injury to the urethra or ureter. Closure should be performed using a water-tight, multilayered repair with absorbable suture. Laparoscopic repair may be performed by those with adequate surgical expertise as long as there is adequate exposure and the ureters and bladder neck are not compromised [95].

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Uterine Leiomyomas

Gregory M. Christman

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22.1 Introduction

Leiomyomas are benign monoclonal tumors of smooth muscle cell origin, the vast majority of which are found within the uterine corpus, although they can occur throughout the body in any structure containing smooth muscle. Leiomyomas are rarely malignant but are responsible for a large number of surgical interventions, including hysterectomy. Although symptoms usually correlate with size, large tumors can be asymptomatic and small tumors can be symptomatic. This chapter will review the pathophysiology and management of leiomyomas. The chapter will clarify the classification terms used to describe the location and relationship of a fibroid to the surrounding uterus and will describe the potential impact of this disorder on the patient. The chapter will postulate how leiomyomas may arise and be influenced by alterations in genetics, lifestyle, endocrinology, and pregnancy. Leiomyomas can be managed by a variety of short-term medical approaches, uterine artery embolization, high-intensity MRI-guided focused ultrasound, and surgery ranging from minimally invasive focal surgical resection to definitive therapy via hysterectomy.

Clinical Case

A 36-year-old women presents with chief complaints of the inability of getting pregnant despite not using contraception for over 1 year and heavy menstrual periods. Prior to this, she used condoms for 18 years, and the only medication she is currently takes is prenatal vitamins. Her menses occur every 30–32 days, are described heavy flow with occasion blood clots and moderate cramping. Her menstrual flow volume and accompanying pain have slowly increased over the last 5 years. She also complains of 2 years of gradually increasing abdominal fullness. Her pain is unrelated to activity. She also complains of urinary frequency during the day, but denies pain with sex or changes in bowel habits. Her past medical, surgical, and social histories are unremarkable. On pelvic examination her uterus is 18 weeks size and irregular. Ultrasound reviews multiple spherical masses contiguous with the uterine fundus consistent with leiomyoma ranging in diameter from 10 to 3 cm.

22.2 Prevalence

Uterine leiomyomas (also known as fibroids or myomas) are the most common benign pelvic tumors found in women. As many as 50% of reproductive-age women have clinically apparent uterine leiomyomas and 25% of women have symptomatic leiomyomas. On pathological examination, 77% of hysterectomy specimens contain one or more uterine leiomyomas [1].

Although generally benign, uterine leiomyomas are associated with significant morbidity, including abnormal uterine bleeding, chronic pelvic pain, impaired fertility, and recurrent pregnancy loss. In the USA, leiomyomas are cited as the primary indication for hysterectomy in over 250,000 cases per year and account for over $5 billion in health-care costs annually [2].

22.3 Classification

Uterine leiomyomas are typically classified into subgroups by their position relative to the layers of the uterus. *Subserosal myomas* occur near the serosal surface of the uterus. They may have either a broad or pedunculated base and can extend between the folds of the broad ligament. *Intramural myomas* originate within the myometrium and may enlarge enough to distort the uterine cavity or the serosal surface. *Submucosal myomas* develop just below the endometrium and, with progression, protrude into the uterine cavity (Fig. 22.1). Subserosal and submucosal myomas can also be either *pedunculated* or *broad-based*. *Cervical myomas*, on the other hand, are derived from cells in the cervix as opposed to the uterine corpus.

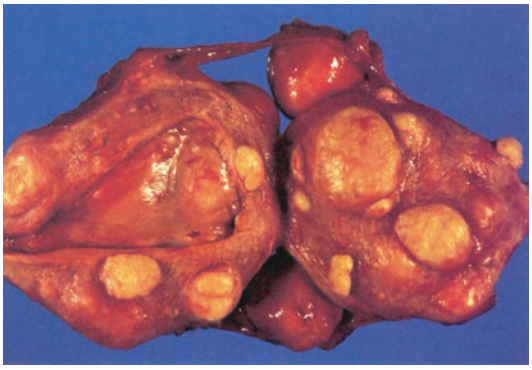

Fig. 22.1 Hysterectomy specimen demonstrating the presence of intramural, submucosal, and subserosal leiomyomas

More recently, uterine myomas have been classified used the International Federation of Gynecology and Obstetrics (FIGO) Classification system [3, 4]. This system categorizes the submucosal leiomyomas as well as the intramural, subserosal, and transmural lesions. Type 0 through 3 myomas are considered to be submucosal: Type 0 myomas are intracavitary lesions attached to the endometrium by a stalk, type 1 fibroids are ≤50% intramural, type 2 are >50% intramural, and type 3 lesions are completely extracavitary immediately adjacent to the endometrium. Type 4 myomas are intramural leiomyomas that are entirely within the myometrium, with no extension to the endometrial surface or to the serosa, whereas types 5 through 7 are subserosal: Type 5 myomas are >50% intramural, type 6 are ≤50% intramural, and type 7 are attached to the serosa by a stalk. Type 8 myomas are neither within or adjacent to the myometrium and include myomas of the cervix, the round or broad ligaments and myomas that rarely occur distant from the uterus.

22.4 Clinical Impact

Although at least 50% of uterine leiomyomas are asymptomatic, many women have significant symptoms that impact their quality of life and warrant treatment. The major clinical manifestations of uterine leiomyoma can be roughly classified into three categories: increased uterine bleeding, pelvic pressure or pain, and reproductive dysfunction.

22.4.1 Abnormal Uterine Bleeding

Abnormal uterine bleeding is the most common symptom reported by women with leiomyomas. The most common types of leiomyomas associated with heavy bleeding are intramural or submucosal myomas. The typical bleeding patterns are heavy menstrual regular bleeding or intermenstrual bleeding. Intermenstrual bleeding can indicate the presence of an intracavitary myoma or specific endometrial pathology; therefore, a more detailed evaluation of the uterine cavity is warranted in these cases. The terms used to describe the abnormal bleeding are reviewed in the chapter on AUB and adhere to the FIGO classification. Heavy vaginal bleeding can lead to problems such as iron-deficiency anemia, which can be severe enough to require blood transfusions, and frequent changes of sanitary protection can cause significant distress in work or social situations.

22.4.2 Chronic Pelvic Pain

Pelvic pain or pressure is the second most common complaint and is frequently described as analogous to the discomfort associated with uterine growth during pregnancy. The pain can occur both during and between bleeding episodes. Posterior leiomyomas may give rise to low back pain, whereas anterior leiomyomas may compress the bladder. Leiomyomas that become large enough to fill the pelvis may potentially interfere with voiding and defecation or cause dyspareunia. Very large leiomyomas can, on occasion, outgrow their blood supply, leading to tissue ischemia and necrosis clinically manifested as acute, severe pelvic pain. Pedunculated leiomyomas can suffer torsion, which can lead to ischemia and acute pain. During pregnancy, leiomyomas have been known to undergo "red degeneration," where hemorrhage occurs within the myoma, leading to acute pain.

22.4.3 Reproductive Function

Uterine leiomyomas are believed to influence reproduction in several ways; however, their direct effect on fertility is still a subject of much debate. The incidence of infertility and uterine leiomyomas increase with advancing maternal age, and no specific data exist to ascertain if the proportion of infertile women with leiomyomas is greater than the proportion of fertile women with leiomyomas.

Yet the indirect evidence is substantial. In one review, pregnancy rates among women with leiomyomas distorting and not distorting the uterine cavity were 9 and 35%, respectively, as compared to 40% among controls with no leiomyomas [5]. Furthermore, the multiple reports of successful pregnancies among infertile women after myomectomy strongly suggest a connection [6, 7].

Though exact physiologic mechanisms for reproductive dysfunction are unclear, many plausible theories exist. There is a potential for reduced fecundity if a myoma occurs in the cornual region of the uterus due to mechanical occlusion of a fallopian tube. It is possible that large leiomyomas may impair the rhythmic uterine contractions that facilitate sperm motility [8]. It has further been documented

that endometrial histology varies in relation to the location of the leiomyoma. Submucosal leiomyomas may be associated with localized endometrial atrophy as well as alterations in the vascular blood flow, which may impede the implantation of an embryo, the delivery of hormones or growth factors involved in implantation, or interfere with the normal immune response to pregnancy [9–11]. Submucosal leiomyomas, which distort the uterine cavity, are associated with first trimester pregnancy loss, preterm delivery, abnormal presentation in labor, and postpartum hemorrhage [12].

In regard to the effectiveness of assisted reproductive technology, submucosal and intramural leiomyomas are generally thought to reduce the effectiveness of assisted reproductive procedures. Early evidence demonstrated that both pregnancy and implantation rates were significantly lower in patients with intramural or submucosal leiomyomas [13, 14]. In one study, the presence of an intramural leiomyoma decreased the chances of an ongoing pregnancy by 50% following in vitro fertilization [15]. Evidence suggests that patients with subserosal leiomyomas have assisted reproductive technology outcomes consistent with patients without leiomyomas [14, 16, 17].

22.5 Epidemiology of Uterine Leiomyomas

The diagnosis of uterine leiomyomas increases with age throughout the reproductive years, with the highest prevalence occurring in the fifth decade of a woman's life. African-American women have a two to threefold greater relative risk of leiomyomas compared to Caucasian women and tend to be diagnosed at an earlier age and have more severe disease (larger leiomyomas and greater incidence of anemia) as compared to Caucasian women [18, 19].

Nulliparous women have higher rates of leiomyomas than multiparous women, and the risk of developing leiomyomas decreases consistently with each subsequent term birth [20]. Early age at menarche is associated with a two to threefold increased risk of developing leiomyomas [21].

Leiomyomas clearly demonstrate their hormonal responsiveness in the fact that they form after puberty, have the potential to enlarge during pregnancy, and regress after menopause. However, studies of exogenous hormone treatments, including oral contraceptives and hormone replacement therapy, reveal conflicting data, and no clear association can be inferred [22].

Twin and family studies suggest a familial predisposition to developing leiomyomas, though further research in genetics of leiomyomas has yet to be done [22]. These studies are hampered by the extremely high incidence of leiomyoma formation in the general population.

According to some studies, an increase in body mass index has been found to increase the risk for uterine leiomyomas by a factor of 2–3, and the evidence suggests that it is adult-onset obesity rather than excessive weight in childhood that infers this risk. However, other studies have not observed similar associations with increased BMI [21].

The majority of epidemiologic studies find that cigarette smokers are at a 20–50% reduced risk for the development of uterine leiomyomas perhaps via a reduction in estradiol levels and that the inverse association was independent of BMI. It is unclear whether this relationship varies as a function of pack years. No clear relationship has been shown between leiomyomas and specific dietary factors or physical activity [21].

22.6 Pathology and Pathophysiology

22.6.1 Genetics

Leiomyomas are defined as monoclonal proliferations of benign smooth muscle [23]. Each monoclonal myoma may be associated with various chromosomal translocations, duplications, and deletions [24]. Many, but not all, myomas contain nonrandom cytogenetic abnormalities, while the myometrium has a normal karyotype. Most of the mutations occur in genes that are involved in cellular growth or are responsible for architectural transcription.

Two hereditary disorders have been reported in which uterine leiomyomas are part of a syndrome complex that demonstrate the potential genetic contribution to myoma formation. The first is hereditary leiomyomatosis and renal cell cancer complex. This is an autosomal dominant syndrome with smooth muscle tumors of the uterus, skin, and kidney. The second is a syndrome of pulmonary leiomyomatosis and lymphangiomyomatosis (LAM) that is the result of mutations in one of the two genes responsible for tuberous sclerosis, a syndrome that results in multiple hamartomas.

22.6.2 Pathology

Grossly, myomas usually appear as discrete, round masses that are lighter in color than the surrounding myometrium, with a glistening, pearly white appearance. Histological features include smooth muscle fibers that form interlacing bundles, with excessive fibrous tissue in between the bundles.

22.6.3 Endocrinology

The influence of steroidal hormones is central to the theory of clonal expansion of leiomyomas. Myomas are responsive to estrogen and progesterone and are therefore more likely to increase in size and cause associated symptoms in women of reproductive age. Serum concentrations of circulating estrogen or progesterone have not been found to be increased.

Tumor initiators and yet undetermined genetic factors are involved in key somatic mutations that facilitate the progression of a normal myocyte into a leiomyocyte responsive to estrogen and progesterone. Estrogen receptor (ER), progesterone receptor (PR), and epidermal growth factor receptor (EGFR) are integral in the development of myoma [25]. Studies have shown that, in comparison with the normal myometrium, myomas have an increased concentration of ER and PR [26, 27].

Aromatase p450 is overexpressed by leiomyomas [28, 29]. Therefore, in addition to circulating estrogen acting on the ER, the local conversion of circulating androgens to estrogens may be important in potentiating the actions of estrogen in the leiomyocyte (Fig. 22.2) [30].

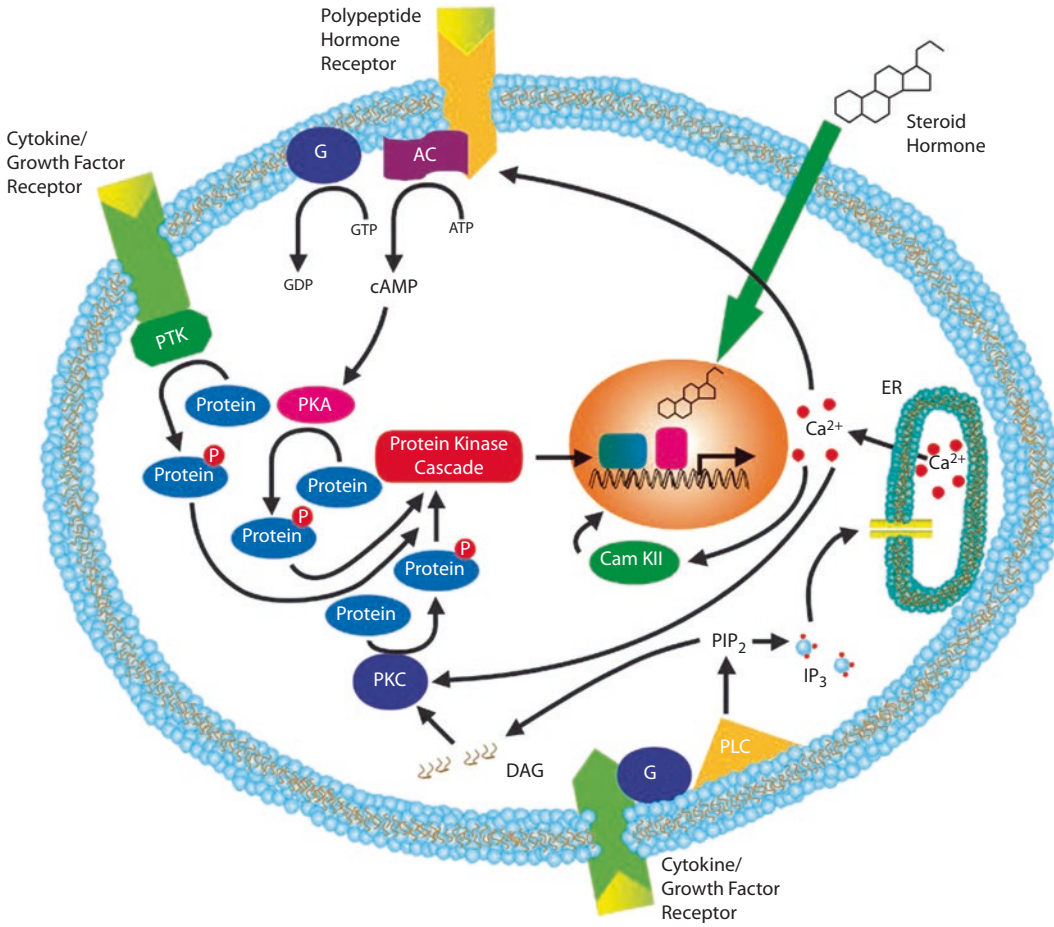

Fig. 22.2 Sex steroid hormone action. Estrogen and progesterone exert action through binding of specific receptors, which then bind to DNA at specific response elements. Binding of estrogen and progesterone at a variety of genes has different effects in various cells. Figure provided to the public via the Internet by Fisher Scientific, Inc

Traditionally, estrogen was thought to be the primary hormonal mediator of myoma growth. Although progestins have been applied for the treatment of bleeding from symptomatic myomas, recent studies have shown that progesterone may play a much greater role as a mediator of myoma growth than previously thought [31]. The antiprogestin RU486 (mifepristone) has been shown to decrease the size of myomas [32, 33], and another study showed that myomas in the secretory phase have increased mitotic counts compared to those in the proliferative phase [34].

Growth of neoplastic tumors is the result of accelerated cellular proliferation that outpaces the inhibitory effect of apoptosis. Apoptosis has been shown to be inhibited in uterine leiomyomas. Progesterone has been shown to increase the anti-apoptotic protein, bcl-2 [35]. Therefore, the stimulation of myoma expansion may be a function of the suppression of apoptosis by progesterone. It has been observed in vitro that the addition of progesterone to cultured leiomyoma cells increased the expression of bcl-2 when compared to controls [35]. Normal myometrium did not express increased levels of bcl-2 in the presence of progesterone.

The complex process of apoptosis involves not only the bcl-2 family but also Fas/FasL and Rb-1 [36]. Martel et al. have described the various apoptotic pathways deficient in leiomyomas and potential corresponding targets for therapy of myomas. The role of apoptosis in the pathogenesis of myomas is a promising area for future research with a great potential for clinical application.

The synergistic interplay between estrogen and progesterone signaling in the pathophysiology of myoma growth has been observed as well. The increase in progesterone receptors as a result of increased estrogen has been well established. An in vitro study showed that progesterone upregulates the expression of EGF, and estrogen also increases the expression of EGFR [25].

22.6.4 Pregnancy and Leiomyomas

The influence of the pregnancy-induced endocrine milieu on a leiomyoma is complex. There are many reports of dramatic leiomyoma growth in pregnancy; however, all prospective studies have shown that most leiomyomas demonstrate no change in diameter from the first trimester to delivery. Essentially, it is impossible to predict which myoma will grow. The main potential complications in pregnancy are pain and pregnancy wastage. Pain can be the result of myomatous degeneration. This phenomenon can be the consequence of necrosis from decreased blood supply to a subserosal or pedunculated myoma. Usually there is localized pain and a cystic or heterogeneous pattern on ultrasound.

Pregnancy wastage is often due to retroplacental myomas that may cause abruptio placenta, bleeding, and premature rupture of membranes. Lower uterine segment myomas may increase the probability of Cesarean section due to obstruction or malpresentation.

22.7 Diagnostic Imaging and Leiomyomas

Imaging has become an integral aspect of the evaluation of leiomyomas. Myoma size and location can be assessed to varying degrees, depending on the imaging technology applied to the evaluation process. Ultrasonography, hysterosalpingography, and magnetic resonance imaging (MRI) are currently the modalities most commonly utilized to image myomas.

22.7.1 Ultrasound

Traditional ultrasound is a cost-effective technology for assessing uterine leiomyomas. The transvaginal approach is more accurate than abdominal ultrasound. However, abdominal ultrasound may be a useful adjunct to transvaginal ultrasound, if a large uterine size warrants such an approach [22]. The presence of myomas may be detected by ultrasound as evidenced by uterine enlargement or a nodular contour of the uterus. They may also appear as discrete, focal masses within the myometrium [37, 38]. Myomas can appear hypoechoic or heterogeneous when compared with the appearance of the myometrium on ultrasound, and they may be characterized by calcification and posterior shadowing [37, 39]. Sagittal and axial views aid in providing information on the location and size of myomas.

Additional information regarding intracavitary masses, such as submucous myomas, may be obtained by means of saline infusion sonohysterography. This imaging technique consists of

real-time transvaginal ultrasound during which sterile saline is injected into the uterine cavity. The saline is injected transcervically via a catheter of small caliber. As the uterine cavity is distended by the saline, intracavitary masses may be visualized as echogenic structures against the echolucent background of the distending media [40]. Intramural myomas within close proximity of the endometrial cavity may also be assessed by sonohysterography. In addition, entities such as endometrial polyps and uterine anomalies such as adhesions may also be detected. Sonohysterography can be used not only to diagnose submucous myomas but also to assess the potential access to surgical intervention [41].

Three-dimensional ultrasound [42] and color Doppler ultrasound [43] are increasingly being applied to the evaluation of myomas for imaging. Color Doppler ultrasound highlights vascular flow, which is usually increased at the periphery of myomas and decreased centrally [42, 43].

22.7.2 Hysterosalpingography

Hysterosalpingography is a screening test for intracavitary anatomic defects and entails injection of iodine contrast dye transcervically, via a catheter, into the uterine cavity with radiologic assessment under fluoroscopy. Hysterosalpingography is performed in the follicular phase of the menstrual cycle in order to avoid interfering with ovulation and/or a potential pregnancy. Since the hysterosalpingography instillation medium contains iodine, an iodine-allergic patient would require premedication with glucocorticoids and antihistamines prior to the procedure [44].

Hysterosalpingography allows visualization of submucous myomas, as the uterine cavity is distended by the contrast medium. The size and contour of the uterus may be altered by submucous myomas. Intramural myomas may enlarge the uterine cavity in a globular manner, and fundal myomas may enlarge the space between the cornua. Subserosal myomas are not typically noted on hysterosalpingography; however, if large enough, they may be detected as a mass effect on the uterine cavity [37]. In cases where a submucous myoma must be differentiated from an endometrial polyp on hysterosalpingography, hysteroscopy and sonohysterography play roles as complementary, potentially confirmatory adjuncts.

22.7.3 Magnetic Resonance Imaging

MRI is increasingly being utilized for imaging leiomyomas. The location of myomas can be accurately documented, more so with MRI than with ultrasound. It is often used to evaluate the precise location for surgical planning or prior to uterine artery embolization mapping.

Disadvantages of MRI include cost, limited availability, and an inability to perform the procedure in patients with morbid obesity or claustrophobia. Traditionally, cost had been more of a disadvantage; however, as the expense of MRI decreases, it is more commonly employed in clinical and presurgical evaluation. MRI is contraindicated in patients with pacemakers, defibrillators, metallic foreign bodies, and in rare cases of allergy to gadolinium [45].

In T2-weighted images, the endometrial stripe is visualized as a central, high signal; the junctional zone is a low signal; and the myometrial areas are an intermediate signal [46]. Leiomyomas are represented by variable signal density. Most of the time, they appear as hypodense, well-demarcated masses; however, increased cellularity [47] and degeneration may be seen as high signal intensity [46].

There is less distinction of the endometrial lining, junctional zone, and myometrium in T1-weighted images. These components are usually homogeneous and, consequently, obscured in appearance. Fatty or hemorrhagic degeneration may be represented by a high signal intensity [48].

22.8 Treatment of Leiomyomas

Treatment of leiomyomas has traditionally been interventional. In patients with a menstrual abnormality, the birth control pill has been used successfully, and the presence of a leiomyoma is not considered a contraindication. If the oral contraceptive fails, typically surgery is offered. However, new medical therapy may potentially change this approach.

The important concept in the management of leiomyomas is that intervention is not required in asymptomatic women. No longer acceptable reasons for surgical intervention include previous suggested indications, such as nonpalpable adnexa and preemptive intervention for asymptomatic fibroids in order to circumvent a potentially more difficult surgery in the future. Rapid growth of a

leiomyoma traditionally was considered a potential sign of malignancy. However, this sign in isolation of other manifestations is not considered prognostic of a leiomyosarcoma.

Surgery is indicated with a history of pregnancy complications. Surgical treatment specifically for infertility is indicated if there is distortion of the uterine cavity. Surgical removal of uterine fibroids is sometimes considered in patients with longstanding infertility and when no other identifiable cause is found, although the latter indication is strongly debated.

22.9 Medical Treatment of Leiomyomas

Medical treatment of leiomyomas is indicated for the treatment of pain or menstrual dysfunction. Medical therapy has not been investigated for the management of infertility or pregnancy-related complications.

22.9.1 Gonadotropin-Releasing Hormone Agonists

Gonadotropin-releasing hormone (GnRH) agonists are an effective means of medically treating patients with symptomatic leiomyomas. After affecting an initial flare of LH and FSH, GnRH agonists down regulate the hypothalamic-pituitary-ovarian axis via action on pituitary receptors. The flare effect is due to an initial stimulation of FSH and LH owing to the binding of pituitary receptors, after which these receptors are desensitized, with a subsequent decrease in FSH and LH secretion [49]. This results in decreased estrogen production.

GnRH agonists have been shown to directly inhibit local aromatase p450 expression in leiomyoma cells [50], thereby presumably resulting in decreased local conversion of circulating androgens to estrogens within the leiomyocyte. Several studies have concluded that GnRH agonists can directly induce apoptosis and also suppress the cellular proliferation of myomas presumably via action on peripheral GnRH receptors.

Maximum reduction of the mean uterine volume occurs within 3 months of GnRH agonist administration. The decrease in volume is usually in the range of 40–80%. However, after the discontinuation of GnRH agonists, myomas will rapidly grow back to their pretreatment size, usually in the span of several months [51].

Advantages of GnRH agonists include their use in the perimenopausal transition with add-back therapy for the goal of avoiding hysterectomy. Additionally, laparoscopic myomectomy may be made more feasible with GnRH-agonist pretreatment, and GnRH agonists can also be beneficial in a patient who is to undergo hysterectomy to facilitate a vaginal approach rather than an abdominal incision. In a randomized clinical trial comparing the study group (patients receiving GnRH agonist and iron) to a control group (iron alone), preoperative hematologic parameters were improved [52].

Although decreased tumor bulk and a decrease in associated symptoms are attained, the potential for unwanted long-term side effects exists; therefore, treatment with GnRH agonist is recommended for no more than 6 months. Common side effects include hot flashes, vaginal dryness, headache, and mood swings. Most importantly, in terms of bone health status, there is a recognized decrease in bone mineral density during therapy [53]. Although add-back doses of steroidal hormones can be used with the aim of decreasing this bone loss; the long-term use of GnRH agonists with add-back is impractical and not recommended, especially in younger patients.

22.9.2 Selective Estrogen Receptor Modulators

Selective estrogen receptor modulators (SERMs) are compounds that bind to the ER and confer an agonist or antagonist effect, depending on tissue specificity. They have been applied to the treatment and prevention of estrogen-responsive breast cancer; examples include the use of tamoxifen and raloxifene. Tamoxifen, a triphenylethylene, has antagonist activity in the breast and displays a desirable agonist activity in the bone and the cardiovascular system as well as a mild agonist activity in endometrial tissue [54, 55]. Raloxifene, a benzothiophene, has a similar profile and the added benefit of not acting as an agonist in the endometrium [56].

In animal models, SERMs have been shown to be effective in decreasing the growth of myomas. Eker rats are a rat strain with a tuberous sclerosis 2

(TS-2) gene defect that can spontaneously develop leiomyomas. In studies, the administration of SERMs was associated with the inhibition of leiomyoma formation in Eker rats [57, 58]. Guinea pigs require long-term exposure to estrogen in order for leiomyoma formation to occur. Two groups of oophorectomized guinea pigs, an estrogen-only group and an estrogen plus raloxifene group, were compared for myoma formation. A decrease in the size of the induced myomas was observed in the estrogen plus raloxifene group [59].

In humans, raloxifene appears effective in decreasing the size of myomas in postmenopausal women [60], but beneficial effects were not significant in premenopausal women [61]. A recent study has demonstrated that a combination of raloxifene and GnRH agonist is more effective in reducing leiomyoma volume [62] and preventing a decrease in bone mineral density [63] than the use of GnRHa alone. A Cochrane review of three studies showed no consistent evidence from a limited number of studies that SERMs reduce fibroid size or improve clinical outcomes and suggested that further studies be conducted to establish benefit [64].

22.9.3 Selective Progesterone Receptor Modulators

This class of compounds has either agonist or antagonist effects on the progesterone receptor, based on tissue specificity [65]. Asoprisnil is a selective progesterone receptor modulators (SPRM) that, along with its major metabolite, J912, has high affinity for the PR, moderately binds growth hormone receptor, and has a very low binding potential for androgen receptors (Fig. 22.3). Asoprisnil has virtually no affinity for ER or mineralocorticoid receptors [66]. It differs from the long-term effect of progesterone on the endometrium in that amenorrhea is rapidly established without breakthrough bleeding [67].

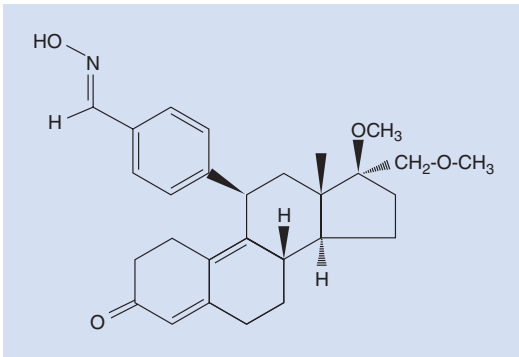

Fig. 22.3 Chemical structure of asoprisnil

The oral SPRM ulipristal acetate has successfully completed phase III clinical trials and is approved for use in the European Union. It has not received FDA approval for use in the USA [68]. A randomized trial of ulipristal acetate vs. placebo for fibroid treatment before surgery has shown that treatment for 13 weeks effectively controlled excessive bleeding and reduced the size of fibroids [69]. Further clinical research is underway to study long-term effects of SPRMs on the endometrium. As SPRMs are studied and tested further in clinical trials, there may be a practical use for this class of medications in the long-term medical treatment of leiomyomas, especially in women with menorrhagia who are interested in avoiding surgery or maintaining future fertility.

22.9.4 Aromatase Inhibitors

Aromatase inhibitors (AI) were originally used for the treatment of breast cancer, and this class of medications is FDA approved for this purpose. In recent years, the use of AI has expanded within the field of reproductive medicine, and its application in the potential treatment of uterine fibroids has been investigated in several studies. The rationale behind AI is due to the finding that aromatase p450 enzyme concentrations are elevated at the local level in leiomyoma tissues [28, 29]. Interestingly, aromatase expression has been shown to be highest among African-American women (83-fold) when compared to that of Caucasian (38-fold) and Japanese women (33-fold) [70]; this higher expression may be one underlying mechanism that may explain the higher prevalence of uterine fibroids among African-American women when compared to women of other ethnic backgrounds.

In a prospective study by Gurates et al., a 3-month course of the AI letrozole at 5 mg per day was found to significantly decrease uterine and leiomyoma volume without changes in lumbar spine BMD or biochemical markers of bone metabolism; in addition, heavy menstrual bleeding associated with leiomyoma was improved [71]. Furthermore, an RCT that compared the effects of AI treatment with GnRHa on myoma

volume and hormonal status in premenopausal women with leiomyomas showed promise as well. Treatment duration for both groups was 12 weeks. Leiomyoma volume was significantly reduced in the AI and GnRHa groups without a difference between groups. The AI group did not have a significant change in hormonal milieu from baseline in contrast to the GnRHa group. The authors concluded that uterine leiomyomata may be successfully managed by the use of AI, which may be most useful in the setting of pretreatment for surgery. Advantages of AI over GnRHa may include rapid onset of action as well as the avoidance of the GnRHa flare [72].

22.10 Surgical Therapy of Leiomyomas

Surgical treatment is the mainstream therapy for leiomyomas. Hysterectomy represents the only definitive curative therapy; however, myomectomy, endometrial ablation, and high-intensity focused ultrasound are increasing in frequency as alternative therapeutic procedures. Indications for surgical intervention include failure to respond to medical treatment, worsening vaginal bleeding, suspicion of malignancy, or treatment of recurrent pregnancy loss. In postmenopausal women with an enlarging pelvic mass and abnormal bleeding, surgery should be strongly considered. In this population, the incidence of a leiomyosarcoma is still very uncommon but higher than the incidence found in the premenopausal population. The incidence of leiomyosarcoma in patients undergoing surgery for uterine leiomyomas is extremely rare (1 case per 2000 procedures) with more than 80% of these patients being menopausal. [73]

22.10.1 Hysterectomy

Hysterectomy has been described as the definitive management for symptomatic leiomyomata. When employed, it is highly effective for the carefully selected patient with symptomatic leiomyomata who does not desire future fertility. Among hysterectomies performed abdominally vs. vaginally for uterine leiomyomas, the abdominal route has been chosen approximately 75% of the time based on data from the late 1980s and early 1990s [74].

Vaginal hysterectomy is associated with a lower complication rate and decreased need for blood transfusion. Another advantage of vaginal hysterectomy is the association with shorter operating times [75]. Myoma size and location, total uterine size, and the surgeon's skill are factors that may determine the feasibility of vaginal hysterectomy.

Laparoscopy-assisted vaginal hysterectomy, total laparoscopic hysterectomy, and laparoscopic supracervical hysterectomy are minimally invasive surgical methods that are associated with decreased postoperative pain and recovery time in comparison to vaginal and abdominal hysterectomy [76]. These surgical modalities may incur increased hospital costs in terms of equipment and operating room time, but they have the recognized advantages listed above. In addition, these options provide the ability to assess the pelvis if the patient also complains of pelvic pain.

22.10.2 Myomectomy

Hysterectomy has long been considered the definitive treatment for symptomatic uterine leiomyomas. Yet as more and more women delay childbearing, the incidence of uterine leiomyomas among patients suffering with infertility increases, and hysterectomy becomes an unacceptable management option. Therefore, abdominal, laparoscopic, and hysteroscopic myomectomies have become increasingly common treatment modalities for women with leiomyomas and infertility.

Myomectomy is the surgery of choice for treating women with symptomatic myomas in those who desire to preserve fertility or to otherwise keep their uteri. It is most useful for subserosal, especially pedunculated subserosal, myomas as well as intramural leiomyomas. Myomectomy entails the surgical removal of myomas by enucleation. It is preferable to use as few incisions as possible to remove myomas from the uterus, in order to minimize adhesion formation as well as to minimize any compromise of the myometrial integrity. The surgeon must be diligent regarding the orientation of the uterus, especially during the repair, in order to preserve the integrity of the endometrial cavity. Several techniques have been described to decrease blood loss with an abdominal myomectomy and include the use of tourniquets around

the lower segment of the uterus to occlude the uterine arteries, the use of dilute vasopressin and misoprostol preoperatively [77].

Methods employed to minimize postoperative adhesion formation include the use of permanent or absorbable barriers and good surgical technique minimizing trauma to the tissues, use of nonreactive suture material, and the avoidance of tissue desiccation or aggressive cautery. Due to the potential risk of uterine rupture, a trial of labor after myomectomy is not recommended by the American College of Obstetricians and Gynecologists (ACOG) [78].

22.10.3 Laparoscopic Myomectomy

Laparoscopic myomectomy offers many advantages for this minimally invasive surgery technique to remove myomas. This procedure, however, requires appropriate training and advanced endoscopic skills from the surgeon. It is most useful in cases in which myomas are easily visualized and readily accessible.

Several studies have shown advantages of the laparoscopic approach to myomectomy. Mars et al. randomized 20 patients undergoing myomectomy to laparoscopy and 20 patients to laparotomy. The laparoscopy group had lower postoperative pain as well as a greater number of patients that were analgesia-free on postoperative day 2, discharged home by postoperative day 3, and fully recovered on postoperative day 15 [79]. Among a group of 131 patients randomly assigned to myomectomy via laparoscopy or laparotomy, Seracchioli et al. found that laparoscopic myomectomy is associated with lower intraoperative blood loss and shorter postoperative length of stay in the hospital. Moreover, no significant difference in subsequent fecundability, spontaneous miscarriage rate, preterm delivery rate, and Cesarean section rate was found between the groups. A lower incidence of postoperative febrile morbidity was yet another advantage found in this study [73].

Adhesion formation was evaluated in a retrospective study of 28 patients who underwent laparoscopy or laparotomy for myomectomy followed by a second-look laparoscopy [80]. A lower incidence of adhesion formation was noted in patients that initially underwent a laparoscopic approach to their myomectomy. Similar observations were made in two other studies [81, 82].

Disadvantages of laparoscopic myomectomy include the lack of opportunity to palpate the uterus intraoperatively, resulting in the potential for a higher rate of subsequent recurrence of myomas [83, 84], which is supported by several studies and refuted by others [85]. An additional disadvantage is operator dependent and involves the technical difficulty in repairing the enucleated leiomyomas, with a potential risk for uterine rupture during future pregnancies if improperly closed.

22.10.4 Robotic-Assisted Myomectomy

The advantages of robotic-assisted myomectomy (RAM) compared with conventional laparoscopic myomectomy include the potential for greater technical feasibility of removing uterine fibroids via a minimally invasive surgical approach [86] due to three-dimensional vision, ergonomic considerations, seven degrees of freedom in terms of wrist movement with distal aspect of the robotic arms, and no fulcrum effect [87]. RAM has been associated with decreased intraoperative blood loss as well as a shorter length of hospital stay as compared with laparoscopic as well as abdominal myomectomy [87]. A large, retrospective, multicenter study showed that women who underwent RAM by experienced robotic surgeons had similar pregnancy outcomes as those who underwent laparoscopic myomectomy; in addition, 11% were found to have pelvic adhesions at subsequent Cesarean section [88].

22.11 Technical Considerations

The surgical technique has been described in detail [89]. Laparoscopic myomectomy and RAM are usually performed with the standard three to four ports. Dilute vasopressin, although not approved by the FDA for this indication, is diluted 20 units in 100 mL of normal saline and injected into the myoma. An incision is then made into the myoma and enucleated bluntly (Fig. 22.4). After enucleation, the defect (Fig. 22.5) is closed in

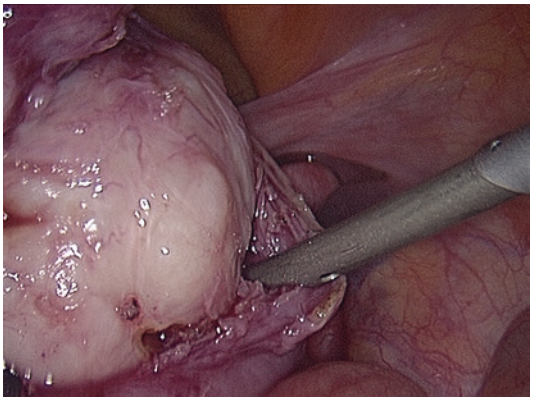

Fig. 22.4 Dissection of the myoma bed is usually accomplished bluntly, as in open cases. Picture provided by Dr. T. Falcone

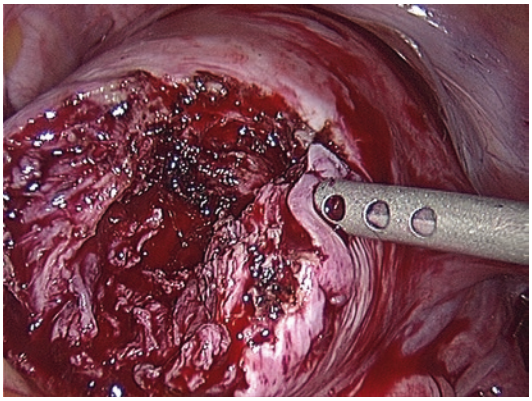

Fig. 22.5 A large myometrial defect is left behind. This requires closure with two to three layers of delayed absorbable sutures. Picture provided by Dr. T. Falcone

two to three layers, typically a deep layer and then a seromuscular layer. Intra and extracorporeal knot tying are acceptable. This step is the most critical part of a laparoscopic myomectomy, and the defect should be closed tightly (Fig. 22.6).

The last step of a minimally invasive myomectomy or hysterectomy is tissue extraction. The issue of when and how to extract tissue by morcellation is very controversial. The use of the uncontained electro-mechanical morcellator has been banned in many institutions. In some institutions it has been completely banned leaving contained or uncontained scalpel morcellation as the only option. Both the American College of Obstetricians and Gynecologists (ACOG) and the AAGL support its judicious use.

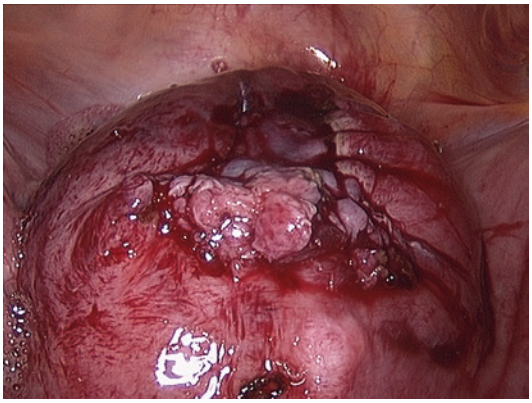

Fig. 22.6 The myometrial defect is tightly closed. Picture provided by Dr. T. Falcone

22.12 Uterine Artery/Leiomyoma Embolization

Uterine artery embolization (UAE), also referred to as uterine fibroid embolization (UFE), is a minimally invasive alternative to hysterectomy and myomectomy that has been applied to the treatment of symptomatic leiomyomas. UAE was initially developed for the control of pelvic hemorrhage and has been utilized for the primary treatment of leiomyomas since 1995 [90]. It has been associated with positive clinical outcomes and a high rate of patient satisfaction. Success rates of over 90% have been reported [91, 92]. Decreases in the size of the uterus and dominant leiomyomas of 45% have been reported [39]. The ACOG has found that UAE is safe and effective [93].

Pre-embolization imaging with MRI aids in precisely locating the position of leiomyomas prior to the procedure. Embolization of the uterine arteries with polyvinyl alcohol or tris-acryl gelatin microspheres involves femoral artery catheterization under fluoroscopic guidance. Typically, uterine fibroid embolization is performed with the patient under conscious sedation [45].

Post-embolization follow-up consists of clinical evaluation as well as a follow-up MRI in order to assess and monitor the final resulting volume of leiomyomas and the uterus. The degeneration and devascularization of leiomyomas can be visualized on MRI as increased signals on T1-weighted and decreased signals on T2-weighted images [94–96]. After uterine fibroid embolization, the

size of leiomyomas can continue to decrease for 1 year or more. The high rate of patient satisfaction [91] is due to the improvements in menorrhagia and pressure symptoms as well as pain relief experienced by a high percentage of women undergoing uterine fibroid embolization.

A systematic review and meta-analysis of 54 studies with a total of over 8000 patient examined the complication rates and effectiveness of UAE in the treatment of uterine fibroids. There were no reported deaths, and rate of major complications was 2.9%. During a 0.25–2-year follow-up, clinical symptomatic improvement ranged from 78 to 90% [97]. A Cochrane review of six RCTs that totaled 732 women found moderately good evidence that patient satisfaction rates at 2 and 5 years were similar between UAE and either hysterectomy or myomectomy. UAE was also found to be associated with a shorter length of hospital stay and a quicker return to daily activities. However, UAE is associated with high rates of minor complications and surgical reintervention [98].

It is important to note that successful pregnancies have occurred following this procedure [99, 100]. There is a recognized risk of inducing premature ovarian failure in older women. Several studies have shown an increased risk of intrauterine growth restriction and placentation problems in pregnancies following uterine fibroid embolization. A systematic review showed that after UAE, there were higher rates of miscarriage, Cesarean section, and postpartum hemorrhage as compared with controls [101]. There are currently no studies that establish a successful pregnancy rate after UAE [102]. Although there are confounding variables in the existing studies and a Cochrane review showed very low evidence suggesting better fertility outcomes with myomectomy over UAE [99], short-term reproductive outcomes favor myomectomy over UAE [103]; for women seeking pregnancy, myomectomy is favored over UAE. Complications of uterine fibroid embolization include angiography-related problems [45], allergic reactions [104], perforation of the uterus [105], and infection [96, 104]. If the collateral blood supply to the ovary is embolized, then infertility, amenorrhea, and premature menopause are potential risks [92, 106]. Sciatic nerve injury leading to buttock claudication is a recognized potential complication of uterine fibroid embolization [105]. Deep venous thrombosis and pulmonary embolus are rare risks, as is death [105, 107, 108], which has a reported 3/10,000 rate with uterine fibroid embolization compared with the 1/1000 rate from hysterectomy [109].

Post-embolization syndrome is relatively common occurrence and consists of nausea, vomiting, pain, and a temporary increased WBC count following the procedure. This syndrome affects most patients to some extent in the first 48 h after the procedure but is severe in approximately 15% of those who undergo uterine fibroid embolization [110].

22.13 MRI-Guided Focused Ultrasound Therapy

Over the last decade, high-intensity focused ultrasound surgery (HIFU), with either MRI or US for guidance, has been used for treating symptomatic uterine fibroids in a noninvasive manner. The therapeutic HIFU component focuses thermal energy in order to ablate leiomyoma tissue in a precise and controlled fashion. By placing an ultrasound transducer on the abdomen of a patient and focusing the ultrasound energy at a specific, controllable depth, and position, leiomyoma tissue is destroyed within the focal zone. The therapeutic ultrasound effect is often monitored by MRI, which precisely records the temperature elevation from the heat generated over time. Once the temperature reaches 57°C for 1 s, tissue is rapidly destroyed within the focal zone. Tissue within 2–3 mm of the focal zone is unaffected owing to the very precise demarcation between normal and destroyed tissue.

MRI-guided focused ultrasound surgery (MRgFUS or MRgHIFU) is considered superior to US guidance due to increased soft tissue resolution as well as capability of tissue temperature mapping; in addition, ultrasound-guided HIFU is not FDA-approved, whereas MRgFUS as a therapy for leiomyomata received FDA approval in October 2004 [111]. The ExAblate System (ExAblate 2000, Exablate 2010; InSightex, Haifa, Israel) is the first medical device approved for the treatment of leiomyomas as its primary indication. General patient selection criteria include leiomyomas between 4 and 10 cm, maximum depth of subcutaneous tissue to the leiomyoma <12 cm, completion of childbearing, premenopausal status, and leiomyomas that can be clearly visualized on MRI. Based on results of a 6-month

follow-up study, the mean leiomyoma reduction in volume after HIFU was 13.5 cm^3, and the mean volume of nonperfused tissue was 51.2 cm^3. Furthermore, 79.3% of patients reported a greater than 10-point reduction in symptom scores and improvement in quality-of-life measures on the questionnaire used in the study [112]. Adverse events included minor skin burns in 4% of patients, worsening menorrhagia in 4% of patients, hospitalization for nausea in only 1% of patients, and nontargeted sonication of the uterine serosa in 1% of patients [112].

Two other studies have assessed longer-term clinical outcomes. In a 24-month follow-up study, improvement in symptoms and moderate volume reductions in types of uterine fibroids with lower MRI image intensity than the myometrium were observed after MRgFUS [113]. In a 12-month study of patients who underwent MRgFUS, relief of symptoms was reported in 86%, 93%, and 88% of patients at 3-, 6-, and 12-month follow-up, respectively [114]. Reintervention rates are low and comparable to those with UAE [114]. There are no randomized clinical trials that have compared this technology with surgical or other radiologic treatment.

The effect that MRgFUS may have on fertility is not clear; however, there have been reports of successful pregnancies after MRgFUS. A published series of all pregnancies after MRgFUS reported to the manufacturer and the FDA, as part of post-approval device monitoring, showed that normal pregnancy outcomes and deliveries are possible. The series reported a 41% live birth rate, 20% ongoing pregnancy rate, 11% rate of elective termination, and 28% spontaneous abortion rate after MRgFUS for the treatment of uterine fibroids [115]. Furthermore, in a retrospective study, seven women who unintentionally became pregnant after ultrasound-guided HIFU continued their pregnancies without complications [116].

22.14 Cryomyolysis

Cryomyolysis of uterine leiomyomas has been performed by laparoscopy, and in recent years, MRI-guided cryomyolysis has been devised as a less invasive approach. Cryomyolysis involves placement of a 2-cm diameter cryoprobe, directly into a uterine leiomyoma. After the cryoprobes are advanced and placed into position, the probes are then cooled by liquid nitrogen instillation or by differential gas exchange, reducing the local temperature within the leiomyoma to less than −90°C, resulting in a 3.5–5-cm ice ball, ultimately resulting in tissue necrosis. Because the temperature at the edge of the ice ball is 0°C and not destructive to surrounding tissue, the imaging of the ice ball can predict the limits of targeted tissue [117]. Laparoscopic cryomyolysis for women with leiomyomas as well as a combination of abnormal uterine bleeding, pelvic pain/pressure, and/or urinary frequency was shown to be effective in a study of 20 patients [118]. MRI-guided cryomyolysis was devised as a less invasive and more precise approach. MRI can be employed to accurately visualize ice-ball formation, which eventually encompasses the leiomyomas appearing black due to the slow or absent hydrogen ion spins of water molecules in the ice.

A report of MRI-guided cryomyolysis used to treat leiomyomas in ten patients showed MRI evidence of marked uterine volume reduction between 48 and 334 days after the procedure. The mean volume reduction was 65%. All patients reported improvement of symptoms, whether they were due to bleeding and/or pressure symptoms. One patient had uterine bleeding for 2 months post-procedure, with subsequent spontaneous resolution. Another patient had a residual submucosal leiomyoma that had to be resected hysteroscopically at a later date. Complications included a patient with laceration of a serosal vessel covering a leiomyoma, which required a laparotomy and open myomectomy for repair. Another complication was peroneal nerve involvement and a mild foot drop that resolved over several months. Nausea and mild abdominal discomfort that was relieved by NSAIDs were reported as minor complications [119].

Another study of 14 women evaluated the efficacy of 2 months of pretreatment with GnRH agonist prior to laparoscopically directed cryomyolysis [120]. The GnRH agonist was discontinued immediately prior to the procedure. Four months after cryomyolysis, the follow-up MRI showed a mean volume decrease of 10% among the frozen leiomyomas, whereas other uterine tissue returned to their size prior to GnRH agonist treatment.

Studies have shown that cryomyolysis can be an effective, minimally invasive means to treat symptomatic uterine fibroids [120, 121]. Myoma volume was reduced by 50%, along with a

corresponding reduction in symptoms, 6 months after the procedure [95]. Follow-up data have shown that at 12 months, further myoma shrinkage up to 62% from baseline occurred and heavy menstrual bleeding was reduced [122]. Because long-term outcomes are necessary and the effects of cryomyolysis on fertility are unknown, although a small series of nine women showed fertility may remain preserved [77], this technique is considered experimental at the present.

22.15 Laparoscopic Uterine Artery Ligation

Laparoscopic uterine artery ligation is yet another potential option for women who opt to preserve their uteri. A study comparing this technique to uterine artery embolization suggests that the uterine volume was slightly reduced at 3 months and stabilized at 6 months, with an average volume reduction of 58.5% [89]. The authors concluded that both laparoscopic uterine artery ligation and UAE are reasonable alternatives to hysterectomy.

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Tubal Disease and Ectopic Pregnancy

Rebecca Flyckt and Jeffrey M. Goldberg

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23

23.1 Introduction

Tubal factor infertility remains a frequently encountered entity in gynecologic practice. Analysis of data from the World Health Organization indicates that tubal factor accounts for approximately 35% of female infertility cases worldwide, with a much higher prevalence in areas such as Africa [1]. The overall incidence of tubal disease in Europe or the USA may be lower. For example, one population-based study of over 700 couples indicated that approximately 14% of couples evaluated by an infertility specialist had tubal disease [2]. This figure remained stable in a more recent survey of a similar cohort [3]. The most common causes of this condition include pelvic infection, endometriosis, and sequelae of ectopic pregnancy. Although the incidence of advanced ectopic pregnancies and some reportable sexually transmitted diseases is decreasing in the USA [4], early and accurate recognition in the diagnostic workup of the infertile couple is essential.

Fortunately, tubal infertility is often amenable to surgical intervention. With increasing success rates of assisted reproductive technologies (ART), patients are opting more frequently for in vitro fertilization (IVF) to circumvent damaged fallopian tubes. However, surgical management is still recommended in cases of proximal occlusion, mild hydrosalpinges, and reversal of tubal ligation.

In this chapter, the etiology of tubal factor infertility will be discussed, with a focus on pelvic inflammatory disease (PID) and ectopic pregnancy. In addition, the assessment of tubal status with **hysterosalpingogram (HSG)** will be reviewed. Finally, the surgical approaches to the treatment of tubal disease will be addressed, with an emphasis on tubal reconstructive techniques and management of hydrosalpinges. The treatment of tubal disease with ART does not differ from other conditions and is discussed separately.

Clinical Case: Part 1

A 26-year-old G0 female is seen for primary infertility evaluation after 1 year of regular unprotected intercourse with her male partner. She has regular cycles and no history of medical illness or prior abdominal surgeries. Her partner has had a normal semen analysis. She has a remote history of chlamydial infection. She has no history of endometriosis or pelvic pain. She denies dyspareunia or dysmenorrhea.

23.2 Mechanisms of Tubal Damage

The fallopian tubes are delicate organs which function in sperm transport, the pickup and fertilization of oocytes, and the transport of embryos to the uterus. The ciliated endothelium is particularly susceptible to damage by infection. However, abnormalities of the fallopian tubes can also follow tubal pregnancy, endometriosis, developmental exposure to teratogens, or iatrogenic causes (Table 23.1).

The most common cause of tubal infertility is PID, which can arise after ascending infection with *N. gonorrhoeae* or *C. trachomatis* [5, 6]. Other infectious agents thought to be deleterious to tubal structure and function include *Mycoplasma* species and tuberculosis; however, a causal relationship has not been substantiated [7]. It is known that the chance of post-inflammatory tubal damage rises with repeat infections and the successful function of the fallopian tubes is directly related to the severity of the damage [5, 8]. According to these landmark studies from Sweden involving thousands of women, the incidence of tubal infertility after laparoscopically diagnosed PID was 10–12% after one infection, 23–35% after two infections, and 54–75% after three infections [8].

Table 23.1 Causes of tubal infertility

Pelvic inflammatory disease
Prior ectopic pregnancy
Surgery/trauma to fallopian tubes
Endometriosis/adhesions
Septic abortion/endometritis/salpingitis
Ruptured appendix
Inflammatory bowel disease
Iatrogenic
DES

Longitudinal data from multiple centers within the USA confirm a twofold increased risk of infertility after recurrent PID [9].

It appears that many women with tubal disease or seropositivity for chlamydial antigen have not had any documented or reported history of prior infection [10]. Therefore, it must be concluded that salpingitis and resulting tubal damage can result even after asymptomatic or subclinical infections [7]. A recent study of women with subclinical PID based on endometrial histology demonstrated decreased fertility compared to controls [7]; these findings were despite treatment for uncomplicated lower genital tract infections.

As might be expected, a close association also exists between PID and ectopic pregnancy. Women with an episode of PID have approximately a 10% chance of developing an ectopic pregnancy in their first pregnancy following documented salpingitis [5]. In one retrospective cohort study examining sequelae of [11] chlamydial infection, the authors found that women with two episodes of infection were twice as likely, and women with three of more episodes of infection were greater than four times as likely, to be hospitalized for ectopic pregnancy [11].

23.3 Imaging of the Fallopian Tubes

Whatever the cause of tubal damage, recognizing tubal factor infertility is essential in the diagnostic workup of the infertile couple. HSG has been the standard initial test for assessing the uterine cavity and fallopian tubes since 1925 [12], although laparoscopic chromopertubation remains the gold standard for diagnosing tubal disease. HSG is a radiographic imaging procedure to image the uterine cavity and demonstrate tubal patency by injecting radiographic contrast media through the cervix. The technique is easy to learn and perform, is relatively low cost, has an acceptable radiation exposure, and has few complications. Indications and contraindications for HSG are listed in Tables 23.2 and 23.3. Risks, benefits, and alternatives of the procedure are outlined in Table 23.4. The most significant additional benefit of HSG is an enhanced post-procedure pregnancy rate. Alternative methods for evaluating tubal condition (such as sonohysterosalpingography or "hystero-contrast-sonography," transvaginal hydrolaparoscopy, falloposcopy, and salpingoscopy) are used far less often.

Table 23.2 Indications for hysterosalpingogram (HSG)

– Basic infertility workup
– Recurrent pregnancy loss
– Evaluation after uterine or tubal surgery
– Suspected uterine anomaly
– Confirming post-procedure tubal occlusion

Table 23.3 Contraindications for hysterosalpingogram (HSG)

– Active pelvic infection
– Cervicitis
– Severe iodine allergy
– Bleeding/menstruation
– Known or suspected endometrial carcinoma
– Pregnancy

Table 23.4 Risks, benefits, and alternatives to HSG

Risks/complications
– Vasovagal reactions
– Post-procedure infection
– Granuloma formation with oil-based contrast
– Oil embolism with oil-based contrast
Benefits
– Guides infertility treatment management
– Fertility enhancement
Alternatives
– Chlamydia antibody testing
– Sonohysterography or sonohysterosalpingography
– Magnetic resonance imaging
– 3D ultrasonography
– Radionuclide HSG
– Laparoscopy
– Hysteroscopy
– Transvaginal hydrolaparoscopy
– Salpingoscopy and falloposcopy

HSG should be considered a screening test and should have a high sensitivity, so as not to miss the opportunity to treat an abnormality, but a low false-positive rate to prevent unnecessary additional testing and treatments. The accuracy of a HSG is highly dependent on technique and interpretation. The technical quality of the HSG is important to limit misinterpretations (i.e., eliminating air bubbles that may be confused with a polyp or myoma or using inadequate contrast volume or injection pressure to demonstrate tubal patency).

23.4 Diagnosing Uterine Cavity Abnormalities

HSG has a high sensitivity but a low specificity for the diagnosis of uterine cavity abnormalities [13]. HSG and diagnostic hysteroscopy performed on 336 infertile women showed that HSG had a sensitivity of 98% but a specificity of only 35% due to difficulties distinguishing between polyps and myomas [14]. Thus, HSG fulfills the requirements as a good first-line screening test for revealing abnormalities of the uterine cavity, though any abnormalities found will likely need further evaluation to make a definitive diagnosis. A uterine septum and a bicornuate uterus cannot be differentiated on a HSG. Evaluation of the external fundal contour by laparoscopy, MRI, or 3D ultrasonography is required to make a definitive diagnosis. Other conditions visualized on HSG are adhesions, diethylstilbestrol (DES) changes, and adenomyosis.

23.5 Diagnosing Tubal Abnormalities

HSG appears to be a highly valid and accurate test for assessing tubal patency in subfertile couples; however, its reliability for diagnosing tubal occlusion is questionable. Tubal blockage on HSG is not confirmed by laparoscopy in up to 62% of patients. Laparoscopy is needed to confirm or exclude tubal occlusion on HSG [15]. Even laparoscopy is imperfect, as 2% of patients with bilateral tubal occlusion subsequently conceived spontaneously [16]. One study noted that 60% of patients with **proximal tubal occlusion (PTO)** on HSG showed patency on repeat HSG 1 month later [17].

Surprisingly, hydrosalpinges may also be poorly diagnosed by HSG. When detected, they may be only mildly dilated with preservation of mucosal folds or massively dilated with complete loss of the normal intratubal architecture (Fig. 23.1). HSG can also diagnose **salpingitis isthmica nodosa**, represented by diverticuli from the mucosa into the muscularis (Fig. 23.2). HSG is also not an ideal test for diagnosing pelvic adhesions because it detects them in only one-half of the cases in which they are present [12]. Adhesions are usually diagnosed on HSG by the presence of loculated spill of contrast.

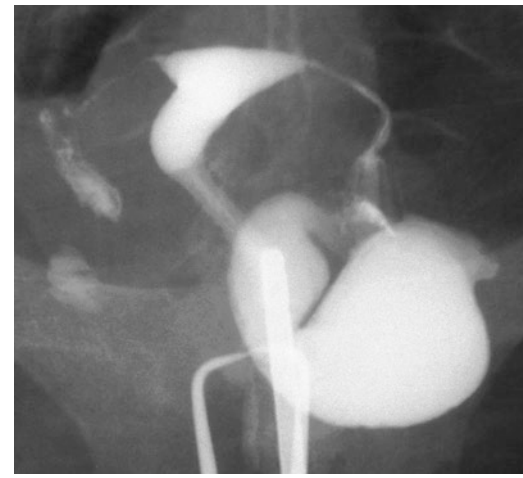

Fig. 23.1 Hydrosalpinx

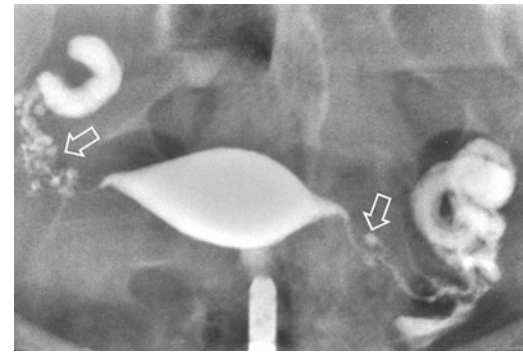

Fig. 23.2 Salpingitis isthmica nodosa

23.6 Technical Considerations

If possible, the gynecologist should perform the study; patients are comforted by having their physician present during this stressful test. At a minimum, the films should be obtained for review as the radiologists' reports vary greatly depending on training and experience. The procedure should be performed during the window between the end of menses but before ovulation. Although most patients experience uterine cramping during the HSG, the duration of the procedure is short and the discomfort generally resolves rapidly. Several studies have noted that NSAIDs do provide significant analgesia for HSG [18–20]. Proponents of water-soluble media (WSM) vs. oil-soluble media (OSM) have been arguing the relative merits of their favored contrast for decades and as yet there is no clear winner. WSM offers better image quality as the higher density OSM tends to obscure fine details in the uterus and tubal mucosal folds. Also, since WSM dissipates quickly, there is no need for delayed films, whereas 1–24 h delayed films are necessary with OSM. OSM also carries increased risks for oil embolism and granuloma formation. OSM has been claimed to have higher post-procedure pregnancy rates, although this finding has been questioned in the current Cochrane analysis [21].

23.7 Technique and Troubleshooting

The HSG cannula is attached to a 20-cc syringe filled with contrast media, which is flushed to expel air and prevent air bubble artifacts. The acorn is advanced so that it is about 1 cm from the end of the cannula. The cannula tip should not go beyond the internal os. Using a lubricated, open-sided bivalve speculum, the cervix is cleansed with an antiseptic solution, a single-tooth tenaculum is applied to the anterior lip, and the cannula tip is inserted into the cervical canal. Gentle upward pressure on the cannula while pulling downward on the tenaculum will seal the cervix and straighten the uterine axis.

Contrast media is then injected slowly. If a filling defect is suspected to be an air bubble, the patient can be rotated to her side (defect side down). A polyp or myoma will remain stationary, whereas a bubble will rise to the elevated side. Only 5–10 mL is usually required to complete the study. Patients should be observed for several minutes afterwards for bleeding and signs of vasovagal or allergic reactions.

While bilateral PTO is usually indicative of anatomical pathology, unilateral PTO is frequently transient due to spasm of the uterotubal ostium, plugging by mucus, debris, or air bubbles. Unilateral PTO is found in 10–24%, but 16–80% are patent on repeat HSG or laparoscopy with chromotubation [22]. Increasing the hydrostatic pressure and rotating the patient may establish patency during HSG showing PTO. The use of antispasmodic agents such as glucagon to prevent PTO has also been recommended, but this practice is supported only by limited anecdotal reports in the literature.

23.8 Surgical Management of Tubal Disease

Tubal disease may be present at multiple sites; however, tubal damage or blockages are often categorized as proximal, mid-tubal, or distal. The success of surgical management will depend on the location and degree of the damage in addition to nonsurgical factors, such as patient age and ovarian reserve. In general, patients with extensive tubal damage will be best served by IVF, whereas those without widespread disease may be good surgical candidates.

Minimally invasive surgical techniques are most appropriate for tubal surgery, but this approach requires some advanced training as well as meticulous dissection and hemostasis to prevent adhesions or further damage to the tubes.

23.9 Proximal Tubal Occlusion

PTO is most often caused by salpingitis isthmica nodosa, chronic pelvic infection, intratubal endometriosis, mucus plugging, or anatomic malformations [23]. Persistent PTO can be treated by therapies such as selective salpingography, tubal cannulation under fluoroscopy, hysteroscopic cannulation, or tubal resection and reanastomosis with a high degree of success.

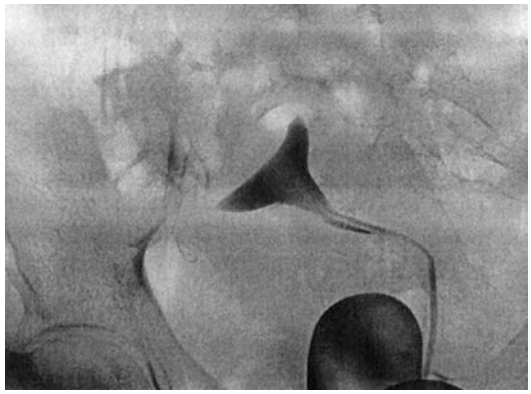

Fig. 23.3 Selective salpingography (reproduced with permission from Al-Fadhli R, Tulandi T. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

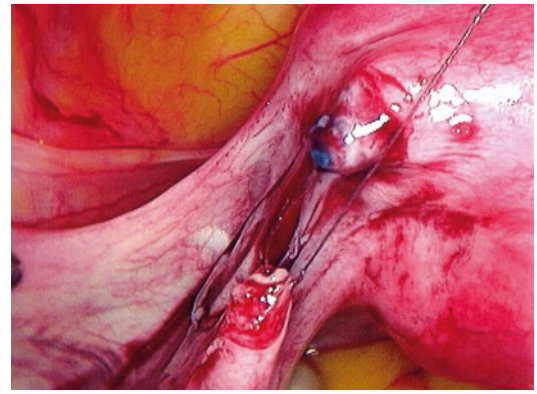

Fig. 23.4 Approximation of tubal lumen (reproduced with permission from Al-Fadhli R, Tulandi T. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

Selective salpingography is performed by injecting contrast media through a transcervical catheter positioned at the tubal ostia hysteroscopically or under fluoroscopic guidance (Fig. 23.3). Patency is confirmed by laparoscopy or fluoroscopy, respectively. Often, the hydrostatic pressure will relieve an obstruction. If unsuccessful, this is immediately followed with the introduction of a smaller catheter with an atraumatic guidewire through the selective salpingography catheter. The inner catheter and guidewire are advanced through the tubal ostia into the proximal isthmus. Chromotubation is then performed though the catheter to demonstrate patency through the fimbria. Although there is a high success rate of 75–95% in terms of achieving patency, reocclusion rates average 30% [23–25]. Despite similar tubal patency rates between hysteroscopic and fluoroscopic tubal cannulation, pregnancy rates are significantly higher with the former technique, 48.9% vs. 15.6% [23]. Tubal perforation occurs 2–10% of the time but is innocuous. Although microsurgical resection and reanastomosis can be used when tubal cannulation fails or when PTO is due to salpingitis isthmica nodosa, IVF is the preferred treatment.

23.10 Sterilization Reversal

Tubal sterilization is the most common contraceptive used by women worldwide [26]. Although it should be considered a permanent birth control option, 5–20% of women experience regret [27], and 1–2% of women seek reversal of sterilization [28]. In a large prospective, multicenter cohort study of over 11,000 women, regret appeared most strongly associated with younger age at the time of sterilization [27]. Compared with IVF, tubal anastomosis offers patients desiring sterilization reversal the advantage of a one-time, minimally invasive treatment with high success rates. In addition, some patients prefer the ability to attempt conception each month as well as to conceive more than once as well as the avoidance of the inconvenience and risks of IVF. The disadvantages are the possibility of bleeding, infection, inadvertent injury to other organs, and anesthetic complications inherent with any surgery. There is also a higher risk for ectopic pregnancy following tubal reanastomosis. Other factors involved in the decision are the IVF program's success rates, surgeon's ability, presence of other infertility factors, cost, and patient preference. A retrospective cohort study reported significantly higher cumulative pregnancy rates for tubal anastomosis compared to IVF for women less than 37 years of age, but there was no significant difference in women aged 37 years or older [29]. Furthermore, the average cost per delivery for tubal anastomosis was almost half that of IVF.

The procedure is performed by first mobilizing and opening the occluded tubal ends. A stitch is then placed in the mesosalpinx beneath the tubal ends to align them and relieve tension on the anastomosis. The anastomosis is accomplished with interrupted sutures through the tubal muscularis (Fig. 23.4). The serosa is also repaired

with interrupted stitches. Transcervical chromotubation is used to confirm tubal patency. A HSG is recommended if the patient has not conceived within six cycles postoperatively. Tubal anastomosis has usually been performed utilizing an operating microscope by laparotomy with an overnight hospitalization, but outpatient minilaparotomy achieves the same success rate with less cost and discomfort.

Cumulative pregnancy rates after tubal reanastomosis in women under age 40 range from 70% to over 90% [28, 30–32]. Even women ages 40–45 years have good success rates of 13–70%, with subsequent ectopic risks of 2–10% [28, 30, 32–35]. Most of the pregnancies occur within the first year of surgery. High success rates were also reported with laparoscopic tubal anastomosis, though few possess the skills to perform this [28, 31]. Robotic surgery is a technique that facilitates laparoscopic tubal reanastomosis. Two small studies comparing tubal anastomosis by robotic-assisted laparoscopy vs. laparotomy or minilaparotomy found no difference in pregnancy rates, but robotic surgery took significantly longer and cost more, even compared with laparotomy with overnight hospitalization, though recovery was quicker after robotic surgery [36, 37].

23.11 Distal Tubal Disease

The most frequent site for tubal infertility is the distal tube, which manifests as hydrosalpinges and **fimbrial phimosis** (agglutination of fimbria leading to a narrowed tubal opening). As described earlier, these most often follow PID but can also be preceded by peritonitis from any cause, trauma from previous surgery, or endometriosis. The decision to attempt repair of distal tubal disease is often made at the time of surgery, and categorizing patients as either favorable or poor prognosis may be helpful in guiding management. Tubal reconstruction should not be considered in patients with both proximal and distal occlusion or for women with severe disease, and patients should be counseled preoperatively about the possibility of **neosalpingostomy/fimbrioplasty** vs. salpingectomy depending on the surgical findings. According to American Fertility Society classifications, a favorable-prognosis patient generally has minimal adnexal adhesions, tubal dilation <3 cm, preservation of normal tubal walls, and endosalpinx [38]. In contrast, poor-prognosis patients may have dense tubal adhesions; significantly dilated tubes; thickened, fibrotic tubal walls; and damaged mucosa.

For favorable-prognosis patients, a neosalpingostomy or fimbrioplasty can be attempted to open up a **hydrosalpinx** or narrowed tubal opening (◘ Fig. 23.5). This is accomplished laparoscopically, with the release of adhesions and incision at the distal end of the occluded or narrowed tube. The mucosa and fimbria are everted and attached to the serosa using fine suture or electrosurgery. Transcervical chromopertubation is used to confirm patency at the completion of the procedure. Depending on the severity of the disease, pregnancy rates can range from 58% to 77% in favorable-prognosis patients, with ectopic rates of 2–8% [39]. As expected, for poor-prognosis patients, pregnancy rates fall to 0–22%, with ectopic rates of 0–17% [39]. It should be noted that although patency can often be achieved surgically in both favorable- and poor-prognosis patients, the irreversible damage caused by pelvic infection to the endosalpinx may account for compromised tubal function after surgery.

23.12 Hydrosalpinx and IVF

The harmful effect of hydrosalpinx on IVF outcomes has been well documented [40–43]. This observation may be explained by toxic effects of the hydrosalpinx fluid on the embryo, flushing of the embryo from the endometrium by hydrosalpinx fluid, or impaired endometrial receptivity. A meta-analysis of over 5500 women showed that the implantation and delivery rate per transfer is halved and the miscarriage rate is increased in women with untreated hydrosalpinx undergoing IVF [44]. Several prospective randomized trials have demonstrated that hydrosalpinges treated with salpingectomy prior to IVF result in restoration of comparable pregnancy rates to controls [45–47]. This finding has been replicated even in patients with unilateral salpingectomy for unilateral hydrosalpinx [48]. A large hydrosalpinx visualized by ultrasound should be removed prior to IVF, as these appear to be associated with the poorest outcomes [44, 45, 49]. An area of greater controversy is whether less pronounced hydrosalpinges (such as those identified by HSG or on

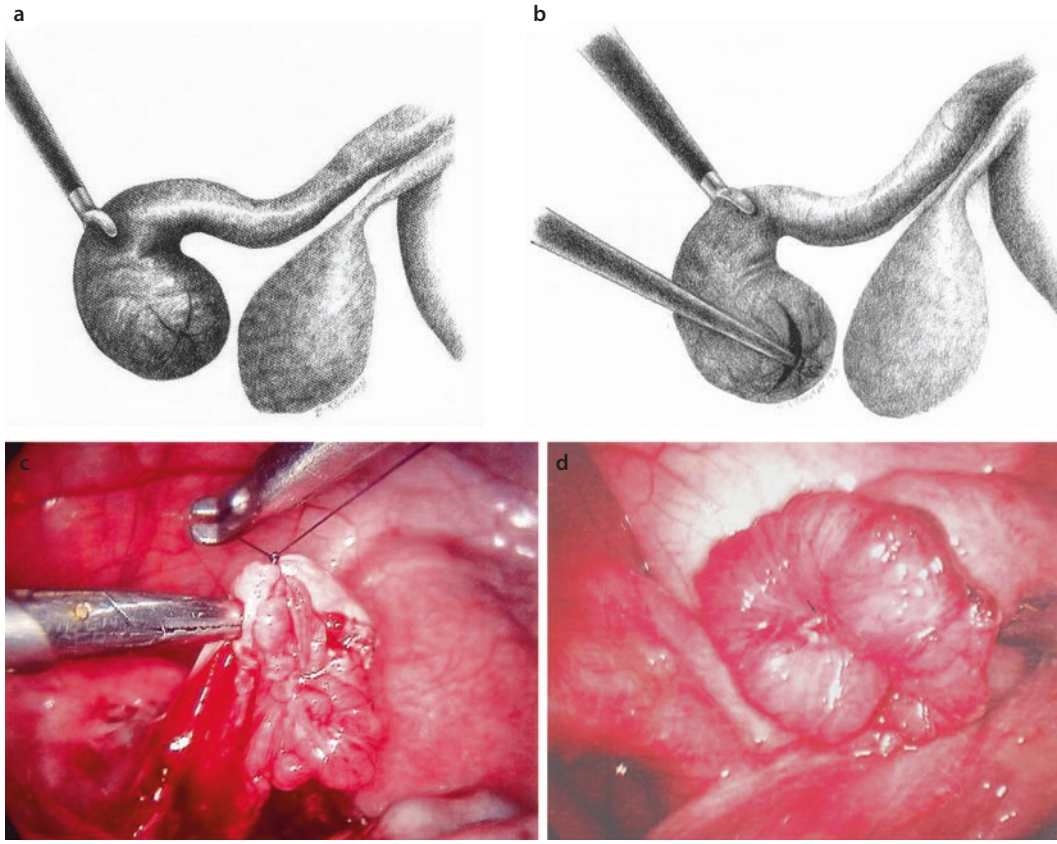

Fig. 23.5 (**a**–**d**) Technique for neosalpingostomy (reproduced with permission from Al-Fadhli R, Tulandi T. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

laparoscopy) should also be removed in this circumstance.

The gold standard for treatment of hydrosalpinx prior to IVF is laparoscopic salpingectomy; however, other techniques such as proximal tubal ligation or occlusion, ultrasound-guided drainage, or salpingostomy have been suggested. Salpingectomy prior to IVF should be performed laparoscopically, taking care to remain close to the tube to preserve the ovarian blood supply and maintain ovarian reserve. PTO has been demonstrated to be an effective alternative to salpingectomy in cases where salpingectomy is technically difficult or surgery is contraindicated [47, 50]. Pregnancy rates following tubal occlusion appear comparable to treatment with salpingectomy [51]. Most recently, several small case series have demonstrated the effectiveness of using tubal inserts to hysteroscopically occlude hydrosalpinges [52, 53]; however, persistent coils within the endometrial cavity may theoretically limit the success of subsequent ART cycles.

Clinical Case: Part 2

The aforementioned patient undergoes laparoscopic bilateral neosalpingostomies for mild hydrosalpinges with distal tubal occlusion discovered on HSG. She conceives spontaneously 3 months after surgery. She presents to her obstetrician with a positive home pregnancy test and light vaginal bleeding. Her last menstrual period was 5 weeks prior. An ultrasound shows a thickened endometrium without evidence of a gestational sac and her quantitative hGC is 2500 mU/mL. There are no adnexal masses.

23.13 Ectopic Pregnancy

Women with a history of tubal adhesions are at an increased risk of ectopic pregnancy. Ectopic pregnancy is defined by the abnormal implantation of

an embryo outside of the endometrial cavity. These pregnancies represent approximately 1.55–2% of pregnancies and most frequently affect the fallopian tube (>90%), although they can uncommonly involve sites such as the abdomen, ovary, cervix, or cesarean scar [54]. Further, within the tube itself, ectopic pregnancies have a predilection for the ampullary portion of the tube where fertilization occurs (70%); an additional 10% occur in the isthmus, 10% in the fimbria, and 2% in the uterine cornu or interstitium [55]. Although rates of ectopic pregnancy in the USA appear to have peaked and plateaued in the 1990s [56], the true incidence is difficult to estimate because these pregnancies are increasingly managed as outpatients and therefore may not be included in hospital databases. It does appear that enhanced awareness and improved detection methods have resulted in more favorable outcomes. Nevertheless, ectopic pregnancies remain the leading cause of maternal deaths in the first trimester [57] and must be recognized and managed promptly. Failure to do so can result in fallopian tube rupture, intraperitoneal hemorrhage, shock, and even death.

Abnormal implantations are thought to result mainly from inflammation or blockage within the tubal lumen. As with infertility, most tubal damage that precedes ectopic pregnancy is caused by infection with *N. gonorrhoeae* or *C. trachomatis*. As many as half of women with ectopic pregnancies will have no identifiable risk factors [58]. Known risk factors for ectopic pregnancy include infection, prior ectopic pregnancy, and prior tubal surgery. Although IUD use does not increase the overall risk of ectopic pregnancy, a positive pregnancy test in an IUD user warrants suspicion for ectopic, as the location of the gestation is most likely extrauterine [58]. A complete list of maternal risk factors is shown in Table 23.5. It has also been theorized that chromosomally abnormal embryos may have a higher rate of inappropriate implantation. However, more recent studies with larger patient numbers are not consistent with earlier case reports showing high percentages of abnormal karyotypes from ectopic pregnancies [59, 60].

Of special consideration is the relationship between ART and extrauterine pregnancies. The ectopic risk is increased in patients undergoing treatments ranging from ovulation induction to IVF, perhaps due to preexisting tubal pathology or the effects of supraphysiologic hormonal levels on tubal motility. Ectopic risk after oocyte retrieval and embryo transfer is 4.5%, significantly higher than what is observed in the general population [61]. The incidence of **heterotopic pregnancy** (i.e., simultaneous intrauterine and extrauterine pregnancies) is similarly increased in ART patients. In contrast to the classic rate of 1:30,000 pregnancies or even more recent rates of 1:4000 pregnancies, heterotopic pregnancies in ART patients are estimated to occur as frequently as 1:100 pregnancies [62, 63].

Table 23.5 Risk factors for ectopic pregnancy[a]

Strong associations
Tubal surgery
Pelvic inflammatory disease
Prior ectopic pregnancy
Weaker associations
Infertility
Cigarette smoking
Increasing age
More than one lifetime sexual partner
Abdominal or pelvic surgery
Sexually transmitted diseases (gonorrhea and/or chlamydia)
Intrauterine device use
No clear association
Oral contraceptive use
Prior spontaneous miscarriage
Prior elective termination of pregnancy
Cesarean section

[a]Reproduced with permission from Seeber B, Barnhart K. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

23.14 Presentation and Diagnosis

Over the past few decades, increased reports of ectopic pregnancy are likely due to earlier and better methods of detection. Most diagnoses are

made based on abnormally rising levels of beta-hCG or characteristic ultrasonographic findings. In recent years, the development of the radioimmunoassay for hCG quantification and the introduction of the specific antiserum to the beta subunit of hCG has improved the clinician's ability to monitor the rise and fall of this hormone in early pregnancy [64]. Similarly, ultrasound technology has progressed to allow higher resolution transvaginal images assisted by Doppler flow to evaluate the adnexa and endometrium.

Although diagnostic methods have recently improved, the typical presentation of ectopic pregnancy outside of the ART setting has remained extremely consistent over time. Pain (99%) and vaginal bleeding (56%) are the hallmarks of confirmed ectopic pregnancies [65]. Pain is thought to result from tubal distention and/or peritoneal irritation from hemoperitoneum. However, the sensitivity and specificity of these clinical indicators is low, and clinicians should be aware that these complaints may be intermittent or absent. An important observation is that neither presenting complaints nor beta-hCG levels correlate well with risk of tubal rupture. Studies have shown that low (<100 IU/L) or even decreasing beta-hCG levels can still be associated with ruptured ectopic pregnancy [66, 67].

The physical exam for ectopic pregnancy should begin with vital signs; the combination of hypotension and tachycardia signifies acute blood loss and the need for prompt surgical intervention and fluid resuscitation. The abdominal exam may reveal signs of peritoneal irritation (e.g., rebound or guarding) or tenderness to palpation. A normal physical exam cannot be used to exclude ectopic pregnancy. The speculum exam should document the presence of blood or products of conception from the cervical os. The adnexa should be gently palpated on bimanual exam; excessive pressure can increase the risk of tubal rupture.

Although suspicion for ruptured ectopic may necessitate a rapid bedside transabdominal ultrasound to verify hemoperitoneum, most ART patients and nonurgent non-ART patients can be investigated further with standard transvaginal ultrasound techniques and beta-hCG levels. An algorithm for diagnosing ectopic pregnancies based on ultrasound and laboratory findings is outlined in Fig. 23.6. A key concept in the evaluation of ectopic pregnancies is the "**discriminatory zone**." This cutoff is defined as the beta-hCG level above which a single intrauterine gestational sac should be visualized, if present, by transvaginal ultrasound [68]. The discriminatory zone varies by institution but is generally between 1500 and 2500 mIU/mL. Care must be taken to differentiate a "pseudosac" (endometrial cavity distended by bleeding from decidualized endometrium) from a true gestational sac. In the presence of multiple gestation, as may be suspected after ART, the discriminatory zone may not be valid; twins and higher-order multiples may have beta-hCG levels above the cutoff value before ultrasound detection is possible [69].

In cases where the beta-hCG is lower than the discriminatory zone, serial beta-hCG measurements will be helpful. Although older gynecologic dogma suggested that beta-hCG levels should rise by 66% in 2 days, newer data indicate that beta-hCG rise in viable gestations may be considerably slower than previously reported [70]. According to these data, an increase in beta-hCG of less than 53% in 48 h confirms an abnormal early pregnancy with 99% specificity. Adhering to this model can minimize the risk of intervening during a potentially viable pregnancy. Serum progesterone levels have limited utility in ectopic pregnancy due to the common scenario of an equivocal progesterone level as well as the long turnaround time for the test at many centers [71]. When an abnormally rising hCG is documented and the distinction cannot be made between failing intrauterine pregnancy and ectopic pregnancy, uterine cavity sampling to look for presence or absence of chorionic villi may be required.

23.15 Medical Management of Ectopic Pregnancy

Methotrexate (MTX) is an antimetabolite that has been used for decades in the treatment of ectopic pregnancy and gestational trophoblastic diseases. MTX acts as a folic acid antagonist that inhibits cellular DNA and RNA synthesis. MTX arrests mitosis in rapidly dividing tissues such as trophoblast, bone marrow, and orogastric/intestinal mucosa. Side effects such as gastrointestinal symptoms (nausea, vomiting, indigestion, abdominal pain, or stomatitis), as well as more unusual severe effects such as gastritis, enteritis, or pneumonitis [68], are uncommon with regimens used for treating ectopic pregnancy. Hepatotoxicity and

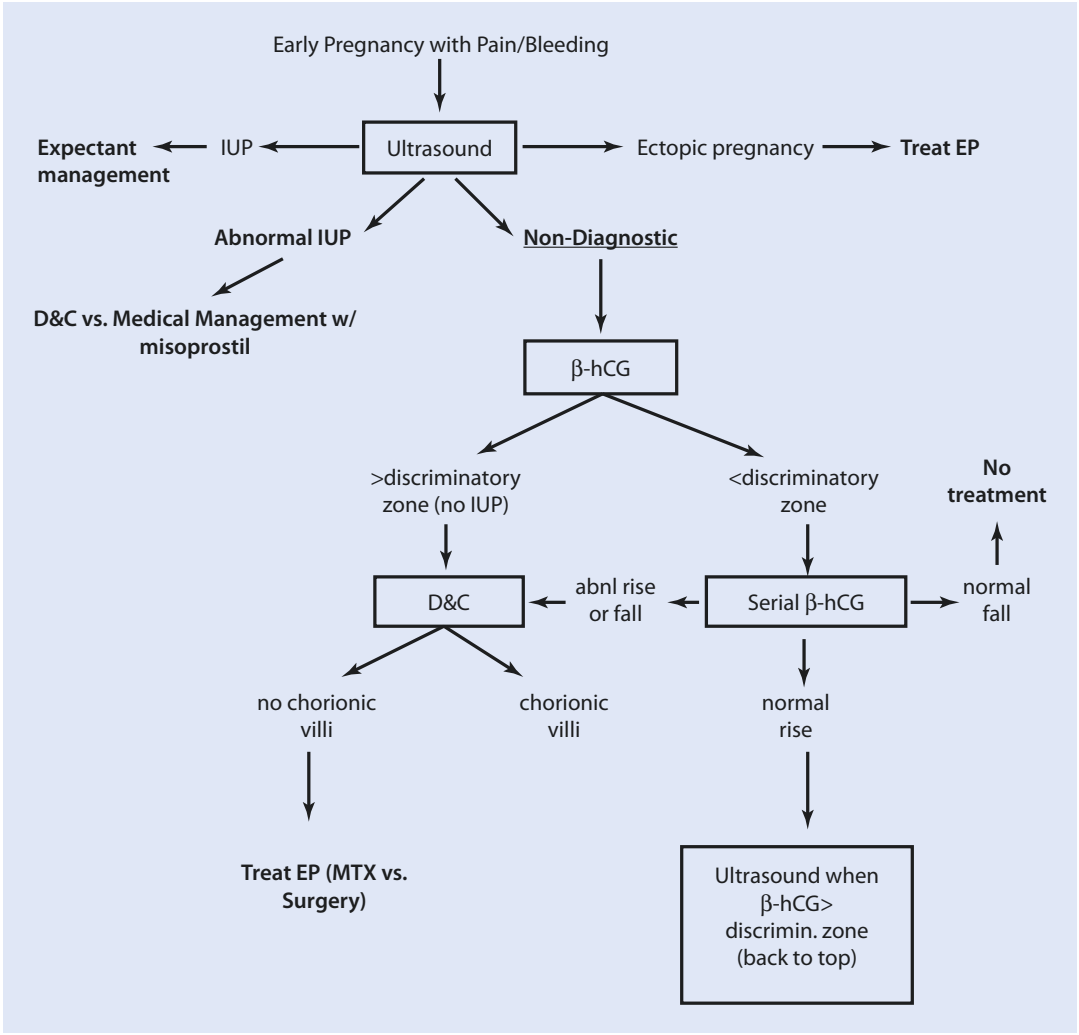

Fig. 23.6 Diagnostic algorithm flowchart (reproduced with permission from Seeber B, Barnhart K. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007; adapted from [78])

bone marrow suppression have also been rarely described. A baseline complete blood count and liver function testing should be obtained prior to MTX treatment. More commonly, abdominal pain and cramping after MTX administration is often reported 2–7 days after the injection and typically represents tubal distention or abortion of necrotic trophoblastic tissue. Without signs of tubal rupture or acute bleeding by laboratory or ultrasonographic criteria, these symptoms can be managed expectantly with reassurance for the patient.

Initial treatment with MTX is appropriate for patients with confirmed or highly suspected unruptured ectopic pregnancies that desire conservative management and are reliable for follow-up. Absolute medical contraindications to MTX therapy are listed in Table 23.6. Relative contraindications for MTX are the subject of some debate; however, many clinicians would restrict MTX treatment to patients with adnexal masses <3.5 cm, no fetal cardiac activity, and beta-hCG beneath a predetermined limit [72]. Although prior hCG thresholds were in the 5000–15,000 mIU/mL range, a recent systematic review has reported a significant and substantial increase in failure rates (14.3% vs. 3.7%) with single-dose MTX when the hCG level exceeds 5000 mIU/mL [73]. A multidose approach or surgical treatment may be preferable in this scenario.

Table 23.6 Absolute contraindications to methotrexate therapy[a]

– Breastfeeding
– Overt or laboratory evidence of immunodeficiency
– Alcoholism, alcoholic liver disease, or other chronic liver disease
– Preexisting blood dyscrasias (bone marrow hypoplasia, leukopenia, thrombocytopenia, significant anemia)
– Known sensitivity to methotrexate
– Active pulmonary disease
– Peptic ulcer disease
– Hepatic, renal, or hematological dysfunction

[a]Reproduced from Seeber B, Barnhart K. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007; adapted from [77]

Several regimens have been outlined for the treatment of ectopic pregnancy with intramuscular MTX. Protocols for the single-dose, two-dose, and fixed multi-dose MTX regimens as outlined by the American Congress of Obstetricians and Gynecologists are shown in Table 23.7 [68]. Under the single-dose and two-dose regimens, MTX is administered at a dose of 50 mg/m^2 based on body surface area, which is calculated as follows:

$$BSA\left(\mathrm{m}^2\right) = \left\{ \begin{bmatrix} \mathrm{Height}\left(cm\right) \\ \times \mathrm{Weight}\left(kg\right) \end{bmatrix} \Big/ 3600 \right\}^{1/2}$$

A fixed multi-dose protocol adds "rescue" with leucovorin (i.e., folinic acid). For all protocols, a beta-hCG decrease of less than 15% between days 4 and 7 represents treatment failure and the need for additional management [68]. Although the single-dose regimen is the least complicated and costly and minimizes side effects, a meta-analysis of multiple case series has shown that the fixed multiple-dose regimen is five times more effective than a single-dose regimen [74]. It should be noted that, regardless of treatment regimen, the overall success for treatment with MTX was high (89%). MTX therapy can be safely pursued in appropriately selected patients; two-dose or fixed multiple-dose regimens should be considered in more advanced gestations or when MTX therapy is pursued in the setting of relative contraindications. All regimens must be followed until beta-hCG levels are <5 mIU/mL to ensure complete resolution of the trophoblastic tissue.

23.16 Surgical Management of Ectopic Pregnancy

Ectopic pregnancies can be treated with either chemotherapeutic agents or surgery. Both strategies have high success rates and maintain the potential for future pregnancies. Indications for surgical management of ectopic pregnancy are listed in Table 23.8. The traditional surgical approach for ectopic pregnancy was exploratory laparotomy with salpingectomy. Today, laparoscopy with tubal preservation (i.e., salpingostomy) is usually possible. Exploratory laparotomy is still used when patients are unstable following tubal rupture, when the laparoscopy would be complex (significant adhesions, extratubal gestation) or contraindicated, or when clinical proficiency in laparoscopy is lacking.

Laparoscopic salpingectomy can be accomplished with either a combined coagulation-cutting device or by sequential use of bipolar cautery and laparoscopic scissors. Care should be taken to stay close to the tube to avoid compromising the ovarian blood supply. The more conservative approach via **linear salpingostomy** involves an incision on the anti-mesenteric surface of the tube using the unipolar needle or scissors, laser, or ultrasonic scalpel. Prior to that, dilute vasopressin is injected in the mesosalpinx beneath the ectopic to improve hemostasis and to minimize tubal damage from excessive cauterization. After tubal incision, the trophoblastic tissue is then either gently teased or irrigated from the tube, taking into consideration that the majority of tissue is usually located on the more proximal aspect of the incision. Failure to remove all trophoblastic tissue can lead to persistent ectopic pregnancy or post-ectopic tubal obstruction; a Cochrane review concluded that persistent ectopic rates after salpingostomy can be reduced when coupled with a prophylactic single injection of MTX [75].

Table 23.7 Methotrexate protocols for ectopic pregnancy[a]

Treatment day	Laboratory tests	Intervention
Pretreatment	β-hCG, CBC with differential, LFTs, creatinine, type and screen	Rule out SAB
		RhoGAM if Rh-negative
Single-dose protocol		
1	β-hCG	MTX 50 mg/m^2 IM
4	β-hCG	
7	β-hCG	MTX 50 mg/m^2 IM if β-hCG <15% decrease day 4–7
Two-dose protocol		
Pretreatment	β-hCG, CBC with differential, LFTs, creatinine, type and screen	Rule out SAB
		RhoGAM if Rh-negative
0	β-hCG	MTX 50 mg/m^2 IM
4	β-hCG	MTX 50 mg/m^2 IM
7	β-hCG	MTX 50 mg/m^2 IM if <15% decline day 4–7. If >15% stop treatment and start surveillance
11	β-hCG	MTX 50 mg/m^2 IM if <15% decline day 7–11. If >15% consider surgical treatment
Multi-dose protocol		
1	β-hCG	MTX 1.0 mg/kg
2		LEU 0.1 mg/kg
3	β-hCG	MTX 1.0 mg/kg if <15% decline day 1–3. If >15% stop treatment and start surveillance
4		LEU 0.1 mg/kg
5	β-hCG	MTX 1.0 mg/kg if <15% decline day 3–5. If >15% stop treatment and start surveillance
6		LEU 0.1 mg/kg
7	β-hCG	MTX 1.0 mg/kg if <15% decline day 5–7. If >15% stop treatment and start surveillance
8		LEU 0.1 mg/kg

Surveillance every 7 days (until β-hCG <5 mIU/mL)
β(beta)-hCG β(beta)-human chorionic gonadotropin, *CBC* complete blood count, *LFTs* liver function tests, *MTX* methotrexate, *SAB* spontaneous abortion
[a]Adapted from [26]

When choosing between salpingostomy and salpingectomy, the most conservative approach possible (i.e., salpingostomy) is advised in patients wishing to preserve their fertility. However, in cases complicated by severe tubal damage, adhesions, or rupture, large ectopic size (>5 cm), or bleeding from the incision site, a salpingectomy may be the only feasible strategy. Additional factors such as previous ectopic in the same tube, prior tubal reanastomosis or other tubal surgery, or undesired future fertility are also important when determining a surgical approach. In a

Table 23.8 Indications for surgical management of ectopic pregnancy

- Hemodynamic instability
- Signs of tubal rupture
- Simultaneous intrauterine pregnancy
- Unable to adhere to follow-up plan
- Contraindications to MTX (see Table 23.6)
- NB: Surgery may also be considered with ectopic size >3.5 cm, presence of fetal cardiac activity, or elevated beta-hCG due to higher risk of MTX failure

multi-study review, linear salpingostomy was associated with a 15% recurrent ectopic rate and 60% successful postoperative pregnancy rate [64]. The risk of persistent ectopic pregnancy with tube-sparing surgery was 3–20%. For salpingectomy, this same review cited a recurrence risk of 10% and a subsequent pregnancy rate of 38%. When possible, tubal conservation is recommended because it may increase future fecundity; however, patients must be carefully followed owing to the concern for persistent ectopic, recurrent ectopic, and post-ectopic tubal obstruction. Serial beta-hCG levels should be performed weekly until they reach non-detectable values to ensure resolution of the ectopic pregnancy after salpingostomy.

23.17 Expectant Management

Increased monitoring and early pregnancy detection now prompt the clinician to intervene when ectopic pregnancy is suspected. However, prior to sensitive hCG assays and high-resolution ultrasonography, it is likely that many early tubal pregnancies resolved spontaneously without adverse outcomes. With close and careful follow-up, patients with low and decreasing beta-hCG levels may be appropriate for expectant management, but these patients must be counseled and accept the risk of tubal rupture and its associated morbidities. In one prospective observational study of over 100 women, spontaneous resolution rates were 88% when the first hCG level was <200 IU/L [76].

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Endometriosis

Dan I. Lebovic and Tommaso Falcone

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24.1 Introduction

Endometriosis is an incorrigible disease that is responsible for a multitude of hospital admissions and operative procedures [1]. When endometrial tissue is found outside the endometrial lining this is called endometriosis. There is debate as to whether or not symptomatology must be present to meet the definition of endometriosis. This disease is typically a chronic, progressive disorder, with pelvic pain and infertility as the hallmark symptoms. Owing to the recognition of more subtle forms of endometriosis and the more liberal use of diagnostic laparoscopy, the prevalence has been increasing. As noted in the accompanying outline for this chapter, it has been organized to lead the reader initially through the prevalence, diagnosis, and classification scheme before then exploring the varied anatomic sites of endometriotic lesions. Predisposing factors are then delineated as a means to expedite a diagnosis in those more prone to having the disease. This then leads into a review of the myriad theories as to the pathophysiology of endometriosis—still inconclusive. We then continue the theoretical mindset by explaining the mechanisms for endometriosis-associated subfertility and pain. At this point treatment options are explored starting with the infertile couple and then pelvic pain—both medical and surgical management. Finally, options are provided for dealing with extragenital endometriosis.

Clinical Case

A 27-year-old woman presents with a 1 year history of infertility. She has a history of dysmenorrhea and dyspareunia. An ultrasound reveals a 5 cm ovarian cyst compatible with an endometrioma. Semen analysis is normal.

24.2 Prevalence of Endometriosis

Endometriosis affects nearly 10% of reproductive-age women between the ages of 12 and 80 years, with the average age at diagnosis around 28 years. Several conditions are associated with higher rates of disease: for instance, up to 50% of those with subfertility or chronic pelvic pain are found to have endometriosis and nearly 70% of adolescents with nonresponsive pelvic pain end up showing endometriotic lesions at the time of surgery. The incidence of ovarian cystic endometriosis (endometriomas) increases with age [2], but its incidence and progression seem to stabilize in patients who are older than 35 years. Based on a study that involved repetitive laparoscopy in baboons, we know that endometriosis appears spontaneously and that new lesions seem to appear and disappear spontaneously [3]. Thus, endometriotic lesions in humans most likely are in continuous evolution as well [4].

24.3 Diagnosis of Endometriosis

The definitive diagnosis of endometriosis can only be made surgically. The classic symptoms are dysmenorrhea, noncyclic pelvic pain, dyspareunia, and infertility. Specific presenting physical signs indicative of advanced endometriosis are listed in Table 24.1. Physical examination during menstruation is more sensitive in the detection of pelvic disease. There is no laboratory test that can identify patients with endometriosis with a high degree of sensitivity and specificity. The search for biomarkers of this disease is robust [5], although further studies are required before clinical use can be recommended. Currently, there is no reliable biochemical marker to diagnose or stage endometriosis.

The microscopic definition of endometriosis implies the presence of endometrial glands and stroma outside the endometrial cavity and uterine musculature. The various typical lesions of endometriosis are referred to as clear, white, red, polypoid, flame-like, powder-burn, brown, blue-black, brown, or yellow lesions (Fig. 24.1). Defects in the peritoneum or peritoneal windows may contain these lesions. The American Society for Reproductive Medicine (ASRM) scoring system for endometriosis is widely used clinically but has significant intra- and interobserver variation (between 38 and 52%) [6].

The positive predictive value of laparoscopic visualization of endometriosis is considered to be approximately 50%. Classic red or black lesions

Table 24.1 Signs and symptoms of endometriosis

Signs
Adnexal mass
Adnexal tenderness
Uterine tenderness
Fixed retroversion
Lateral cervical displacement
Cul-de-sac
Tenderness
Nodularity
Mass
Uterosacral ligament
Tenderness
Nodularity
Vaginal lesions
Cervical lesions
Symptoms
Reproductive tract
Infertility
Dysmenorrhea
Dyspareunia
Noncyclic pelvic pain
Gastrointestinal
Diarrhea and/or constipation
Tenesmus
Abdominal cramps
Cyclic rectal bleeding
Urinary
Low-back pain

have a visual accuracy between 90 and 100%. White lesions are associated with endometriosis less often. The main pathologies that can be confused with endometriosis are endosalpingiosis, fibrosis, mesothelial hyperplasia, carbon deposits from previous surgery, and malignancy. Hemangiomas, adrenal rests, and splenosis can also rarely be confused with endometriosis. For this reason, diagnostic laparoscopy should be accompanied with biopsy for lesions that are not clearly endometriosis.

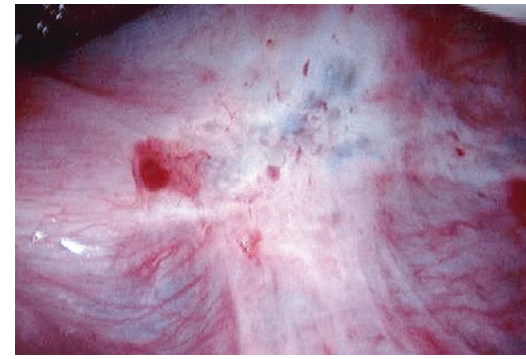

Fig. 24.1 Typical *black* and *white* lesions of the pelvic peritoneum with dense fibrosis and retraction

Ovarian endometriosis, as opposed to non-ovarian disease, can be detected pre-operatively with a high degree of accuracy using ultrasonography. There may be a role for magnetic resonance imaging (MRI) in the identification of deeply infiltrating lesions that involve the cul-de-sac or lesions in uncommon locations such as the sciatic nerve. Diagnostic tests for patients with gastrointestinal symptoms such as colonoscopy or barium enema radiography are typically normal or may occasionally show stricture. Patients with significant urinary symptoms should have a urologic evaluation to rule out interstitial cystitis or potentially endometriosis of the bladder wall.

The more common differential diagnoses of patients with chronic pain and potential endometriosis are adhesions, chronic pelvic inflammatory disease, interstitial cystitis, irritable bowel syndrome, and musculoskeletal problems such as myofascial pain or neuralgias.

24.4 Classification of Endometriosis

The classification of endometriosis has been an evolving process. In 1978, the American Fertility Society (AFS, now called the American Society for Reproductive Medicine) classified four stages (stages I-IV) and used an arbitrarily weighted point score that included assessment of the extent of endometriosis in two dimensions and the presence and extent of adhesions in the peritoneum, ovaries, and tubes. It also took into account whether the endometriosis was unilateral or

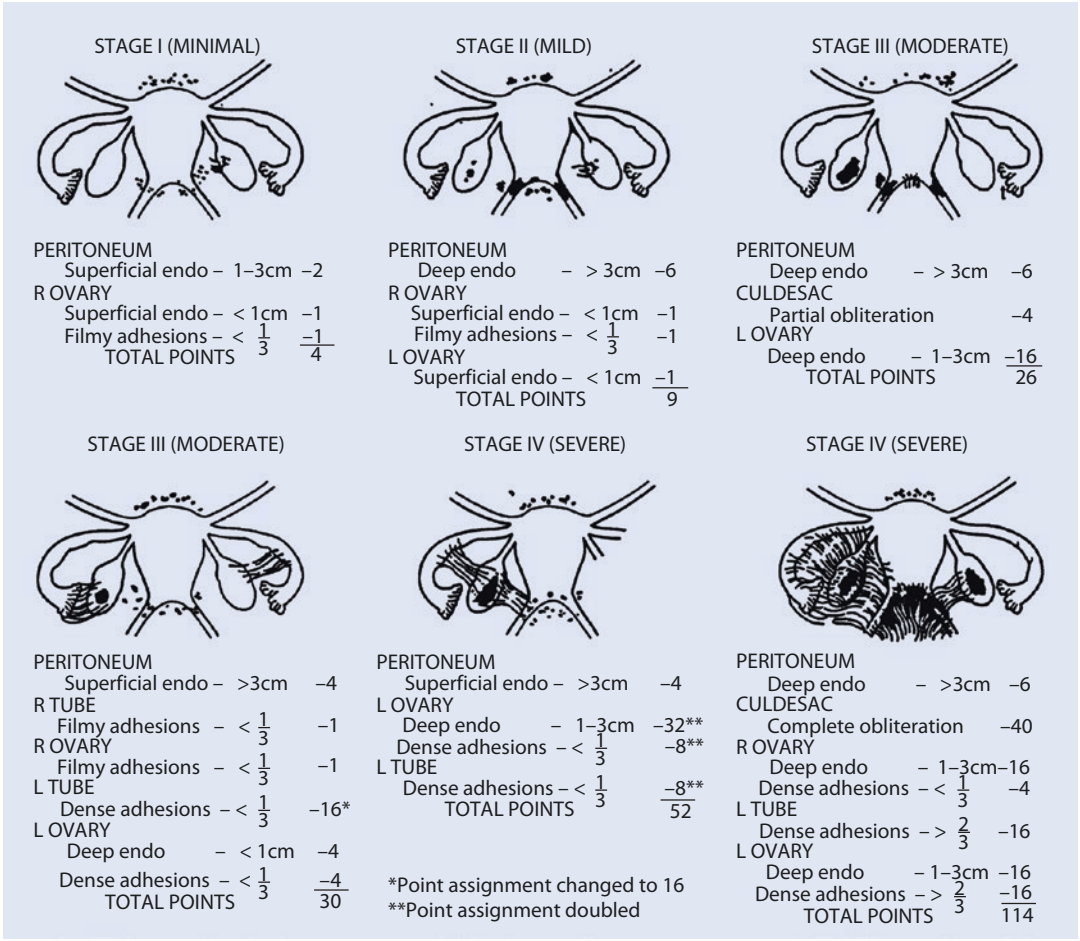

Fig. 24.2 ASRM revised classification of endometriosis

bilateral. The size of the endometrioma was considered along with the presence of filmy vs. dense adhesions. In 1985 and again in 1996 (R-AFS), further revisions were made to the original AFS classification [7] (Fig. 24.2). An endometrioma that is larger than 3 cm in diameter is at least stage III disease. Despite the improvements, the correlation between the stage of endometriosis and the likelihood of pregnancy or degree of pain is poor, and future improvement in the classification is both warranted and likely.

24.5 Associated Disease Processes

Surveys of endometriosis patients report increased incidence of atopic disease and other autoimmune phenomena such as thyroid disease, fibromyalgia, and chronic fatigue syndrome. Endometriosis is associated with increased incidence of non-Hodgkin's lymphoma, dysplastic nevi, and melanoma. Ovarian cancer (endometrioid and clear cell) is higher, although the overall lifetime risk is still quite low: 1.5% compared to 1% in the general population.

24.6 Anatomic Sites of Endometriosis

Endometriosis is most commonly found in the posterior pelvis compartment [8]. The following are the most common locations, in descending order: ovaries, cul-de-sac, broad ligament, and uterosacral ligaments. The left hemipelvis is the most common location—64% compared to the right hemipelvis. More endometriosis is found on the left as opposed to the right ovary, possibly because the sigmoid colon alters intraperitoneal fluid movement.

The bowel is the most common extragenital location of endometriosis. Bowel locations, in decreasing order of frequency, are the sigmoid colon (>65% of cases), rectum, terminal ileum, appendix, and the cecum. Most bowel lesions are superficial and limited to the serosa. Occasionally, transmural involvement of the bowel occurs, which may cause cyclic diarrhea, rectal bleeding, abdominal distension, and, rarely, bowel obstruction. The urinary tract is involved in only 1% of cases, and it most often affects the bladder (84%). Symptoms for vesical endometriosis are similar to those associated with recurrent cystitis. Endometriosis of the urinary tract should be suspected if cyclical urinary symptoms, such as urgency, frequency, and suprapubic pain with or without hematuria, occur.

24.7 Ovarian Endometriosis

Ovarian endometriosis or endometriomas increase with age and are generally associated with a more advanced stage of the disease [2]. This form of endometriosis can be diagnosed with a high level of accuracy by serial ultrasounds. They may be confused with a hemorrhagic corpus luteum, which will disappear over the course of a few months. These ovarian forms of endometriosis often have associated peritoneal implants (77% of the time and 85.4% of these women experienced pelvic pain whereas only 38.3% of those with an isolated endometrioma experienced pain [9]). Only a small percentage of patients with peritoneal implants will eventually develop an endometrioma. Endometriomas can be uniloculated or multiloculated. They are more commonly localized in the left ovary, as with peritoneal implants, likely due to the natural peritoneal fluid flow subsequent to menstrual regurgitation.

24.8 Deep Endometriosis

Invasion of endometriotic cells deeper than 5 mm has been associated with increased pain [10]. A rectovaginal exam during the menstrual period in the office setting or a thorough exam under anesthesia prior to laparoscopy may alert the surgeon to the presence of these types of lesions. In a study looking at 93 women with deep infiltrating peritoneal endometriosis, 61% had concomitant superficial implants and 51% had endometriomas. Deep nodules were the only form of the disease in just 7% of the women.

24.9 Extra-Pelvic Endometriosis

Cutaneous endometriosis has been reported in abdominal scars following cesarean sections, hysterectomy, appendectomy, and laparoscopy. Rare lung cases of endometriosis leading to cyclical hemoptysis or even catamenial pneumothorax have been reported and imply that hematogenous and/or lymphatic dissemination of endometrial cells is possible. This mechanism can also explain the possible spread to rare locations, such as the brain, liver, pancreas, kidney, vertebra, and bones.

24.10 Predisposing Factors for Endometriosis

Endometriosis is mainly present during the reproductive years (average age of 28) and usually regresses during menopause, suggesting that the development and growth of endometriosis is estrogen dependent. Accordingly, the Nurses' Health Study prospectively assessed predisposing factors for endometriosis and observed an association with early age of menarche, shorter length of menstrual cycles during late adolescence, and nulliparity. Furthermore, women with low estrogen levels and low body mass index, who use alcohol, who are infertile smokers, and who exercise intensely appear to be at decreased risk [11].

Heredity is an important predisposing factor for endometriosis since the prevalence is increased sevenfold among first-degree relatives. In monozygotic twins, the prevalence increased 15-fold. Exposure to pollutants, especially endocrine-disrupting compounds such as dioxins or polychlorinated biphenyls (PCBs), might also play a role in the predisposition to endometriosis.

Data from the Nurses' Health Study II suggests that specific dietary fat consumption may influence the risk of developing endometriosis—long-chain omega-3 fatty acids were protective, whereas *trans*-unsaturated fats led to a greater risk.

24.11 Pathophysiology

Managing endometriosis receives the lion share of attention, although investigation into the genesis of the disease does not lag far behind. In fact, it is likely that only with the discovery of the true pathogenesis of endometriosis will more efficacious therapy emerge as well as preventative measures for younger women. The three different endometriosis entities—endometriomas, implants, and retrocervical septum disease—could develop along distinct routes, but overlapping mechanisms are probably at play for at least some of these. The disease has multitudinous theories for pathogenesis, yet only a handful continue to be proffered as valid: (1) retrograde menstruation (Sampson's theory), (2) metaplastic transformation (Meyer's theory), (3) lymphatic or hematogenous embolization (Halban's theory), (4) tissue relocation (i.e., iatrogenic surgical displacement of endometrium during laparoscopy or cesarean section), and (5) immune dysregulation leading to deficient clearance of ectopic endometrial tissue.

Over the years each theory has received indirect corroboration. Retrograde menstruum from the fallopian tubes into the pelvis and beyond has been supported by identifying menstrual tissue refluxing from the fallopian tubes during surgery and the identification of fresh endometrial lesions during menstrual phase laparoscopy. In addition, the baboon model of endometriosis is, in effect, iatrogenic retrograde menses invariably leading to the development of scattered lesions [12]. Lastly, a greater frequency of lesions in the right subphrenic region and left hemipelvis/ovary supports retrograde menses, since these locations follow the natural tendency of intra-abdominal peritoneal flow and obstruction via the falciform ligament. Unclear, however, is why endometriosis is not then found in all women, given the ubiquitous nature of retrograde menstruation.

Metaplasia of the coelomic epithelium seems equitable given that both peritoneal and endometrial tissues emanate from coelomic cells. Zheng et al. have shown histologic, morphologic evidence of transitioning ovarian surface epithelium into endometriotic cells, corroborating a metaplastic process [13]. A corollary to this postulate is that of the embryogenetic theory or Müllerianosis: misplaced endometrial tissue during the embryologic period of organogenesis. Signorile et al. demonstrated the presence of ectopic endometrium in 9% of 101 human female fetuses [14]. With endogenous estradiol stimulation later in life, this tissue could grow and thus present as ectopic implants. Theoretically, deep retrocervical septum lesions could derive from such abnormal embryogenesis.

Newer, exciting pathophysiology theories borrow from the traditional theories and, most significantly, build upon these premises in order to better grasp the true etiology. For instance, stem cells originating from the bone marrow (Meyer's theory) have been found to populate eutopic endometrium (Halban's theory) that may then be shed (Sampson's theory) into the peritoneal cavity. Vercellini et al. provided another concept for the development of endometriomas when they described how a hemorrhagic corpus luteum may progress to an endometrioma [15]. If this were truly an endometrioma, then retrograde menses (Sampson's theory) would be a prerequisite to seed the cyst contents with endometrial cells.

Underlying virtually all of these theories is the molecular underpinnings of the disease and, in particular, the inherent immune dysfunction that could at once promote and sustain endometriosis [16]. The aberrant immune factors found in women affected with endometriosis could explain why some develop the disease while others do not. The chronic inflammatory milieu can impair normal clearance of endometrial tissue and encourage adherence/invasion, angiogenesis, and nerve fiber innervation [17].

24.12 Mechanism of Infertility

Even though there is a purported association between infertility and endometriosis, the mechanism of this association remains complex and is not completely understood [18]. A population based cohort study using record linkage comparing 5375 women with surgically confirmed endometriosis with outcomes in 8710 women without endometriosis revealed an increased risk of miscarriage [19]. The following factors may explain a diminished fecundity:

- *Anatomical changes*. Endometriosis, when moderate or severe, will often lead to peritubal or periovarian adhesions, thus compromising tubal motility and ovum capture.

- *Immunological factors*. The peritoneal fluid of women with endometriosis has an abnormal level of cytokines, prostaglandins, growth factors, and inflammatory cells, which are likely to participate in the etiology and/or sustenance of endometrial implants. These alterations negatively affect sperm motility, oocyte maturation, fertilization, embryo survival, and tubal function.
- *Effect on embryo development and implantation*. Patients with stage I and II endometriosis have high levels of anti-endometrial antibodies, which may reduce implantation. IL-1 and IL-6 are elevated in the peritoneal fluid of patients with endometriosis and are embryotoxic. Expression of HOXA10 and HOXA11 genes, which are usually upregulated during the secretory phase of the menstrual cycle, is not upregulated in patients with endometriosis. These genes regulate the expression of α(alpha)vβ(beta)3 integrin, which plays a crucial role in the embryo's ability to attach to the endometrium. A decrease in αvβ3 and L-selectin expression has been reported in patients with endometriosis, which might explain the decrease in implantation.

24.13 Mechanism of Pain

Pain associated with endometriosis is quite complex. Pain associated with advanced disease can be caused by extensive adhesions, ovarian cysts, or deeply infiltrating endometriosis. Expression of nerve growth factor is associated with endometriosis pain. Sensory nerve fibers have been found more frequently in the functional layer of the endometrium of women with endometriosis than those unaffected by the disease. Finally, discrete changes in the central pain system (i.e., regional gray matter volume) may contribute to chronic pain in women with endometriosis [20].

Even patients with early-stage disease (few scattered implants) can experience severe pain. This pain can be explained in part by the increase in prostaglandins. In contrast to the normal endometrium (referred to as eutopic endometrium), ectopic endometrium (endometriosis) is the site of at least two molecular aberrations that result in the accumulation of increasing quantities of estradiol and prostaglandin E2 (PGE2). With the first aberration, activation of the gene that encodes aromatase increases, leading to increased aromatase activity in endometriotic tissue. This activation is stimulated by PGE2, which is the most potent inducer of aromatase activity in the endometriotic stromal cells. The second important molecular aberration in endometriotic tissue is the increased stimulation of COX-2 by estradiol, which leads to increased production of PGE2. This establishes a circular event leading to accumulation of PGE2 in the endometriotic tissue.

24.14 Treatment of Endometriosis in the Infertile Couple

It is estimated that in an infertile couple with stage I or II endometriosis, the monthly fecundity rate is 3% per cycle. Medical suppressive therapy with an oral contraceptive agent or gonadotropin-releasing hormone agonist (GnRHa) does not improve the pregnancy rate prior to trying non-assisted reproductive technology.

24.15 Surgical Treatment

Surgical treatment of minimal or mild (stage I-II) endometriosis improves the spontaneous pregnancy rate; however, the absolute benefit is small. A meta-analysis of the two randomized clinical trials investigating this question showed a mild improvement with a number needed to treat (NNT)—that is, the number of persons that would need to be treated surgically to achieve an extra pregnancy—of 12 (95% CI, 7, 49).

Postoperative suppressive medical therapy after surgical treatment does not improve fertility. The only value of medical suppressive therapy (i.e., GnRHa) may be before in vitro fertilization (IVF) [21]. In these cases, the use of GnRHa for 3–6 months prior to IVF increases the clinical pregnancy rate by a factor of 4 (OR 4.28, 95% CI, 2, 9.15). There are few studies along these lines with significant design heterogeneity giving one pause as to its validity. Moreover, it is not clear for the moment if a specific disease severity may have a better response to such suppressive therapy.

If the patient does not wish surgical therapy, then the next step is either IVF or treatment with

superovulation and intrauterine insemination (IUI) followed by gonadotropins and IUI. There is insufficient evidence to recommend surgery prior to IVF.

In advanced disease, surgical management improves fertility. However, this surgery is complex and requires meticulous dissection. If an initial surgery for advanced disease fails, subsequent surgery is less successful than IVF in establishing a pregnancy and should be reserved for patients who require management of pain.

24.16 In Vitro Fertilization

In a retrospective cohort study, the diagnosis of endometriosis (without endometriomas) was associated with similar IVF pregnancy rates [22] compared with tubal factor infertility. IVF offers the best fecundity rate for those with endometriosis. Admittedly, cost and fewer supernumerary embryos may be a limiting factor.

24.17 Endometriomas

Endometriomas larger than 4 cm should be removed to confirm they are benign. In a randomized trial, excision of the endometrioma was associated with less recurrence and higher spontaneous fertility rates than fenestration and bipolar coagulation [23]. IVF outcomes have been reported to be similar in patients with and without endometriomas. However, the number of oocytes, fertilization rates, and the number of embryos obtained were decreased in women with endometriomas compared with those without endometriomas. The preponderance of evidence suggests that in symptomatic women, one can safely remove endometriomas without compromising ovarian function or the success of assisted reproduction. Accumulating studies reflect a detrimental impact from surgery on ovarian reserve as assessed by anti-Müllerian hormone levels [24]. Accidental puncture of an endometrioma during oocyte retrieval may cause infection or contamination of follicular fluid. Histologic confirmation of the benign nature of a large endometrioma may be prudent.

Interestingly, in a retrospective chart review, the higher the rate of endometrioma recurrence, the greater the antral follicle count in the affected ovary. This may be a consequence of less ovarian trauma at the time of cystectomy [25]. Simple drainage of an endometrioma has been shown to be ineffective.

24.18 Treatment of Patients with Chronic Pelvic Pain

24.18.1 Medical Management for Pain

Several classes of drugs have traditionally been used to manage pain associated with endometriosis (Table 24.2). Progestins or combined oral contraceptives and nonsteroidal anti-inflammatory drugs are used as first-line therapy for chronic pain associated with endometriosis. In a prospective, randomized controlled trial comparing combined oral contraceptives with GnRHa, both treatment arms led to similar pain relief [26].

24.18.2 Progestins

Subcutaneous medroxyprogesterone acetate (Depo-subQ provera 104, Pfizer, New York, NY, USA) administered every 12–14 weeks subcutaneously was approved by the US Food and Drug Administration (FDA) for the treatment of endometriosis-related pelvic pain. The bone loss seems to be less pronounced than with the use of GnRHa without add-back therapy. However, there are no data yet with the prolonged use of Depo-subQ, and the recommendation is not to use the drug for more than 2 years unless other methods are unacceptable. Note that the rate of abnormal vaginal bleeding while on Depo-subQ was 17%.

There are several other progestins that have been used for the treatment of endometriosis-associated pelvic pain. The FDA also approved norethindrone acetate (NETA) 5 mg daily with a GnRHa. Through NETA's estrogenic activity, there is a beneficial effect on both bone mineral density and vasomotor symptoms; 5 mg of NETA is equivalent to 20–30 μg of oral ethinyl estradiol. The levonorgestrel intrauterine system has been successfully used for symptomatic endometriosis. Trials have demonstrated pain relief and decreased menstrual blood loss.

Table 24.2 Drugs used for the treatment of endometriosis[a]

Class	Drug	Dosage
Androgen	Danazol[b]	100–400 mg PO twice a day
		100 mg per vagina daily
Aromatase inhibitor	Anastrozole[c]	1 mg PO daily
	Letrozole[c]	2.5 mg PO daily
Estrogen–progestin combinations	Monophasic estrogen/progestin[b]	Low ethinyl estradiol dose continuously
Gonadotropin-releasing hormone agonist	Goserelin[b,c]	3.6 mg SC monthly (10.8 mg IM every 3 months)
	Leuprolide depot[b,c]	3.75 mg IM monthly (11.75 mg IM every 3 months)
	Nafarelin[b,c]	200 μg intranasally twice a day
Gonadotropin-releasing hormone antagonist	Cetrorelix	3 mg SC weekly
Progestin	Depo-subQ provera 104[b]	104 mg/0.65 mL SC every 3 months
	Dienogest	2 mg PO daily[d]
	Etonogestrel-releasing implant	1 for 3 years
	Levonorgestrel-releasing IUS	1 for 5 years
	Medroxyprogesterone acetate	30 mg PO daily for 6 months, followed by 100 mg IM every 2 weeks × 2 months, then 200 mg IM monthly × 4 months
	Norethindrone acetate[b]	5 mg PO daily

SC subcutaneously, *IM* intramuscularly, *IUS* intrauterine system
[a]Adapted from [4]
[b]FDA approved for endometriosis
[c]With add-back therapy, that is, norethindrone acetate 5 mg daily + vitamin D 800 IU daily + calcium 1.25 g daily
[d]Dienogest is a 19-nortestosterone derivative that is approved in the European Union for the treatment of endometriosis. It is not available in the USA as a separate drug. It is only available in the oral contraceptive Natazia (Bayer Pharmaceuticals, Montville, NJ, USA) (estradiol valerate/dienogest), which is a newer four-phasic pack that contains dienogest

24.18.3 Gonadotropin-Releasing Hormone Agonists

GnRH agonists can be given via an intramuscular (leuprolide acetate), subcutaneous (goserelin), or nasal (nafarelin) route. After an initial increase in gonadotropins during the first 10 days, there is a decrease in pituitary secretion secondary to GnRH receptor downregulation. These drugs are typically given for an initial 6-month course. The majority of patients (75–80%) in clinical trials responded. However, many patients will have recurrence of pain within 5 months. A main concern is the progressive loss in bone mineral density. Menstrual periods return between 2 and 3 months after the last monthly injection, but recovery of bone mineral density takes more time.

Add-back therapy with estrogens or progestins was introduced as a way to reduce the hypoestrogenic side effects of GnRHa, especially the loss in bone mineral density, but also vasomotor symptoms and atrophic vaginal mucosa. Other symptoms such as insomnia, mood disorders, and cognitive dysfunction may occur. The mean decrease in bone mineral density after 1 year of GnRHa treatment without add-back is between 3 and 7%.

Add-back therapy has been proposed for both short-term (less than 6 months) and long-term use (more than 6 months). Many studies have shown that the efficacy is not reduced. Norethindrone (5 mg orally daily) is the most commonly used add-back regimen. Low-dose estrogen (conjugated equine estrogen 0.625 mg) can also be added to the norethindrone without loss of benefit in symptom control. Higher doses of estrogen (i.e., combined oral contraceptives) are associated with diminished efficacy in relieving pain symptoms.

24.18.4 Aromatase Inhibitors

Aromatase inhibitors have been successfully used in a limited number of cases of persistent disease after hysterectomy and bilateral oophorectomy as well as in patients with intact pelvic organs. The putative mechanism is by suppression of locally produced estrogens from aromatase activity expressed by the endometriotic cells. Typically, an aromatase inhibitor such as letrozole 2.5 mg or anastrozole 1 mg daily is given with NETA (5 mg daily) to prevent ovarian cyst formation and a possible decrease in bone mineral density.

24.18.5 Surgical Management for Pain

Two prospective randomized controlled studies have clearly shown that surgical therapy is superior to no treatment for relief of pain from endometriosis [27]. One randomized controlled trial with only 16 women did not show a statistically significant difference in pain relief [28].

Several observations can be surmised from these studies: (1) surgery is more effective than simple diagnostic laparoscopy in the treatment of pain associated with endometriosis; (2) there is a significant placebo effect associated with surgery, especially early on (3 months), that persists in approximately 20% of patients; (3) between 20 and 40% of patients will not respond to surgery and will continue to experience pain and (4) surgery is least effective for early-stage disease.

It is unclear if excision of endometriosis is superior to simple ablation by cautery or laser. Postoperative treatment with suppressive therapy with a GnRHa for 6 months may delay recurrence of symptoms.

24.18.6 Neurectomy

Interruption of the cervical and uterine sensory nerves by transection of the uterosacral ligaments, a uterosacral nerve ablation, has been shown not to have any long-term benefit [29]. A presacral neurectomy has been shown to be beneficial in patients with chronic pelvic pain and endometriosis when performed with surgical treatment of the endometriosis lesions [30]. The procedure is associated with greater relief of midline pain rather than lateral pain. The success of the procedure is dependent on excision of the superior hypogastric plexus (presacral nerve) before extensive branching has occurred. The most common postsurgical complications that have limited its use are constipation and urinary urgency.

24.18.7 Hysterectomy

Hysterectomy with or without bilateral salpingo-oophorectomy can be considered in patients whose disease fails to respond to conservative management and who do not desire future fertility. Most studies have shown significant pain relief from definitive surgery. Caution should be used in recommending oophorectomy in women less than 40 years of age [31].

24.18.8 Post-Hysterectomy Recurrence

Endometriosis can recur in 5–10% of patients after hysterectomy and bilateral salpingo-oophorectomy. The role of hormone replacement therapy after surgical castration is controversial. There is a possibility of symptom or disease recurrence (3.5%). On the other hand, there is the real possibility of severe vasomotor symptoms and osteoporosis. Hormone replacement therapy is not contraindicated, and the risks and benefits should be discussed with the patient.

24.18.9 Retrocervical Septum Endometriosis

The management of retrocervical septum endometriosis is extremely difficult and usually involves the rectosigmoid. Patients usually have severe

symptoms that may involve the gastrointestinal tract such as constipation, diarrhea, and painful bowel movements. However, some patients with retrocervical endometriosis are asymptomatic. These patients do not need treatment.

24.19 Management of Endometriosis on Extragenital Organs

24.19.1 Gastrointestinal Endometriosis

Although gastrointestinal symptoms are quite common in women with endometriosis, the overall incidence of bowel involvement is reported to be around 5%. Endometriosis of the gastrointestinal system typically involves the rectum or rectosigmoid. Recurrence of disease after hysterectomy and oophorectomy more commonly involves the bowel. Excision of disease or intestinal resection can be performed by laparotomy or by laparoscopy. Rectovaginal fistula and abscess formation are the most serious complications reported.

24.19.2 Respiratory System

Diaphragmatic endometriosis can be asymptomatic and noted incidentally at diagnostic laparoscopy (Fig. 24.3). Symptomatic patients often report right-sided chest pain or shoulder pain in association with menstruation that occasionally radiates into the neck or arm and dyspnea. Asymptomatic diaphragmatic lesions do not need treatment. Electrosurgery, laser, or surgical excision should be performed carefully because the thickness of the diaphragm ranges between 1 and 5 mm.

Thoracic endometriosis most commonly presents as a right-sided catamenial pneumothorax but can also be manifested by hemothorax, hemoptysis, or pulmonary nodules. The typical symptoms are chest pain and dyspnea. Approximately 30% of these women have pelvic endometriosis at the time of surgical management of the thoracic disease. A chest CT scan may demonstrate pulmonary or pleural nodules, especially if performed during a menstrual period. Chemical pleurodesis is associated with a lower recurrence rate of catamenial pneumothorax than hormonal treatment. However, initial treatment with hormonal therapy is indicated.

24.19.3 Genitourinary System

Endometriosis often involves the peritoneum over the ureter. However, direct ureter involvement is uncommon and has been reported in less than 1% of patients; it is predominantly left-sided (63%) when this is observed. Ureteral involvement can be the result of extrinsic compression from extensive endometriosis that surrounds the ureter with significant fibrosis. The majority of the patients have significant involvement of the retrocervical septum with nodules that are often greater than 3 cm. Preoperative imaging studies such as MRI with contrast should be used to evaluate the renal system preoperatively in patients with retrocervical disease. Medical therapy has

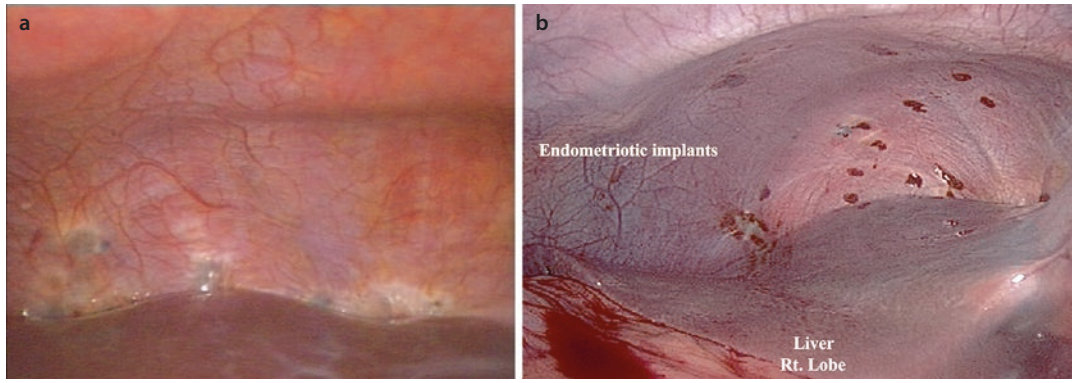

Fig. 24.3 **a** This figure shows fibrotic-type endometriosis involving the right hemidiaphragm. The lesions are seen above the liver. Most of the lesions are obscured by the liver. **b** Hemorrhagic-type endometriosis lesions of the right hemidiaphragm

been used successfully in a limited number of patients. Most cases of ureteral endometriosis can be treated with excision of the periureteral fibrosis and active lesions without ureter resection.

24.19.4 Sciatic Nerve Involvement

Patients with endometriosis of the sciatic nerve can present with hip pain, which is usually localized to the buttock. The pain radiates down to the back of the leg, and numbness occurs in areas innervated by the sciatic nerve. The symptoms typically occur in association with a menstrual period but then extend into other times of the cycle. Progressive leg and foot muscle weakness with electromyogram studies showing denervation can be demonstrated. MRI typically shows a lesion infiltrating the sciatic nerve. CT-guided biopsy can be used to confirm the diagnosis. Two-thirds of cases are localized to the right side. Most cases have pelvic endometriosis associated with this disease. Treatment with a GnRHa and add-back therapy have been shown to reverse the neurologic abnormalities.

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Contraception and Sterilization

Jaclyn M. Grentzer, Caitlin Parks, and Jeffrey F. Peipert

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25.1 Introduction

Unplanned and teen pregnancies have been major public health problems in the USA for decades. Unplanned pregnancies are high-risk pregnancies; they are more likely to result in low birthweight, preterm birth, and other adverse outcomes. In addition, women who experience unplanned pregnancy are more likely to experience socioeconomic deprivation, abuse, and are less likely to seek prenatal care.

A recent update from the National Survey of Family Growth has demonstrated a reduction in the rate of unintended pregnancy from 54 per 1000 women ages 15–44 years of age to 45 per 1000 in 2011 [1]. This 18% decline is the first significant decline in decades. Changes in contraceptive use, including use of the most effective contraceptive methods such as the intrauterine device (IUD) and implant, is believed to be at least partially responsible for this decline. Despite this reduction, the USA still has one of the highest rates of unplanned pregnancies among developed countries.

In this chapter, we will review how to discuss and counsel women regarding contraceptive methods including long-acting reversible contraception or LARC. A brief description of the Center for Disease Control (CDC) Medical Eligibility Criteria (MEC) will be presented. We then will review the major risks and benefits of common contraceptive methods used in the USA, including both reversible methods and sterilization.

Clinical Case

A 17-year-old female presents for advice regarding contraception. She is a high school senior, currently sexually active with a male partner, and is using condoms for contraception. Her body mass index is 34. She desires a more reliable and effective contraceptive method.

25.2 Addressing Contraception with Patients

25.2.1 Contraceptive Counseling

Contraceptive counseling requires a highly individualized discussion based on a woman and her partner's values, priorities, experiences, medical conditions, and reproductive life plan. Reproductive life planning can be helpful in guiding the range of options for each woman. A model of shared decision-making in which the provider effectively offers expert, evidence-based information, while the patient is able to be an autonomous decision-maker should guide the contraceptive counseling session.

Frameworks for contraceptive counseling include tier-based, where methods are described from most to least effective, and preference-based, where methods are described based on what is most important to the woman. Often, the two significantly overlap, as method effectiveness is commonly one of the most important factors in a woman's contraceptive decision-making [2]. The framework of long-acting reversible contraception (LARC) first puts emphasis on intrauterine devices (IUDs) and contraceptive implants as first-line contraceptive methods, as these are the most effective reversible methods and have high satisfaction and continuation rates, as well as important non-contraceptive benefits [3]. When counseling women about contraceptive effectiveness, it is important to discuss that this is generally estimated based on typical use, as opposed to perfect use, in order to give patients accurate estimates of what they may experience.

25.2.2 Recommendations for the Use of Contraception by Women with Medical Conditions

Many women have medical conditions that must be considered when choosing a contraceptive method. However, in these women the potentially greater risks associated with pregnancy must be weighed against those associated with contraceptive use. The Centers for Disease Control (CDC) and World Health Organization (WHO) provide evidence-based guidance for the use of each contraceptive method based on current or previous medical conditions. Updated in 2016, the United States Medical Eligibility Criteria (U.S. MEC) guidelines classify the risk of each contraceptive method/condition combination into categories that health-care providers can use when assessing the safety of contraceptive method use. The accompanying *Selected Practice Recommendations* also addresses timing of initiation, when a

back-up method is needed, and recommendations for the management of side effects and other problems with contraceptive method use [4]. U.S.MEC definitions are as follows:

- Category 1 comprises conditions for which no restrictions exist for use of the contraceptive method.
- Category 2 indicates the method generally can be used because the benefits outweigh the risks, but careful follow-up may be required.
- Category 3 indicates that the use of that method usually is not recommended unless other, more appropriate methods are not available or acceptable.
- Category 4 comprises conditions that represent an unacceptable health risk if the method is used.

Classification of risk is primarily related to the hormonal composition of the contraceptive method. Methods are generally categorized as combined hormonal contraceptives (CHCs), progestin-only methods, and non-hormonal methods. Combined hormonal contraceptives (CHCs), including the combined hormonal oral pill, transdermal patch, and vaginal ring, contain a synthetic estrogen and progestin. Progestin-only contraceptives include progestin-only pills (POPs), injectable depo-medroxyprogesterone acetate (DMPA), the etonogestrel-containing contraceptive implant, and the levonorgestrel-containing IUDs (LNG-IUS). Non-hormonal methods include the Copper IUD, barrier methods, fertility-based awareness methods and sterilization procedures.

Cardiovascular Risks

There is a pro-thrombotic effect of estrogen that is believed to be additionally modulated by the combination with progestin to produce the cardiovascular risks associated with CHCs. However, progestins alone are not believed to have an impact on the clotting system; therefore, progestin-only methods are good options for women at high risk for thrombotic events. The main risks related to CHCs are venous thromboembolism (VTE), myocardial infarction (MI), and stroke. These risks are primarily of concern in women with additional risk factors for these conditions (e.g., older age, hypertension, smoking, obesity, diabetes, etc.)

Myocardial Infarct/Stroke

Modern formulations of CHCs do not increase the risk of MI or stroke in otherwise healthy, non-smoking women under the age of 35. The risks for MI in CHC users increase with age, but are greatly magnified by the combination of age, smoking, and hypertension. Smoking and hypertension also increase the risk of stroke, especially in women over 35 [5, 6]. Therefore, the use of CHCs in women over age 35 who smoke and women of any age with hypertension, multiple risk factors for cardiovascular disease, or current/past ischemic heart disease or stroke is not recommended (U.S. MEC 3 or 4). Stroke has also been found to be more common in CHC users who experience migraines, with higher risk among those experiencing migraines with aura and those over age 35 (U.S. MEC 3 or 4) [7].

Venous Thrombo-Embolism

The relative risk of VTE in CHC users is increased relative to non-users, but the absolute risk is still very low (Table 25.1). However, this risk is higher in women with other risk factors for VTE. Therefore, CHC use is not recommended in women with a history of VTE, known thrombophilia, including lupus with positive or unknown antiphospholipid antibody status, known thrombogenic mutations, or active cancer (U.S. MEC 3 or 4). However, studies have shown that universal screening for thrombogenic mutations before initiating CHCs is not cost-effective because of the rarity of the conditions and the high cost of screening [13]. Obese CHC users are more likely than non-obese CHC users to experience VTE, but the absolute risk in otherwise healthy obese women of reproductive age is small [14–15]. Limited evidence suggests that obese women who use CHCs do not have a higher risk for acute myocardial infarction or stroke than do obese nonusers [16–17]. Therefore, CHC use is not restricted in obese women (U.S. MEC 2), but caution is advised in women who have other risk factors for VTE or cardiovascular disease.

Weight Gain

The only contraceptive method shown to be associated with weight gain is DMPA. One randomized trial of DMPA vs. placebo showed no increase in weight gain over 3 months [18]. However, another study found that 25% of DMPA-users gained more than 10% of their

Table 25.1 Comparative risks of venous thromboembolism [8–12]

Population	Incidence per 10,000 women/year
General population of women <35 years old	1–5
Oral contraceptive pills (OCPs) users	3–10
Second generation progestin-containing OCPs	6
Drospirenone-containing OCPs	10
Pregnant women	5–20
Immediate postpartum women	45–65
Leiden mutation carriers	30–80
Leiden mutation carriers PLUS OCPs	50–100
Homozygous Leiden mutation carriers	400–800

baseline weight [19]. However, it is unclear what causes the weight gain (e.g., increased appetite versus change in metabolism), and it is difficult to predict who will be affected. Some reports have found that higher baseline weight may predict greater weight gain while using DMPA; however, this finding is not consistently reported [20]. Data about weight gain while using the contraceptive implant is mixed, but the mean increase was less than 5 pounds over 2 years [21]. CHCs and IUDs have not been found to be associated with weight gain [22].

DMPA

Limited evidence suggests that among women with uncontrolled hypertension, those who used DMPA had a small increased risk for cardiovascular events compared to women using non-hormonal methods. However, these risks were significantly less than among CHC users [23]. Therefore, DMPA is listed as U.S. MEC 3 in women with uncontrolled hypertension, ischemic heart disease, multiple risk factors for cardiovascular disease, lupus with positive or unknown antiphospholipid antibody status or antiphospholipid syndrome, or current or past ischemic heart disease or stroke. However, risks of using DMPA must be weighed against the risks of pregnancy in women with uncontrolled hypertension or other comorbidity.

Breast Cancer

Because breast cancer is a hormonally sensitive tumor, there is concern that the prognosis for women with current or recent breast cancer might worsen with the use of estrogen or progestin-containing methods. Therefore, the use of any hormonal method of contraception is not recommended in women with current or past breast cancer (U.S. MEC 3 or 4). Given the lower systemic concentrations of progestin associated with the LNG-IUS, there is less concern related to this method, but the data are insufficient to make a definitive recommendation. Given the paucity of data, decision-making should be individualized, ideally within a multidisciplinary team. Current evidence does not suggest that any contraceptive method further increases the risk for breast cancer among women with either a family history of breast cancer or a known breast cancer susceptibility gene [24]. Therefore, no contraceptive method is restricted among these women (all methods U.S. MEC 1).

Depression

There is no increased risk of clinical depression in women using CHCs; in fact, Keyes and colleagues noted that CHC use may even be associated with lower rates of depressive symptoms among young women [25]. Women who report mood changes with the use of CHCs often

experience this during the hormone-free periods, indicating that estrogen withdrawal may be responsible. In these cases, continuous or extended use formulations may be helpful. If mood changes occur during use of active pills, switching to a lower dose estrogen is reasonable. Data evaluating the impact of DMPA on depression are limited and conflicting, with few studies showing a small increased likelihood of depression in DMPA users and most others showing no association. However, it is reasonable to discontinue any method if a woman reports worsening depression with use. Given the risks of uncontrolled depression in pregnancy, an alternative method of contraception is essential, as well as ensuring adequate mental healthcare.

Intrauterine Devices (IUDs)

IUDs are first line-contraceptive options, and there are few contraindications to their use. Medical conditions of concern with the use of IUDs are limited, and mostly relate to infections and abnormalities of the genital tract. Having an IUD does not significantly increase the risk of acquiring sexually transmitted infections (STIs) or pelvic inflammatory disease (PID) [26]. Therefore, being at high risk for STIs, a history of STI, or immunocompromise does not preclude the use of an IUD. However, there is an increased risk of PID when an IUD is placed in the setting of current purulent cervicitis or a known chlamydial or gonorrheal infection (U.S. MEC 4). In these cases, treatment should be initiated prior to insertion.

Significant distortion of the endometrial cavity due to fibroids, synechiae, or uterine anomalies may make IUD placement challenging, and is not recommended (U.S. MEC 4); however, determination of cavity distortion should be based on ultrasonographic findings, rather than physical exam alone. IUDs should not be initiated in women with cervical or endometrial cancer. However, the use of the LNG-IUS for women with low-grade endometrial cancer desiring fertility preservation is currently under study. Based on theoretical data, placing an IUD in the setting of gestational trophoblastic disease (GTN) with decreasing or undetectable bHCG level is generally not recommended (U.S. MEC 3). GTN with persistently elevated bHCG or malignant disease is MEC category 4.

25.2.3 Special Populations

Postpartum Women

The duration of postpartum infertility is variable and unpredictable. In non-breastfeeding women the first ovulation occurs as early as 25 days postpartum, but fertile ovulation generally does not occur until at least 42 days postpartum. In women who are exclusively breastfeeding and experience amenorrhea, infertility may extend up to 6 months postpartum, but timing of return to fertility is also unpredictable [27]. Regardless of breastfeeding status, postpartum women should not use combined hormonal contraceptives during the first 3 weeks after delivery because of concerns about increased risk for VTE (U.S. MEC 4). Limited evidence suggests that postpartum women with additional risk factors for VTE (i.e. age >35 years, obesity, cesarean delivery, smoking, etc.) generally should not use CHCs until 6 weeks after delivery (U.S. MEC 3). Due to concerns of decreased milk supply, CHCs are also not recommended in breastfeeding women until at least 4 weeks postpartum, when milk supply is generally established. All progestin-only and non-hormonal methods have been shown to be safe while breastfeeding and can be initiated immediately postpartum (U.S. MEC 1 or 2) [28].

Adolescents

Adolescents have high failure and discontinuation rates with the pill, patch, ring, DMPA, and condoms.

In addition, studies have demonstrated a twofold increased risk of contraceptive failure in young women (<21 years of age) using combined hormonal methods. Based on a large body of evidence demonstrating safety, including no evidence of increased risk of infertility, ACOG has recommended that clinicians consider LARC as first-line methods for adolescents [29]. Many young adolescents (age 14–17 years of age) seem to prefer the implant over an IUD, while older adolescents (18–20 years) favor the IUD. When consistently offered to adolescents and young women, these methods have been shown to have high rates of initiation, satisfaction, and continuation, and lead to a decrease in the risk of unintended pregnancy among adolescent women [30].

25.3 Contraceptive Methods

25.3.1 Long-Acting Reversible Contraception

Long-acting reversible contraceptive (LARC) methods include contraceptive implants and IUDs. LARC methods are highly effective (<1% failure rate), safe, and have high user continuation and satisfaction [31–33]. Effectiveness of these methods does not depend on user adherence, and thus, typical use effectiveness is similar to perfect use effectiveness.

Contraceptive Implants

Contraceptive implants consist of one or more rods containing a progestin that are inserted subdermally. Currently, the only contraceptive implant available in the USA is an etonogestrel (ENG)-releasing single-rod implant, Nexplanon®. Etonogestrel is a metabolite of desogestrel, a third generation progestin with low androgenicity. The ENG implant is FDA-approved for 3 years of use; however, emerging data indicate that it may be effective for a longer period of use. The ENG implant is the most effective contraceptive method currently available with a 0.05% annual failure rate. The implant provides contraceptive protection through suppression of ovulation and thickening of the cervical mucus. Some non-contraceptive benefits of the implant include improved acne, dysmenorrhea, and relief of pelvic pain due to endometriosis. The implant is associated with a rapid return to fertility, with resumption of ovulation within 3 weeks of removal. One study evaluating 200 parous implant users found that 96% of women using no contraception following removal of their implant conceived within 12 months [34]. The primary reason for discontinuation reported by users of the implant is irregular and unpredictable bleeding, where bleeding patterns can range from amenorrhea to frequent or prolonged bleeding. Bleeding patterns are highly variable, but the most commonly reported patterns are amenorrhea and infrequent bleeding (see Fig. 25.1). Other less commonly reported reasons for discontinuation include weight gain, emotional lability/mood changes, headaches, and breast discomfort [35].

As stated above, the implant can be used in women who are postabortion, postpartum, and lactating. There is no evidence that the implant significantly impacts breastfeeding or newborn growth. The ENG implant is also safe and effective in special populations of women including adolescents, overweight and obese women, and women with contraindications to estrogen use.

Intrauterine Devices

Intrauterine devices (IUDs) are plastic T-shaped devices, often containing an additional active ingredient, with a string attached to one end of the device. IUDs are inserted into the uterus by a trained clinician. There are five type of IUDs currently used in the USA: 4 levonorgestrel (LNG)-containing IUDs (Mirena®, Liletta®, Kyleena® and Skyla®) and one copper (Cu)-containing IUD (ParaGard®).

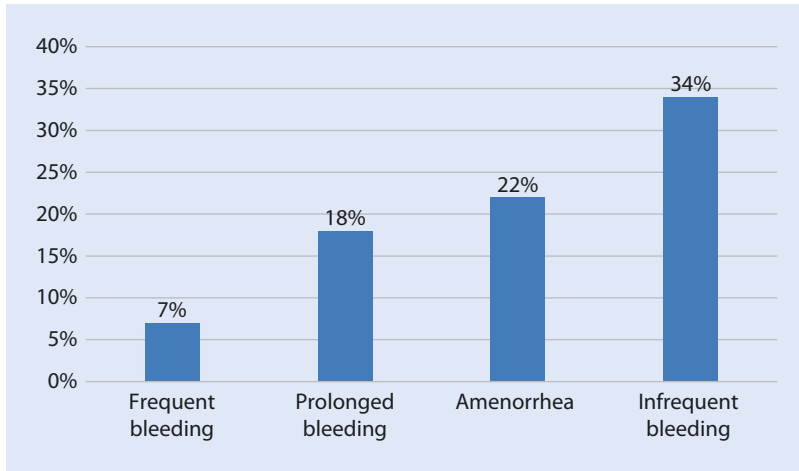

Fig. 25.1 Bleeding patterns associated with the single-rod etonogestrel -containing subdermal contraceptive implant [36]. Data source: Mansour D, Korver T, Marintcheva-Petrova M, & Fraser IS. The effects of Implanon® on menstrual bleeding patterns. Eur J Contracept Reprod Health Care, 2008; 13(Suppl 1):13–28

25.3.2 Contraceptive Effects

Hormonal IUDs (also called LNG-IUS: levonorgestrel-releasing system) prevent pregnancy by thickening the cervical mucus, suppressing the endometrium, impairing sperm function, and partially suppressing ovulation. They have a 0.1–0.2% yearly failure rate [31]. The LNG-containing IUDs have low levels of systemic absorption of levonorgestrel. Thus, these methods do not typically produce progestin-like side effects. Both the Mirena® & Liletta® IUDs contain 52 mg of levonorgestrel, while the Kyleena® and Skyla® contain lower doses (19.5mg and 13mg, respectively) and have smaller frames. Mirena® and Kyleena® are FDA-approved for 5 years (but appear to be effective for at least 6–7 years), while the Liletta® and Skyla® are approved for 3 years. Liletta® follow-up studies are still on-going, but there is no reason to suspect it will have a shorter efficacy period than the Mirena® IUD IUD, which appears to be effective for 6-7 years. The Cu IUD primarily prevents sperm function and has a 0.5–0.8% yearly failure rate. In addition, the Cu IUD is the most effective method of emergency contraception available [37].

IUDs can be placed immediately postabortion or postpartum (ideally within 10 minutes after delivery of the placenta), and are safe for women who are breastfeeding [28]. They are also safe and well tolerated in special populations of women, including adolescents. Fertility returns promptly after removal of the hormonal or copper IUD, and in the majority of women attempting pregnancy after removal of IUD will conceive within 3–6 months after IUD removal. There are no long-term effects on fertility after long-term use of an IUD [38]. There is an approximately 5–10% rate of IUD expulsion, with higher rates reported with immediate postpartum placement.

25.3.3 Bleeding Patterns

The two most common side effects and cause for discontinuation of all IUDs are changes in menstrual bleeding pattern and pain or discomfort. Immediately after IUD insertion, many women experience cramping and irregular bleeding. This typically resolved in days to weeks, but sometimes lasts for longer periods of time. If pain symptoms persist, an evaluation to ensure correct positioning of the IUD is warranted.

LNG-IUS

Non-contraceptive benefits of the LNG-IUS include a decrease in dysmenorrhea, menstrual blood loss, and improvement in bleeding associated with adenomyosis and leiomyomas. Due to this, the LNG-IUS is increasingly being used as the primary mode of conservative management for abnormal uterine bleeding (AUB) due to a variety of etiologies. In many patients, especially those with AUB, amenorrhea may be a beneficial side-effect. Amenorrhea rates have been shown to vary from 15 to 20% by 1 year of use. Women with light or moderate menstrual flow prior to LNG-IUS use are more likely than those with heavier bleeding to report amenorrhea [39]. However, most women report improvement in the amount of bleeding they experience. Because the bleeding experienced may be irregular and unpredictable and some women may not like being amenorrheic, change in bleeding profile can be undesirable and lead to discontinuation.

Copper IUD

Cu IUD users can experience heavier, longer, and/or more painful menses for the first few cycles after insertion. However, cramping and bleeding tends to improve over time. Women using the copper or hormonal IUD report similar long-term satisfaction and continuation rates. Both types of IUDs, copper and hormonal, are associated with a decreased risk of endometrial cancer.

25.3.4 Injectable Contraceptives

Injectable contraceptives are used worldwide and come in many formulations with periods of efficacy ranging from one to 3–4 months. Some require administration every month, others require administration every 3 months. Depot medroxyprogesterone acetate (DMPA), a depot form of a pregnane progesterone very similar to naturally occurring progesterone, is the only injectable available in the USA. DMPA comes in both intramuscular and subcutaneous injections that are administered every 12 weeks (however, contraceptive effectiveness typically lasts 16 weeks). The primary mechanism of action is ovulation inhibition. With typical use, DMPA has a 6% yearly failure rate; however, this rate is much lower with consistent injections every 3 months. Non-contraceptive benefits of DMPA include a decrease in menstrual

blood loss, dysmenorrhea, luteal phase symptoms, discomfort from endometriosis and myomas, endometrial cancer, and sickle cell crises.

Side Effects

The most common reported reason for DMPA discontinuation is change in bleeding pattern, which can range from amenorrhea to prolonged and/or irregular bleeding. Other common causes for discontinuation include weight gain and headaches. For some women, a major disadvantage to DMPA use is its association with a delayed return to fertility. Studies have shown that time to first ovulation is variable, and on average occurs 14–49 weeks from the last injection, with the median delay to conception being 9–10 months after the last injection [40].

Bone Mineral Density

Historically, concern has been raised regarding the association of DMPA use with decreased bone mineral density (BMD). However, studies have demonstrated that DMPA users experienced a temporary and reversible loss in bone density but no increased risk of fracture [41]. As such, the benefits of preventing unintended pregnancy outweigh the theoretical risks associated with decreased BMD, and no restrictions should be placed on the use of DMPA by adolescents or on the duration of DMPA use. The only absolute contraindication to DMPA use is current breast cancer.

25.3.5 Combined Hormonal Contraceptives (CHCs)

Combined hormonal contraceptives (CHCs) are methods containing both an estrogen and progestin. CHC formulations currently available include the combined oral contraceptive pill, transdermal patch, and contraceptive vaginal ring. Evidence indicates that the patch and the ring provide comparable safety and pharmacokinetic profiles to pills with similar hormonal formulations. Therefore, much of the evidence related to the benefits, risks, and side effects are extrapolated from pills to other methods.

Hormonal Composition

Pill formulations are based on the dosage of ethinyl estradiol, the type of progestin, and the length of the pill-free interval (see Table 25.2 for

Table 25.2 Examples of currently available pill formulations

	Name	Estrogen (EE = ethinyl estradiol)	Progestin	Cycle length (hormone/hormone-free intervals)
Usual dosing regimens	Lo Loestrin®	10 mcg EE	1 mg norethindrone	21/7
	Alesse®	20 mcg EE	0.1 mg levonorgestrel	21/7
	Loestrin®	20 mcg EE	1 mg norethindrone	21/7
	Yasmin®	30 mcg EE	3 mg drosperinone	21/7
	Desogen®	30 mcg EE	0.15 mg desogestrel	21/7
	Sprintec®	35 mcg EE	0.25 mg norgestimate	21/7
	Ovral®	50 mcg EE	0.5 mg norgestrel	21/7
Extended dosing regimens	Lo Seasonique®	10 mcg/20 mcg EE	0.1 mg levonorgestrel	84/7
	Yaz®	20 mcg EE	3 mg drosperinone	24/4
	Mircette®	20 mcg EE	0.15 mg desogestrel	26/2
	Seasonale®	30 mcg EE	0.15 mg levonorgestrel	84/7
	Natazia®	2 mg/3 mg estradiol valerate	2 mg/3 mg dienogest	26/2
Progestin-only pills	Micronor®	None	35 mcg norethindrone	28/0

examples). Ethinyl estradiol is a synthetic estrogen formulated to impede first-pass metabolism by hepatic degeneration, thus creating a more bioavailable oral formulation. The most commonly used pills have 30–35 mcg of ethinyl estradiol, but estrogen dose can be as high as 50 mcg or as low as 10 mcg. Doses less than 30 mcg may further decrease the risk of VTE. However, doses lower than 30 mcg result in greater breakthrough bleeding and may have less effect on prevention of ovarian cyst formation and other non-contraceptive benefits of CHCs. The only pill currently available in the USA with an estrogen other than ethinyl estradiol is *Natazia*¸ which contains estradiol valerate and dienogest. This formulation may be superior to ethinyl estradiol preparations in the treatment of heavy menstrual bleeding, with effects similar to the hormonal IUD [42].

Progestins are categorized based on generation of development. First generation progestins include norethindrone, have short half-lives, and typically cause breakthrough bleeding. Second generation progestins, including norgestrel and levonorgestrel, have longer half-lives, but more androgenic side effects. Thus, spurring the development of third generation progestins, including desogestrel, which have the advantage of being anti-androgenic. The contraceptive patch and ring contain ethinyl estradiol and third-generation progestin formulations. Drospirenone is the only progestin derived from spironolactone and is the most anti-androgenic. This benefit is derived from its high binding affinity for progesterone and mineralocorticoid receptors, but low affinity for androgen receptors. This makes drospirenone-containing pills useful for the non-contraceptive benefits of the treatment of acne, hirsutism, and premenstrual dysphoric disorder (PMDD).

25.3.6 Contraceptive Efficacy

The primary mechanism of action of all CHC methods is based on the progestin effects. These mechanisms include thickening of the cervical mucus to prevent sperm entry, and ovulation suppression by negative feedback to suppress luteinizing hormone release through the hypothalamic-pituitary system. Progestins also prevent fallopian tube motility, thus inhibiting sperm transport. The estrogen effect is primarily to stabilize the endometrium and minimize breakthrough bleeding, but it also effectively inhibits ovulation, especially at higher doses. Efficacy rates for all CHC methods are also similar, with 5–9% of women experiencing a pregnancy within the first year of typical use. The significant difference between CHC and LARC effectiveness is due to the daily, weekly, or monthly user-dependence of these methods. There is some evidence that the efficacy of the contraceptive patch may be lower in women weighing over 90 kg (198 pounds) [43]. Therefore, counseling about this risk is important. Continuation rates and satisfaction with various CHC formulations are similar, with 50–55% of users continuing the method for 12 months and 40–43% continuing for 24 months. Continuation and satisfaction with CHC methods is significantly less than with LARC methods [33].

25.3.7 Side Effects

The most commonly reported side effects of CHC methods are headaches, nausea, and breast symptoms, including increase in size, discomfort or pain. Most side effects decrease over time or may be improved by switching to a pill with a lower dose of estrogen. Although many women complain of change in sex drive when using CHCs, there has been no definitive evidence to link this complaint to CHC use and other possible causes should be evaluated. Similarly, although women often perceive weight gain with CHC use, there is no conclusive evidence that CHC use is associated with weight gain.

25.3.8 Non-Contraceptive Benefits

Many of the non-contraceptive benefits of CHCs are mediated through their suppression of hypothalamic gonadotropin-releasing hormone (GnRH) and pituitary gonadotropin secretion. Suppression of follicle-stimulating hormone (FSH) secretion prevents ovarian folliculogenesis, and prevention of the midcycle luteinizing hormone (LH) surge prevents ovulation. In addition, estrogen and progestin work to thin and stabilize the endometrium. These mechanisms mediate the approximately 50% reduction in the risk of ovarian and endometrial cancer seen in CHC users. Additionally, the risk reduction seen begins after only 1 year of use and persists for up to 20 years after use. CHCs also produce

multiple menstrual-related benefits, including regulation or suppression of menses and decreased dysmenorrhea, heavy menstrual bleeding, premenstrual syndrome (PMS), premenstrual dysphoric disorder (PMDD), menstrual migraines, and symptoms of endometriosis. They have also been shown to decrease the frequency of sickle cell crises and catamenial seizures, porphyria, and asthma attacks. Because many of these conditions are related to periods of estrogen withdrawal and bleeding days, extended or continuous use to decrease the number of withdrawal episodes has been found to be more effective than cyclic use. In addition, many of the side effects attributed to CHCs, which are often related to estrogen withdrawal during the hormone free interval (i.e., while taking the placebo pills), can be improved with either shortening of the hormone-free interval or continuous use [44]. The contraceptive ring contains sufficient hormone to last 28 days, and therefore continuous use with monthly replacement is possible with similar contraceptive efficacy. CHCs also inhibit ovarian and adrenal androgen secretion, block conversion of testosterone to dihydrotestosterone, and induce production of serum sex hormone-binding globulin (SHBG). These actions mediate the benefits of improvement in acne and hirsutism associated with CHCs. The use of CHCs has also been shown to decrease the risk of developing and inhibit progression of benign breast conditions, including fibrocystic changes, cysts, and fibroadenomas.

25.3.9 Progestin-Only Pills (POPs)

For women who desire to use contraceptive pills but have contraindications to, or experience side effects with combination estrogen and progestin pills, progestin-only pills (POPs) are a good alternative. POPs available in the USA contain norethindrone, a first-generation progestin, but there are other progestin-only pill formulations available internationally. The primary contraceptive mechanism of action is thickening of the cervical mucus, reduced ciliary function in the fallopian tube, and alteration of endometrium to inhibit implantation. Inhibition of ovulation is variable. POPs are taken daily throughout the cycle without any hormone-free interval. One disadvantage is that POPs contain less hormone and, therefore, requires stricter adherence to daily dosing to maximize efficacy. However, direct comparison of efficacy to CHC pills has been limited and inconclusive. Additionally, POP users have more unpredictable bleeding than CHC users, including irregular cycles and intermenstrual bleeding. POPs may be ideal non-LARC methods for women who experience migraines with aura, and there is some evidence that they may result in improvement in migraine frequency.

25.3.10 Barrier Methods

Barrier methods are non-hormonal methods that are used with each act of intercourse to prevent contact between the sperm and the egg. These include male and female condoms, diaphragm, cervical cap, and spermicides.

Male Condom

Male condoms are the most commonly used barrier method because they are inexpensive, easy to use, and available without a prescription. Most often made with rubber latex (however lambskin or synthetic condoms are also available), and available with or without spermicide, condoms consist of a flexible covering that is applied to a man's erect penis prior to intercourse. Barrier methods can serve the dual function of sexually transmitted infection (STI) and pregnancy prevention; however, barriers are not highly effective contraceptive methods. With typical use, 18% of women will become pregnant each year. For young women and women at high risk for STIs, dual contraceptive method use (use of a highly effective contraceptive method plus barrier method for STI prevention) should be encouraged. Some disadvantages of condom use include lack of spontaneity and the need to use the method with each act of intercourse. Additionally, in couples where one person has latex allergies, traditional latex condoms cannot be used. In these situations, synthetic condoms are good alternatives.

Female Condom

The female condom is a polyurethane sheath with a flexible ring on either end. The closed end inserts into the vagina and the wider ring remains outside of the vagina after insertion. Like the male condom, the female condom is a single use device that is available without a prescription. It is not intended for use with a spermicidal agent. The major benefit of the female condom over the male condom is that it allows the woman to have control over both contraception and STI prevention.

Diaphragm

The diaphragm is a flexible, dome-shaped rubber device that is filled with spermicide and inserted into the vagina prior to intercourse. In proper position covering the external cervical os, the diaphragm sits anteriorly behind the pubic symphysis and extends posteriorly to the posterior fornix. There are two types of diaphragms currently available in the USA. The first is the traditional diaphragm that requires size fitting by a clinician and a subsequent prescription. The second is a new, one-size-fits-most, diaphragm called Caya®. Caya® requires a prescription, but based on its design, does not require fitting by a clinician [45]. Once in place, the diaphragm with spermicide provides up to 6 hours of contraception. After intercourse, the diaphragm is left in place for at least 6 hours. With typical use, it is associated with a 12% yearly failure rate.

Cervical Cap

There is only one cervical cap currently available in the USA—the FemCap®. FemCap® is a device that is shaped to fit over the cervix. Similar to the contraceptive diaphragm, spermicide should be used with the cervical cap. As a barrier method, the cervical cap creates a physical barrier; spermicide adds a chemical barrier at the cervical os. The FemCap is available in three sizes (22, 26, and 30 mm), and there is a strap attached to the cap for removal.

Spermicides

Spermicides are most often used in conjunction with condoms or the diaphragm. However, they can be used alone for contraception. They decrease the risk of unintended pregnancy by preventing sperm from reaching the upper genital tract and subsequently fertilizing an egg. They come in different formulations: gels, creams, foams, films, sponges, and suppositories. The most common spermicide is nonoxynol-9. Spermicide must be used with each act of intercourse, and is associated with a 12–28% yearly failure rate.

25.3.11 Fertility Awareness-Based Methods

The Fertility Awareness-Based (FAB) methods, sometimes referred to as "natural family planning" or "rhythm methods," involve detecting the most fertile days during the menstrual cycle and abstaining from intercourse during these days. There are several FAB methods, including those that require a woman to count the days in her menstrual cycle in order to determine which days she is most fertile (Standard Days Method and Calendar Rhythm Method) and those that involve assessing signs of fertility like changes in basal body temperature or cervical mucus (Two Day Method, Billings Ovulation Method, and Symptothermal Method). With typical use, FAB methods have a 12–24% yearly failure rate. Irregular menstrual cycles (e.g., due to breastfeeding or polycystic ovarian syndrome) may make FAB more difficult to follow.

Withdrawal

Also called "coitus interruptus" or the pull out method, withdrawal is a technique used by a surprisingly high percentage of sexually active adults and teens in the USA. In fact, more than 35 million people worldwide use the withdrawal method. Withdrawal may be one of the world's oldest methods of contraception. The technique is defined as the removal of the penis prior to ejaculation. Many are not aware that sperm may leak out of the penis prior to ejaculation, thus, the 26% failure rate (◘ Table 25.3) with typical use.

25.3.12 Female and Male Sterilization

Sterilization, including both female and male sterilization, is the most commonly used contraceptive method in the USA with 33% of contracepting women in the USA utilizing these methods.

Female Sterilization

Female sterilization involves the occlusion or resection of the fallopian tubes using a variety of methods with the intent to prevent the sperm from fertilizing the egg. Overall, for all female sterilization methods there is a 0.5% failure rate within the first year of use and a 10-year cumulative failure rate of 18.5 per 1000 procedures. There are three commonly used methods of tubal sterilization currently in use: (1) laparoscopic, (2) transcervical (or hysteroscopic), and (3) postpartum.

Laparoscopic tubal sterilization can be achieved using bipolar cautery, permanent (Filshie) clips, silicone (Falope) rings or salpingectomy. Effectiveness of tubal sterilization is not reported

in a standardized fashion. 10-year cumulative failure rates per 1000 procedures for bipolar cautery and silicone rings as reported in the CREST study are 24.8 and 17.7, respectively, whereas studies of permanent clips show a crude failure rate of 36.5 per 1000 women [46]. New data suggests that complete salpingectomy may prevent the development of some types of ovarian cancer; however, there is limited data regarding the effectiveness of this procedure as a contraceptive method. The benefits of a laparoscopic procedure include small incisions and immediate effectiveness. Some disadvantages include need for general anesthesia and a procedure in an operating room.

Transcervical, or hysteroscopic, tubal occlusion has more recently been developed. Currently there is only one method of hysteroscopic tubal occlusion available in the USA, Essure®. For this procedure, a hysteroscope is inserted into the uterine cavity and polyethylene terephthalate fibers surrounded by steel/nickel titanium coil is used to induce fibrosis and subsequent occlusion of the proximal fallopian tubes. Post-market studies and effectiveness models suggest that contraceptive failure of the procedure ranges from 1/1000 procedures performed to as high as 5.7% per year [47]. The benefit of this procedure is that it can be done in an office setting and requires only minimal anesthesia. The drawback to this procedure is that failure to place the coils bilaterally can range from 5–15%, and patients must use a backup method of contraception for 3 months post-procedure until an imaging study confirms occlusion or correct placement of the coils.

Postpartum sterilization is performed either at the time of cesarean section or after vaginal delivery through an infraumbilical minilaparotomy. The most common technique is via resection of a segment of the fallopian tube with ligation of the cut ends. In the CREST study, postpartum partial salpingectomy was the most effective method with a 10-year cumulative failure rate of 7.5 per 1000 procedures [46].

Table 25.3 Contraceptive effectiveness: proportion of 100 women who will experience a pregnancy over 12 months with typical use of the method [31, 50–52]

Method	Proportion
Etonogestrel implant	0.05
Male sterilization (vasectomy)	0.15
Intrauterine device (IUD)	
Hormonal	0.2
Copper	0.8
Female sterilization	
Tubal ligation	0.5
Hysteroscopic (e.g., Essure)	5.7
Depot medroxyprogesterone acetate (DMPA)	3–6
Contraceptive pills, patch, or ring	5–9
Diaphragm	12
Sponge	12 (nulliparous)–24 (parous)
Male condom	18
Female condom	21
Fertility awareness methods	24
Spermicides	28
No method	85

When counseling about female sterilization it is important to address several issues. Studies suggest that the cumulative probability of regret is 13%, with women who received a postpartum procedure and women less than age 30 to have the highest risk of regret [48]. In women who have a sterilization failure, the risk of an ectopic pregnancy is 30%. Finally, all patients should be counseled that a sterilization procedure is considered to be irreversible.

Male Sterilization

Vasectomy, or male sterilization, is a safe, effective, and minimally invasive approach to permanent sterilization. The first-year failure rate is 0.15%. Compared to female sterilization, vasectomy is safer and less expensive. Another advantage is that it can be performed as an in-office procedure with local anesthesia. Some disadvantages of vasectomy include that it requires the couple to use a backup method of contraception for 3 months until azoospermia is confirmed on semen analysis. An additional disadvantage is that women must rely on her partner's truthfulness in self-reporting vasectomy (■ Table 25.3).

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Surgical Techniques for Management of Anomalies of the Müllerian Ducts

Marjan Attaran

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26.1 Introduction

Malformations of the Müllerian ducts and the external genitalia can have significant impact on both reproductive potential and sexual function. When a patient presents with such an abnormality, it is important to put significant thought and time into determining the correct diagnosis and subsequent treatment.

The literature reports rates of female congenital anomalies between 0.2% and 0.4% of the general population [1]. But the prevalence of female congenital anomalies may be as high as 7% when using some of the newer diagnostic methods [2]. These rates are much higher when looking at subgroups of patients with recurrent pregnancy loss and infertility. However, in many instances these anomalies are asymptomatic.

This chapter will begin with a review of the diagnostic and presurgical evaluation of these anomalies. The majority of the chapter will deal with the basic principles of surgical techniques used to correct these anomalies.

Clinical Case

A 20-year-old woman requests information about creation of vagina. She is in a stable relationship and wishes to be sexually active. She was diagnosed at 16 years of age with absence of a vagina, uterus, and cervix. On examination she has Tanner V pubic hair and breast development. She has approximately 2 cm depth of vagina.

26.2 Classification

The classification of Müllerian anomalies helps with both the diagnosis and the comparison of outcomes after various modes of management. However, there is no single classification that encompasses all anomalies that have been reported in the literature [3–5]. Although the direct cause of the majority of these anomalies is not known, on the basis of our embryological knowledge, the pathogenesis of most of these anomalies can be understood. On the basis of pathophysiology, Müllerian anomalies can be broadly classified into problems according to the developmental mechanism whose failure gave rise to the malformation. Anomalies can usually be classified as being related to (1) agenesis, (2) vertical fusion defects, or (3) lateral fusion defects [6].

Agenesis of the uterus and vagina is a relatively common abnormality. Agenesis of other Müllerian structures is extremely rare. Vertical fusion defects are usually the result of abnormal canalization of the vaginal plate and result in defects such as a transverse vaginal septum and imperforate hymen. Lateral fusion defects can be symmetrical or asymmetrical and include septum of the uterus and vagina, as well as unicornuate and bicornuate uteri and related abnormalities.

There are endless variations to Müllerian and vaginal anomalies. It is impossible to note these variations effectively in any one classification system. Consequently, many investigators are still searching for that elusive classification system that can not only encompass all the anomalies noted in the vagina, cervix, uterus, and adnexa but also translate into in-sync comprehension and visualization of the defect by other colleagues [7, 8].

The most accepted classification of uterine anomalies, published by the American Society of Reproductive Medicine (ASRM), places uterine anomalies into distinct groups based on anatomic configuration (Table 26.1) [9]. Since vaginal anomalies are not included in this classification, they must be described along with the uterine anomaly. This classification does not give insight into pathophysiology but is an effective way to communicate observations for purposes of treatment and prognosis.

26.3 Müllerian Agenesis

Clinical Presentation

Müllerian agenesis (i.e., Mayer-Rokitansky-Kuster-Hauser syndrome) was first described in 1829. Its incidence is reported to be 1 in every 5000 newborn females [10]. Because the vagina and associated uterine structures do not develop with this disorder, it is an ASRM Class IA Müllerian anomaly. Patients typically present during their adolescent years with complaint of primary amenorrhea. As a cause of primary amenorrhea, Müllerian agenesis is second only to gonadal dysgenesis [11].

Table 26.1 ASRM classification of Müllerian anomalies

1. Hypoplasia/agenesis	a. Vaginal
	b. Cervical
	c. Fundal
	d. Tubal
	e. Combined
2. Unicornuate	a. Communicating
	b. Noncommunicating
	c. No cavity
	d. No horn
3. Didelphys	
4. Bicornuate	a. Complete
	b. Partial
5. Septate	a. Complete
	b. Partial
6. Arcuate	
7. DES related	

Patients with Müllerian agenesis will present with normal onset of puberty and appropriate secondary sexual characteristics but apparently delayed menarche. They do not complain of cyclic pelvic pain like patients with obstructive Müllerian anomalies. The external genitalia appear completely normal, with normal pubic hair growth and normal-sized Labia minora, which is in contrast to patients with complete androgen insensitivity syndrome. Hymeneal fringes may be evident but the vaginal opening is absent. No pelvic masses suggestive of hematocolpos will be evident, which is in contrast to cases of complete transverse septum.

Since these patients have a 46XX karyotype, normal ovaries will be present in the pelvis. Ovulation can be documented as a shift in basal body temperature. These patients' hormonal levels are normal, and their cycle length, based on hormonal studies, varies from 30 days to 34 days [12]. In addition, they may experience the monthly pain (mittelschmerz) that is indicative of ovulation.

26.4 Associated Anomalies

Some, but not all, anomalies described can be extrapolated from the embryology [13–17]. Hearing difficulties have been reported in patients with Müllerian agenesis [14, 15]. A higher rate of auditory defects has been noted in general in patients with Müllerian anomalies compared to those with normal Müllerian structures [17].

Müllerian agenesis is associated with renal and skeletal system anomalies. Renal abnormalities are noted in 40% of these patients. These include complete agenesis of a kidney to malposition of a kidney to changes in renal structure [18]. Skeletal abnormalities are noted in 12% of patients and include primarily spine defects followed by limb and rib defects [19]. Patients with Müllerian agenesis should be actively assessed for these associated anomalies.

26.5 Etiology

The etiology of Müllerian agenesis remains unknown. It appears to be influenced by multifactorial inheritance, and rare familial cases have been reported. It does not appear to be transmitted in an autosomal dominant inheritance pattern, since none of the female offspring of women with Müllerian agenesis (born via in vitro fertilization and surrogacy) have shown evidence of vaginal agenesis [13].

26.6 Diagnosis

26.6.1 Imaging

The diagnosis of Müllerian agenesis is confirmed via imaging techniques. Abdominal ultrasonography will demonstrate the lack of uterus and existence of ovaries. The presence of a midline mass consistent with a blood collection usually indicates an obstructive Müllerian anomaly. The distinction between Müllerian agenesis and obstruction is

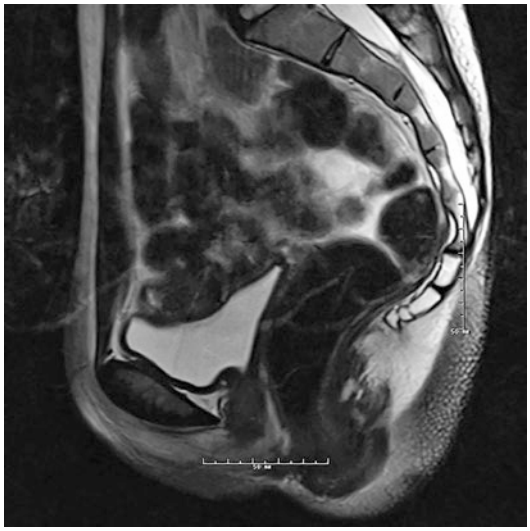

Fig. 26.1 Magnetic resonance image of Müllerian agenesis. Typical findings in the pelvis include normal ovaries and fallopian tubes and usually small Müllerian remnants attached to the proximal portion of the fallopian tubes that may be solid or have functioning endometrial tissue

extremely important since an incorrect diagnosis can seriously jeopardize appropriate management.

With the advent of magnetic resonance imaging (MRI), laparoscopy is no longer considered necessary to make this diagnosis (Fig. 26.1). Typical findings in the pelvis include normal ovaries and fallopian tubes and usually small Müllerian remnants attached to the proximal portion of the fallopian tubes that may be solid or have functioning endometrial tissue.

Direct communication with the radiologist about the differential diagnosis prior to imaging studies is important. On occasion, the unsuspecting radiologist may interpret the small uterine remnants as a uterus. Careful attention to the very small dimensions of this structure will alert the physician to this possibility.

26.6.2 Explaining the Diagnosis to the Patient

The diagnosis, usually made in early adolescence, must be explained to the patient with great sensitivity. At a time when being like her peers is extremely important, knowledge of this diagnosis can be psychologically devastating. Each patient must be reassured that her external genitalia appear normal and that they will be able to have a normal sex life after the creation of a normally functioning vagina. Although usually not voiced, the inability to subsequently bear children is a major disappointment to teenagers. Fortunately, with ART procedures, including IVF and surrogacy, having her own genetic child will be an option for many of these young women.

26.6.3 Creation of a Neovagina

The first goal of treatment of Müllerian agenesis is the creation of a functional vagina to allow intercourse. Frank first proposed vaginal dilatation with the use of a dilator as a means of creating a neovagina in 1938 [20]. However, the surgical techniques of vaginoplasty remained the preferred methods for many years. The success of any technique depends in large part on the emotional maturity of the patient. Pretreatment counseling and continued support during treatment are important.

26.6.4 Vaginal Dilation

The simplicity and ease of vaginal dilation and its significantly lower complication rate than surgical techniques dictate its use as an initial form of therapy for most patients with Müllerian agenesis. The American College of Obstetrics and Gynecology has released a committee opinion that recommends nonsurgical management of Müllerian agenesis as the first mode of treatment [21].

Frank's technique of dilation involves actively placing pressure with the dilators against the vaginal dimple (Fig. 26.2). Not only the patient is in an awkward position but the hand applying the pressure can become tired. In 1981, Ingram proposed the concept of passive dilation, where pressure is placed upon the dilator by sitting on a bicycle seat [22].

Roberts reported a success rate of 92% in women who dilated the vagina via the Ingram technique for 20 min 3 times a day [23]. The average time of the creation of a functional vagina was 11 months. This series demonstrated that an initial dimple <0.5 cm was all that was necessary to achieve adequate dilation. Interestingly, failure of this technique was not related to the length of vaginal dimple but rather more closely associated with the patient's youth. Failure of this technique was more common in patients younger than 18 years of age.

Fig. 26.2 Examples of vaginal dilators of different sizes (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

26.6.5 Procedure

When the patient expresses a desire to proceed with therapy, she is shown the exact location of her vaginal dimple. The axis of dilator placement is also demonstrated (Fig. 26.3). The process is initiated by placement of the smallest dilator against the dimple. Pressure is kept upon the distal aspect of the dilator by sitting upon a stool while leaning slightly forward. When the dilator fits comfortably, she moves to the next size dilator. The patient is instructed to use this technique a minimum of 20 min a day, 2–3 times a day. In motivated patients, a functional vagina can be created in as short as 12 weeks.

Counseling and psychological support is integral to successful treatment [24–26]. Patients are requested to return to the office frequently so that the physician can monitor their progress, and provide guidance and an opportunity to answer questions. Intercourse may be attempted when the largest dilator fits comfortably.

Multiple types of graduated dilators, made of various materials, are present on the market. None have been found superior to the others. Patients may stop and reinitiate the dilation at any time without any negative long-term sequelae. Although most patients appear interested in initiating this therapy the summer before college, when they are mature enough and motivated to create the vagina, the timing of therapy is purely dependent on the patient's desires. The median age of starting treatment is 17 years of age. For appropriately selected motivated patients, the reported success rates are as high as 95% [27].

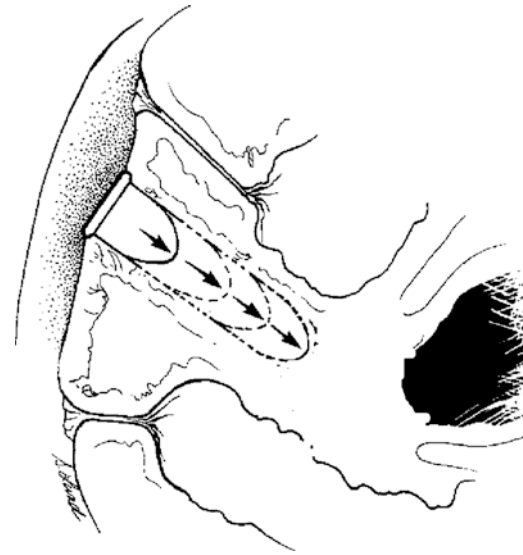

Fig. 26.3 Schematic drawing of angle of dilator placement. The patient is viewed in lithotomy position, and the axis is directed away from the bladder (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

26.7 Vecchietti Procedure

Giuseppe Vecchietti described this method of creating a neovagina in 1965 [28]. Similar to vaginal dilatation, this method avoids the use of a graft. The Vecchietti procedure is a one-step procedure in which a neovagina is created in 7 days by continuous pressure on the vaginal dimple using an acrylic "olive" connected by retroperitoneal sutures to a spring tension device on the lower abdomen. Although the original description of the Vecchietti procedure utilized laparotomy, this technique is currently performed laparoscopically [29, 30].

26.7.1 Procedure

The first step in this procedure is to laparoscopically observe placement of an applicator through the perineum and the vesicorectal septum into the peritoneal cavity. Attached to this applicator are threads that are tied to a dilator otherwise known as a "dummy." Two curved applicators are placed through the abdominal wall and guided down retroperitoneally on the right and left side walls until the needle tip approaches the midline threads. These threads are then loaded via laparoscopic graspers into these applicators and gently withdrawn through the abdominal wall. The threads are connected to a traction device and placed under tension. When the threads are tightened the dummy is pulled and within days a vaginal cavity is created. The duration of canal formation is about 4.5 days, thus patients do need to be under analgesia as the dummy is being pulled into the vesicovaginal space. The average length of vagina created in such a manner is 9 cm. Patients must continue to use the dilator after the full vaginal length is developed.

Brucker and colleagues have reported on a series of 240 patients with a success rate of 98% [31]. Very few operative complications were reported and included bladder hematoma, urethral necrosis, and urinary tract infections. Several smaller studies have been reported by other surgeons using the Vecchietti laparoscopic technique with similar outcomes [32–34].

26.7.2 Vaginoplasty Techniques

The traditional surgical management of vaginal agenesis is to create a vaginal space followed by placement of a lining to prevent stenosis. Multiple tissues and at least one man-made material have been used to line this cavity with varying degrees of success in preventing subsequent stenosis of the neovagina (Table 26.2).

Table 26.2 Surgical methods of creating a neovagina

Dissection of a perineal space	Split-thickness skin graft (McIndoe)
	Full-thickness skin graft
	Peritoneum (Davydov), buccal mucosa
	Tissue engineering
	Muscle and skin flap
	Adhesion barriers
	Tissue expansion
Bowel vaginoplasty	Sigmoid colon
Vulvovaginal pouch	Williams vaginoplasty
Traction on retrohymeneal fovea	Vecchietti

26.7.3 McIndoe Procedure

The most widely used surgical technique for the creation of a neovagina is the McIndoe operation. The first step of the procedure is obtaining the split-thickness skin graft. The plastic surgery team typically acquires the skin graft from the buttock area, a location usually covered by clothing. The patient is placed in the prone position and the site is cleansed with an antiseptic solution and then soaked with an epinephrine saline gauze to allow vasoconstriction of small punctuate bleeding sites. Mineral oil is applied to the donor site and the skin is held taught while the electrodermatome device is used to obtain a thick split-thickness skin graft. The skin graft should be 0.015–0.018-in. thick. After application of antiseptic solution, the

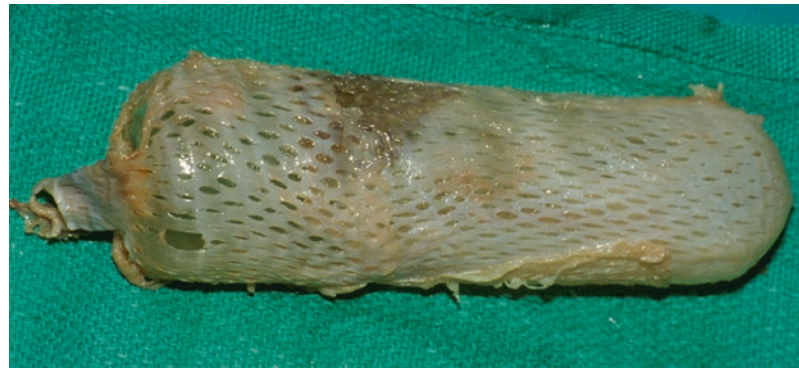

Fig. 26.4 Skin graft is sutured around a mold (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

donor site is covered with OpSite, which is fixed in place by several stitches. Within 2–3 weeks the area heals with acceptable scarring.

The skin graft is placed through a 1:5 ratio skin mesher. The purpose of meshing the skin is not to stretch the skin but rather to allow escape of any underlying blood clots or serous fluids. This skin graft is sutured around the mold with 4–0 absorbable suture (Fig. 26.4). The mold is covered completely because any uncovered sites, whether due to lack of enough tissue or a gaping hole in the line of suture, tend to result in the formation of granulation tissue. Thus, great care must be taken to obtain sufficient amount of graft for this procedure.

The patient is placed in the dorsolithotomy position. A transverse incision is made in the vaginal vestibule, between the rectum and urethral openings (Fig. 26.5). In a patient who has not had prior surgery or radiation in the area, areolar tissue is now encountered. This tissue is easily dissected with either fingers or a Hegar dilator on either side of a median raphe (Fig. 26.6). The dissection is continued for at least the length of the mold without entering the peritoneal cavity. By cutting the median raphe, the two channels are then connected. If the dissection is performed in this manner, minimal amount of bleeding is encountered. Any bleeding sites must be controlled meticulously to avoid lifting of the graft from the newly created vaginal wall and subsequent nonadherence and necrosis.

After creation of the vaginal space, the mold covered with the skin graft is placed inside the cavity (Fig. 26.7). At the introitus, the skin graft is attached with several separate 3–0 absorbable stitches. To hold the mold in place, several loose, nonreactive sutures, such as 2–0 silk, are used to approximate the Labia minor in the midline.

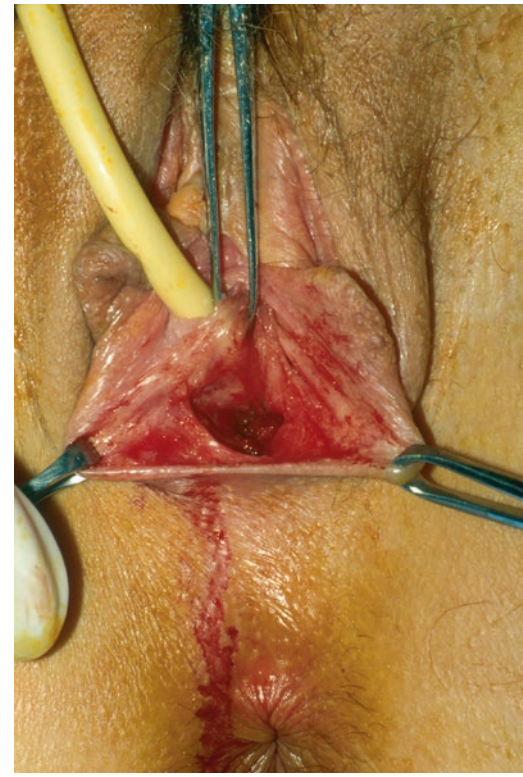

Fig. 26.5 The initial transverse cut was made on the fibrous tissue and an initial space developed (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

During the ensuing week, the patient is maintained on bed rest, broad-spectrum antibiotics, a low-residue diet, and an agent to decrease bowel motility. She also has an indwelling urinary catheter. Upon return to the operating room in 1 week, the mold is carefully removed. The graft site is carefully assessed for any signs of necrosis or underlying hematoma after the vaginal cavity is

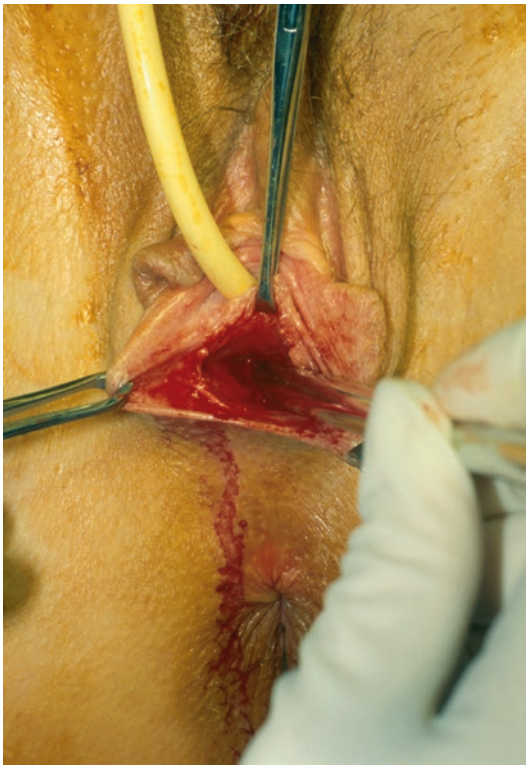

Fig. 26.6 Placement of Hegar dilator to create space for the graft. The direction of the Hegar dilators is posterior (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

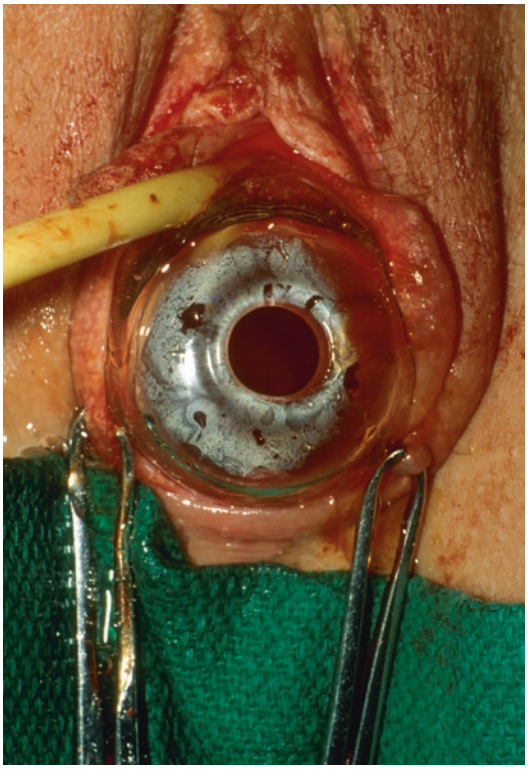

Fig. 26.7 The mold with the graft is placed in the space. Notice that the space to be created must accommodate the mold completely (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

irrigated with warm saline. Another soft mold is then reinserted and kept in place for the next 3 months except during defecation and urination. Nighttime usage of the mold is recommended for the next 6 months. To prevent contracture of the vagina, the patient is instructed to reinsert the mold during extended times of sexual inactivity.

Difficulty in dissecting the neovagina and increased probability of bleeding and fistula formation are encountered in the patient with a prior surgical procedure. Other problems that may be encountered include narrow subpubic arch, strong levators, shorter perineum, prior hymenectomy, and congenitally deep cul-de-sac [35, 36].

Because of concern regarding tissue necrosis from mold pressure and subsequent fistula formation, both rigid and soft molds have been used for this procedure (Figs. 26.8 and 26.9). Theoretically, soft molds decrease the risk of fistula formation that can result from avascular necrosis. A soft mold can be created by covering a foam rubber block with a condom [36]. The foam is able to expand and fit the neovaginal space, thereby providing equal pressure throughout the canal. However, a report on the use of a rigid mold on 201 patients who underwent the McIndoe operation demonstrated a fistula formation rate of less than 1% [37]. There is no study comparing the outcomes of soft vs. rigid molds in this operation. Typically, a rigid mold is used initially, but the patient is sent home with a soft mold in place.

An 80% success rate has been reported with this procedure [38]. Since success rates are highest in those patients who have not undergone prior vaginoplasty, patients must be counseled extensively prior to surgery regarding the need for prolonged use of the mold. Indeed, part of the presurgical assessment involves determination of patient maturity and motivation concerning the use of dilators. Lack of compliance with the postoperative use of dilators will lead to contracture and diminishment of vaginal length.

Fig. 26.8 Hard glass mold (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

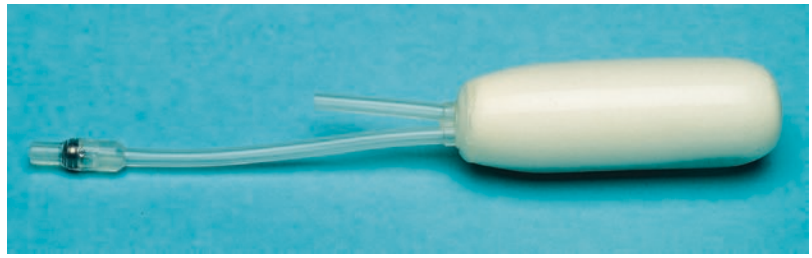

Fig. 26.9 Adjustable vaginal mold by Mentor, Minneapolis, MN (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

Surgical complications include postoperative infection and hemorrhage, failure of graft and formation of granulation tissue, and fistula formation. In general, the incidence of complications is low: rectal perforation rate of 1%, graft infection of 4%, and graft-site infection of 5.5% [37]. In a review of 50 patients, two rectovaginal fistulas and one graft failure were reported [35]. Five patients required an additional operative procedure. Eighty-five percent of these patients considered their surgery to be a success.

Long-term data on the McIndoe procedure, while limited, consistently indicate an improvement in quality of life. In a series of 44 patients who underwent a surgical procedure to create the vagina, 82% achieved a functional satisfactory postoperative result [39]. Vaginal length varied from 3.5 cm to 15 cm. In another long-term study of women who underwent a McIndoe procedure, 79% of the patients reported improved quality of life, 91% remained sexually active, and 75% regularly achieved orgasm [40].

The newly created vagina must be inspected at the time of the yearly pelvic examination. Hair growth has been reported to be a problem with some skin grafts. Transformation to squamous cell carcinoma from skin graft has been described [41, 42].

26.7.4 Peritoneal Graft: Davydov Procedure

Use of the peritoneum to line the newly created vaginal space was popularized by Davydov, a Russian gynecologist, and first described by Rothman in the USA in 1972 [43–45]. In his original description, a laparotomy is performed after creation of the vaginal space as described above with the McIndoe operation.

A cut is made on the peritoneum overlying the new vagina. Long sutures are applied to the anterior, posterior, and lateral sides of this peritoneum. The sutures are then pulled down through the vaginal space, thus pulling the peritoneum to the introitus. The edge of the peritoneum is then stitched to the mucosa of the introitus. Closing the peritoneum on the abdominal side then forms the top of the vagina. Several investigators have also described the laparoscopic modification of this procedure [46, 47].

This procedure may have several advantages compared to the traditional McIndoe. Contrary to skin grafts that leave visible scarring at the donor site, there is no outward sign of using a graft in the Davydov procedure. There appears to be no danger of lack of graft takes and no problem with hair growth.

In Davydov's first reported series, sexual intercourse was initiated within several weeks of surgery in all but 1 of his 30 patients. On follow-up, the length of the vagina was noted to be 8–11 cm. In a series of 18 patients who underwent the laparoscopic modification of this procedure, 85% reported being sexually satisfied during an 8–40-month follow-up. Although there was one report of a rectovaginal fistula 18 months after the surgery, there was no evidence of vault prolapse or enterocele formation. Minor granulation tissue was noted at the vaginal cuff, but the vault was primarily covered with squamous epithelium tissue.

26.7.5 Adhesion Barrier Lining

Jackson first described the use of an adhesion barrier to line the neovagina in 1994 [48]. Oxidized regenerated cellulose (INTERCEED; Johnson and Johnson Patient Care Inc., New Brunswick, NJ) forms a gelatinous barrier on raw surfaces and thus prevents adhesion formation. After creation of the vaginal space, sheets of cloth-like oxidized regenerated cellulose are wrapped around the mold and placed in the vagina in a manner similar to the McIndoe. The neovaginal space must be free of any bleeding. Epithelialization is noted to occur within 3–6 months. Small areas of granulation tissue may be seen at the apex of the vagina and resolve after application of silver nitrate. Average vaginal depth ranges from 6 cm to12 cm. Continuous use of mold is encouraged until complete epithelialization has occurred.

A case series assessed the outcome of this technique on 10 patients with vaginal agenesis [49]. Complete squamous epithelialization was noted within 1–4 months. When compared to a normal vagina, fern formation was noted and the vaginal pH was always acidic. However, none of the women complained of vaginal dryness or foul-smelling discharge. Patients who were sexually active did not report any problems.

The advantages of the use of oxidized regenerated cellulose include avoidance of any scars, readily available product, and low expense. In addition, the surgical procedure is simplified into a one-stage procedure. Although the reported data appear encouraging, confirmatory studies are required before the use of oxidized regenerated cellulose can be recommended without any reservations.

26.7.6 Buccal Mucosa

Buccal mucosa has been used by urologist for several decades in urethral reconstruction and repair of complex hypospadias. It was first reported for use in vaginoplasty in 2003 [50]. Some of the properties that make this an excellent graft choice are: thick elastic lubricating epithelium, thin lamina propria, and same color and texture match to native vagina. In addition the harvest site is hidden and heals very quickly. Once the perineal space is created, the buccal mucosa from both cheeks is harvested, placed over a vaginal stent, and then sown into the new space. Postoperative vaginal lengths have measured 8–10 cm in length and 4–5 cm in width [51].

26.7.7 Tissue Engineering

The first case in which in vitro cultured vaginal tissue was utilized to line the neovagina was published in 2007 [52]. A 1-cm^2 biopsy was performed from the vulvar vestibule. Autologous keratinocyte cultures were created from this biopsy. The McIndoe procedure was utilized to create the vaginal space, and the autologous in vitro cultured tissue was used to line the cavity. The length of the vagina is reported to be normal, as is its depth. Of course, long-term data are currently lacking, as is information on vaginal stenosis. But if proven to be effective, this method of creating a vagina may lead to the increasing popularity of the McIndoe procedure, given the lack of concomitant scars on the skin.

26.7.8 Muscle and Skin Flap

These approaches are not procedures of choice for women with vaginal agenesis. However, they may be used for those who require vaginal reconstruction after exposure to radiation or multiple surgical procedures. The advantage of

using a full-thickness skin flap is that it avoids the problem of contracture encountered with split-thickness grafts.

The use of gracilis myocutaneous flaps and rectus abdominus myocutaneous flaps for vaginal reconstruction has been reported [53, 54]. This approach has been associated with a conspicuous scar and a higher failure rate. Wee and Joseph in Singapore designed flaps that maintained good blood supply and innervation [55]. Known as a "pudendal-thigh flap vaginoplasty," this technique has been particularly successful in patients with vulvar anomalies [56].

The patient's own labia majora and Labia minora have also been used to create a vagina [57]. Tissue expansion has also been advocated to create labiovaginal flaps, which are then used to line the neovagina [58, 59]. Other modifications of this procedure have been reported [60, 61].

26.7.9 Bowel Vaginoplasty

This is not a procedure of choice in women with vaginal agenesis. For this procedure, also known as a colocolpopoiesis, a portion of large bowel with its preserved vascular pedicle is sutured into the neovagina. In recent years, sigmoid colon use has been recommended.

Continuous use of dilators is not considered necessary, although constriction has been noted when ilium has been used. Success rates of up to 90% have been reported. Reported complications include profuse vaginal discharge, prolapse, introital stenosis, bowel obstruction, and colitis [62, 63]. Finally, there is a report of a mucinous adenocarcinoma arising in a neovagina lined with the sigmoid colon [64].

A laparoscopic modification of this procedure has also been described [65, 66]. Given the increased complication rates, it seems appropriate to reserve this treatment modality for complex situations in which a prior vaginoplasty technique has failed or when there are multiple urogenital malformations.

26.7.10 Obstructed Rudimentary Uterine Bulbs

Patients with Müllerian agenesis commonly have Müllerian remnants noted on MRI or during a laparoscopy. The MRI has the added value of determining if any endometrial tissue exists within these remnants (Figs. 26.10 and 26.11). Patients with functional endometrial tissue may present after many asymptomatic years with cyclic pelvic pain secondary to monthly endometrial shedding, and development of endometriosis has been reported in these patients. Symptomatic Müllerian bulbs should be removed either via laparotomy or laparoscopy.

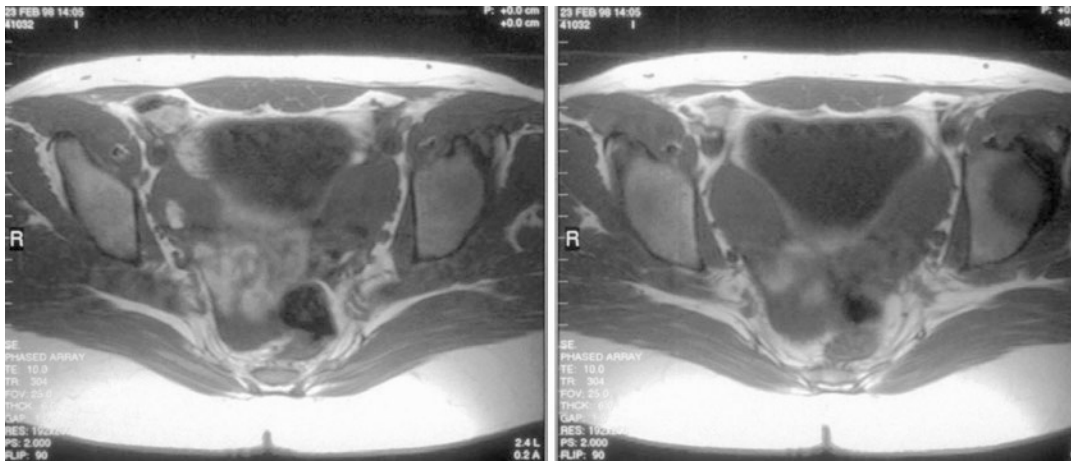

Fig. 26.10 Magnetic resonance image of functioning rudimentary bulbs (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

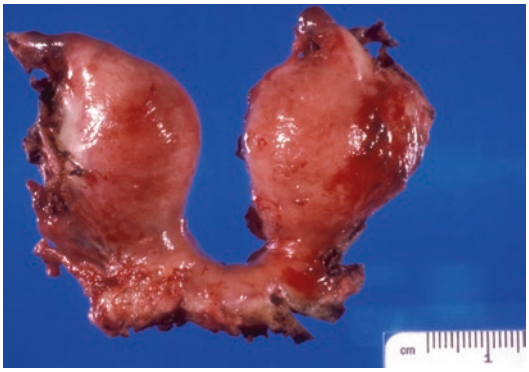

Fig. 26.11 Pathologic specimen of the extirpated rudimentary bulbs (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

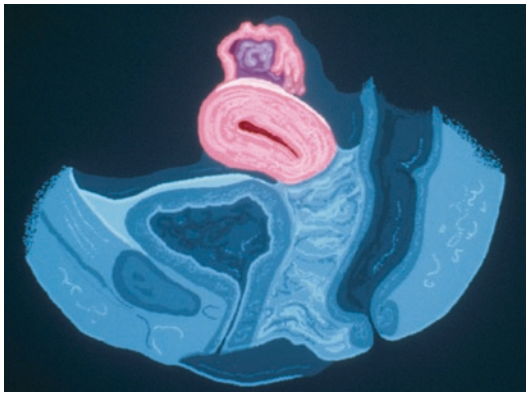

Fig. 26.12 Schematic representation of cervical agenesis (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

26.7.11 Surgical Technique

The procedure is started by placing traction on the ipsilateral uterine bulb. The round ligament is grasped and cut and the peritoneum incised anteriorly, thereby creating a bladder flap. The retroperitoneal space is entered, the ureters identified, and the utero-ovarian ligaments transected. The dissection continues with identification and coagulation of the uterine arteries. Finally, the uterine remnants and the fibrous tissue connecting them are incised.

26.8 Cervical Agenesis

Cervical agenesis is a rare Müllerian anomaly whose true incidence remains unknown despite many case reports in the literature [67] (Fig. 26.12). Various degrees of cervical abnormalities, ranging from dysgenesis to agenesis, have been described [68]. The vagina may or may not be present in patients with cervical agenesis. In a series of 58 patients with cervical atresia, 48% had isolated congenital cervical atresia with a normal vagina [69]. The rest of the patients had either a vaginal dimple or complete atresia.

26.9 Diagnosis

Unlike some of the other Müllerian anomalies, patients with cervical agenesis present very early in adolescence. Typically, patients present between the ages of 12 and 16 with a primary complaint of pelvic pain secondary to obstruction of flow from the uterus. Initially, the pain is cyclic, but it may evolve with time into continuous pain. It is not uncommon for such patients to have been evaluated by their pediatricians for other causes of abdominal pain. Although these girls have amenorrhea, this symptom fails to raise a red flag since the patients are so young at presentation that lack of menses is not concerning. Continuing menstruation in an obstructed uterus forms a hematometra and possibly hematosalpinx, endometriosis, and adhesions in the pelvis.

Imaging of such a pelvis can easily lead to a misdiagnosis. Such patients have been taken to surgery for pain thought to be secondary to a pelvic mass, only to find that they have a congenital anomaly. While ultrasound may be helpful in looking for a cervix, one's clinical suspicion must be communicated directly to the radiologist performing the procedure. MRI is very helpful in visualizing the cervix and can accurately determine its presence or absence [16, 70, 71] (Fig. 26.13).

Patients with cervical agenesis who lack a vagina must be differentiated from those with a high obstructing vaginal septum. MRI is very helpful in making this differentiation by showing accumulation of blood in the upper vagina and a cervix in patients with a high transverse septum. Lack of a hematocolpos helps make the diagnosis of cervical agenesis or dysgenesis. Theoretically,

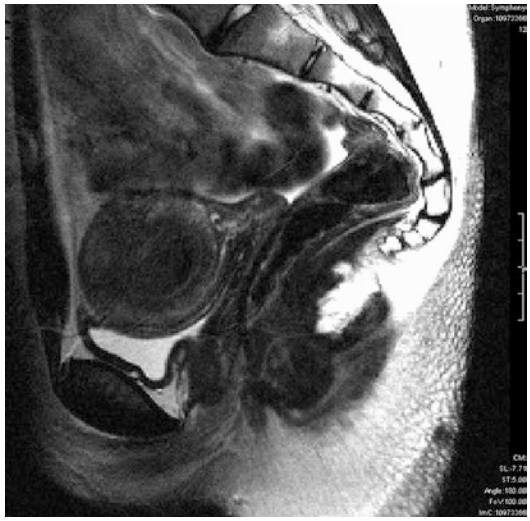

Fig. 26.13 Magnetic resonance image of cervical agenesis. The uterine cavity is distended with clots and no cervix is identified (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

MRI should detect absence of the cervix, but is unable to clearly differentiate among the various degrees of cervical dysgenesis.

26.10 Management

26.10.1 Pain Control

Pain control should be the first goal of treatment. Although analgesia may be required, severe pain will resolve within several days. Suppressive therapy is used to prevent further endometrial shedding until definitive surgery can be performed. Agents that are commonly used to achieve this suppression are continuous oral contraceptive pills, norethindrone acetate, depo-medroxyprogesterone acetate, and GnRH agonists or antagonists.

Most adolescents are neither emotionally ready nor equipped to decide on a surgical course of action, which may be as invasive as hysterectomy. Thus, if suppressive therapy with oral contraceptives or depo-medroxyprogesterone has provided pain relief, many may choose to hold off on definitive surgical therapy until they can fully understand the possible consequences. In addition, this alleviates the burden placed upon parents to make a decision regarding their daughter's reproductive future.

26.10.2 Surgical Approach

There are no specific guidelines in the literature to determine the correct surgical procedure. It is clear, however, that each patient should be assessed individually. The definitive and safest treatment of cervical agenesis is a total abdominal hysterectomy. A hysterectomy would diminish continued physical pain and discomfort. In addition, with the advent of surrogate pregnancy, an early hysterectomy would potentially preserve more ovarian tissue, which may be used to achieve a pregnancy via surrogacy and in vitro fertilization. On the other hand, it is a daunting decision to have a hysterectomy at a young age.

The other surgical options for management of cervical dysgenesis are cervical canalization or uterovaginal anastomosis. There are many reports in the literature of cervical canalization and stent placement [72–74]. Although success has been reported with establishment of menses, many patients will require reoperation secondary to fibrosis of the cervical tract and obstruction. In addition, pregnancy rates are very low. In a review of patients with cervical agenesis, 59% of those that underwent cervical canalization achieved normal menstruation. Four of the 23 who achieved cervical patency required multiple surgeries [69]. The task is even more daunting if there is also a vaginal anomaly that requires reconstruction.

There are several forms of cervical dysgenesis that are sometimes confused with agenesis [75]. A hysterectomy may be considered in those patients with no evidence of a cervix, but canalization/uterovaginal anastomosis may be considered in those with a vagina and an obstructed cervix. Although preserving fertility is ultimately the goal of canalization procedures, sepsis and death have been reported subsequent to canalization [76]. In addition, subsequent pregnancy rates are very low [73, 77].

The poor pregnancy rates may be attributed to several factors. Prolonged delay in diagnosis can lead to extensive endometriosis and scarring in the pelvis. Also, while canalization and stent placement may maintain an open pathway for menstruation, the lack of epithelialization of the fistula not only increases risk of fibrosis but also may impede sperm migration into the uterus. The only pregnancy that was achieved in Rock's series was one in whom a full-thickness skin graft was

used in the canualized area [75]. More recently, the use of bladder mucosa to line the newly created cervical canal was reported [72].

After preoperative assessment and formation of operative plan, the surgeon must be prepared to proceed with a concomitant vaginoplasty, if indeed the patient suffers from vaginal atresia. The patient should have been preoperatively counseled regarding the need for prolonged use of a mold following surgery.

Advances in artificial reproductive techniques have led to reports of pregnancies in patients with cervical agenesis. Thus, many patients are likely to choose effective continued endometrial suppression over a surgical solution in hopes of keeping a glimmer of reproductive hope. With passage of time and attainment of adulthood, such patients may be better able to accept the diagnosis and its consequences.

26.10.3 Uterine Fusion Defects

Uterine fusion defects include septate, bicornuate, and didelphic uteri. Patients with these isolated uterine anomalies are asymptomatic. The diagnosis is usually made during evaluation of infertility, recurrent pregnancy loss, or an obstetric complication.

Correct diagnosis of these anomalies is of utmost importance, since their management varies significantly [78]. The diagnosis is typically made based on evaluation of anatomy via imaging techniques including ultrasonography, hysterosalpingography, and MRI or via laparoscopy and hysteroscopy. Although radiologic guidelines exist to differentiate these entities, the multitude of varieties of these malformations can pose a significant challenge in making the correct diagnosis.

26.11 Septate Uterus

Reproductive difficulties are encountered far more commonly in women with a **septate uterus** than any other uterine fusion defect. The septate uterus is associated with the highest spontaneous abortion rate of uterine fusion defects.

The management of uterine septum detected after an evaluation for recurrent pregnancy loss is hysteroscopic resection of the septum. However, the management of a septum found during an infertility investigation is less straightforward [78]. While the septum does not appear to cause infertility, the concern regarding possible spontaneous abortion after undergoing infertility treatment may be enough to justify hysteroscopic removal of the septum prior to infertility treatment.

On a historical note, in the distant past a uterine septum was sometimes removed along with the midline section of the uterus via laparotomy using a Jones or Tompkins metroplasty procedure. These relatively extreme approaches have been completely supplanted by the hysteroscopic septoplasty described.

26.12 Bicornuate Uterus

The most frequently diagnosed uterine malformation is the **bicornuate uterus** [79]. This anomaly is usually discovered incidentally during an investigation for infertility or recurrent pregnancy loss.

It is important to differentiate a bicornuate from a septate uterus. Hysterosalpingogram (HSG) alone cannot differentiate these entities, because this imaging approach cannot evaluate the external contour of the uterus. While laparoscopy was used primarily for this purpose in the past, modern imaging techniques including 3D ultrasonography and MRI can adequately differentiate these two entities.

Imaging criteria to differentiate septate and bicornuate uteri have been developed [80, 81]. A septate uterus has a flat or convex fundus or a fundal indentation <10 mm. The septum should be relatively thin such that the angle between the medial borders of the hemicavities is <60°. A bicornuate uterus has two distinct fundi with an intervening fundal indentation of at least 10 mm. In most cases, the angle between the medial borders of the hemicavities will be >60°.

On MRI, a septate uterus will fail to show an intervening myometrium between the T2-hypointense septum that separates the endometrial cavities [80, 81]. In contrast, a bicornuate uterus will show two T2-hyperintense endometrial cavities, each with a junctional zone and myometrial band of intermediate signal intensity.

Conception does not appear to be a problem in women with bicornuate uterus [78]. However, higher rates of preterm delivery (19%) and spontaneous abortion (42%) have been reported [82].

Uteroplacental insufficiency and cervical incompetence may play roles in the higher obstetric complications. Thus, the treatment options for bicornuate uterus may include the Strassman metroplasty and cervical cerclage. Since the benefit of metroplasty has never been established in a controlled study, it is typically considered only after multiple pregnancy losses and complications [83].

26.13 Surgical Technique: Strassman Metroplasty

The Strassman metroplasty is the procedure of choice in the uncommon case where unification of a bicornuate uterus is indicated. Via laparotomy, a transverse incision is made across the fundus of the bicornuate uterus. The opened cavity is then repaired in an anteroposterior fashion. Since the subsequent length of gestation appears to increase after each pregnancy loss in patients with a bicornuate uterus (due to myometrial stretching or unknown factors), metroplasty is always the procedure of last resort.

26.13.1 Didelphic Uterus

The **didelphic uterus** is defined as two completely separate uteri and cervix (Fig. 26.14). It accounts for 10% of all uterine anomalies [79]. On ultrasonography, the two bulbs of the uterus are distinctly noted and can be followed down to their individual cervices.

Surgical correction is not indicated. In a long-term follow-up of 49 patients with didelphic uteri, 89% of those desiring pregnancy had at least one living child [84]. The spontaneous miscarriage rate was 21%, and only one ectopic pregnancy occurred. The most common problem was preterm delivery, which occurred in 24% pregnancies. Fortunately, only 7% of the infants weighed

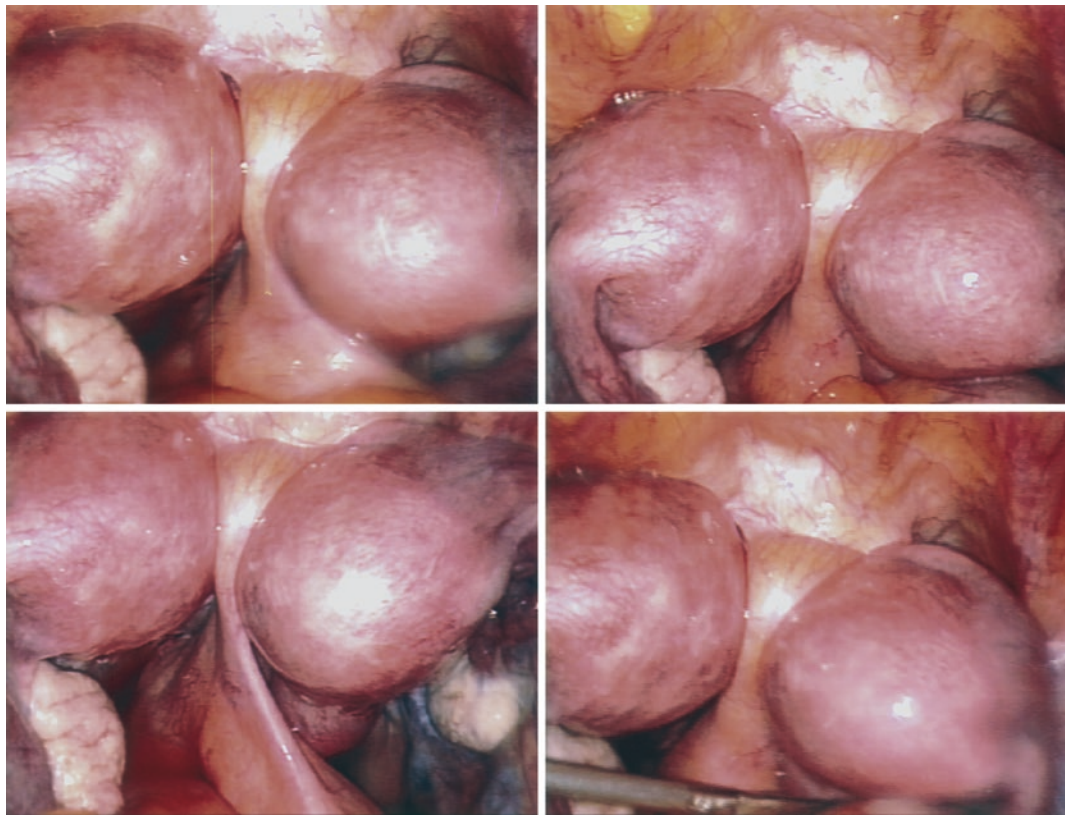

Fig. 26.14 The appearance of didelphic uteri at laparoscopy. Note the peritoneal band in between the two horns (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

<1500 g at birth. Breech presentation was noted in 51% of the infants; thus, the cesarean section rate is increased.

26.14 Unicornuate Uterus and Rudimentary Horn

A **unicornuate uterus** may be associated with a communicating or noncommunicating rudimentary horn. In either case, patients will have monthly regular periods. If a rudimentary horn is a communicating or a noncommunicating horn but nonfunctioning, the patient is unlikely to have severe dysmenorrhea. The diagnosis in these patients is usually made at the time of investigation for infertility and obstetric problems (including recurrent pregnancy loss) or at the time of cesarean section. In contrast, if a noncommunicating rudimentary horn is functioning, most patients will have severe dysmenorrhea unresponsive to medical therapy (Fig. 26.15).

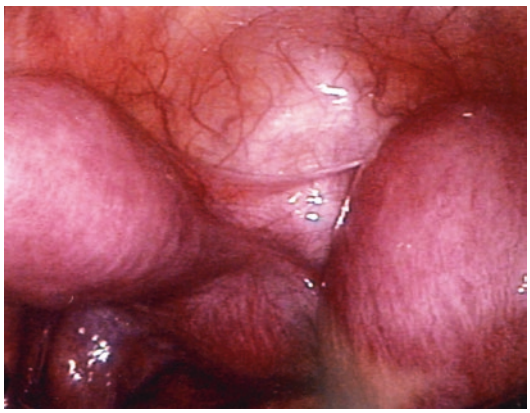

Fig. 26.15 Noncommunicating uterine horn seen at laparoscopy (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

26.14.1 Diagnosis

Pelvic examination may reveal a deviated uterus or an adnexal mass. Ultrasonography will be consistent with a unicornuate uterus on one side, while the other side may be interpreted as a rudimentary horn, a pedunculated leiomyoma, or an ovarian endometrioma. An HSG will show a unicornuate uterus and will often, but not always, demonstrate the presence of a communication with the rudimentary horn when present. Both 3D ultrasonography and MRI are often useful in making a definitive diagnosis.

26.14.2 Management

Management depends on whether the rudimentary horn is functional and/or communicating. A nonfunctioning, noncommunicating rudimentary horn does not need to be removed, as it will be asymptomatic and not put the patient at any risk. In contrast, a functioning, noncommunicating rudimentary horn should be removed upon diagnosis to alleviate the patient's often severe dysmenorrhea and to avoid the risk of rupture, should a pregnancy occur in this horn [85]. It would seem that a functioning, communicating rudimentary horn could be left in situ, since it is unlikely to be symptomatic for the patient. However, there is a risk of developing a pregnancy in such a horn, leading to subsequent rupture if undiagnosed [86]. Therefore, surgical removal is recommended prior to attempting pregnancy.

26.14.3 Surgical Technique: Removal of a Rudimentary Horn

A rudimentary uterine horn can be removed via laparotomy or laparoscopy using similar techniques, depending on surgical experience [87]. After gaining access to the pelvis, the round ligament of the rudimentary horn is identified, ligated, and divided. Access is gained into the retroperitoneal space, the ureter is identified, and the bladder is dissected off the lower border of the rudimentary horn.

The rudimentary horn should be removed together with the corresponding fallopian tube to avoid a future ectopic pregnancy in a blind residual tube via sperm transmigration. After disconnecting the tube from the mesosalpinx, the utero-ovarian ligament is transected so that the ovary can be spared.

The rudimentary horn may share myometrial tissue with the unicornuate uterus or be attached by a band of fibrous tissue [87, 88]. In cases where the uterine horn is attached to the uterus with fibrous band, the blood supply is found within this band. Coagulation and transection of the band are all that is required.

In cases where the rudimentary horn is attached to the uterus via shared myometrium, the blood supply cannot be easily identified and thus the uterine artery ascending beneath the rudimentary horn should be identified and ligated. It may be difficult to find a plane of dissection between the horn and the uterus, but care must be taken to avoid entry into the cavity of the unicornuate uterus or compromising the integrity of the myometrial thickness. After this dissection, the myometrial defect should be carefully re-approximated with interrupted or continuous sutures to minimize the risk of uterine rupture during a subsequent pregnancy.

26.15 Longitudinal Vaginal Septum

A **longitudinal vaginal septum** can be either non-obstructing or obstructing. A non-obstructing vaginal septum is often asymptomatic and discovered at the time of a pelvic exam or childbirth. A woman with an obstructing vaginal septum often presents with an increasingly severe dysmenorrhea and a unilateral vaginal mass.

26.15.1 The Non-obstructing Longitudinal Vaginal Septum

Non-obstructing longitudinal vaginal septa account for 12% of the malformations of the vagina. Although most are asymptomatic, some patients complain of continued vaginal bleeding despite placement of tampon, difficulty removing a tampon, or dyspareunia. These septa may be complete or partial and can exist in any portion of the vagina (Fig. 26.16). The communication can be extremely small, and a septum can easily be missed during physical examination, especially if there is a dominant vaginal canal.

Once the diagnosis is made, both the uterus and renal anatomy should be assessed for associated anomalies. In one study, 60% of patients with longitudinal vaginal septa were found to have a bicornuate uterus [89]. Other investigators have noted a predominance of didelphic uteri in such cases [84].

A longitudinal vaginal septum should be removed in patients with complaints of dyspareunia and those who desire to be able to effectively use a tampon. In cases of didelphic uteri, removal of the septum may be necessary to allow sufficient access to each cervix for pap smears.

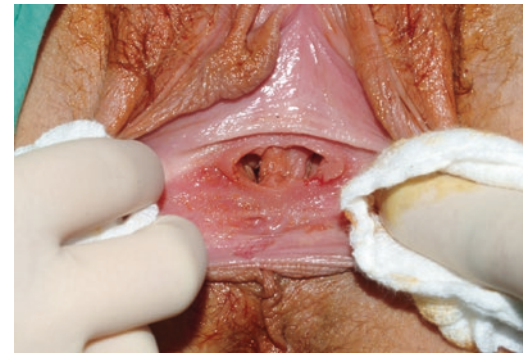

Fig. 26.16 Non-obstructing longitudinal vaginal septum (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

Some obstetricians advocate the removal of a longitudinal vaginal septum prior to delivery to avoid potential dystocia and laceration of the septum [89]. The number of patients with vaginal septa who have had successful vaginal deliveries remains unknown. However, emergent resection of a vaginal septum at the time of delivery to resolve dystocia has been reported [90]. It seems reasonable to remove a thick longitudinal septum prior to pregnancy, or prior to delivery, if discovered during pregnancy.

26.15.2 Surgical Technique

The goal of surgery is the removal of a wedge of tissue without damaging the cervix, bladder, or rectum. A Foley catheter is placed in the bladder. Since longitudinal vaginal septa are well vascularized, the anterior border of the septum, followed by the posterior border, is removed using unipolar electrosurgery. Care must be taken not to remove the septum too close to the vaginal wall, as this will leave larger mucosa defects. The edges of these mucosal defects are re-approximated with 2–0 absorbable suture. Postoperative use of a vaginal mold is not necessary.

26.15.3 The Obstructing Longitudinal Vaginal Septum

Women with an obstructing longitudinal septum usually present with normal-onset menarche and increasingly severe dysmenorrhea. These patients are most likely to have a didelphic uterus. One of

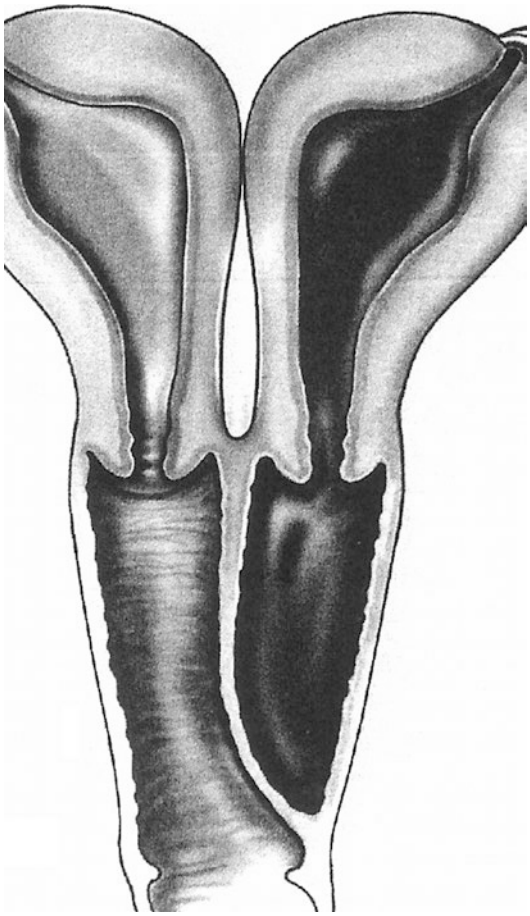

Fig. 26.17 Schematic of obstructing longitudinal septum (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

the uteri has a patent outlet, and the other is obstructed (Fig. 26.17).

If the obstruction is low in the vagina, eventually a bulge may be noted upon examination of the lower canal. However, a higher obstruction may be completely missed with just visual inspection, which is frequently the case in an adolescent. Digital examination may reveal a tense bulge in the vaginal wall (Fig. 26.18). In many instances, the bulge is towards the anterior portion of the vagina between the 12 o'clock and 3 o'clock positions or 9 o'clock and 12 o'clock positions due to the rotation of the two cervices.

Ultrasonography of the pelvis will usually show a pelvic mass, which can be misleading unless a vaginal septum is considered in the differential diagnosis. MRI is the best imaging mode for definitively diagnosing this abnormality. Like

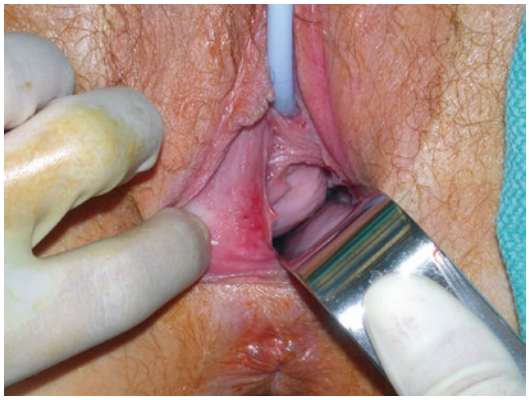

Fig. 26.18 An obstructing longitudinal septum usually presents as a bulge in the vagina (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

other Müllerian anomalies, a longitudinal vaginal septum is associated with renal abnormalities, including absent kidneys, pelvic kidneys, and double ureters [91].

Some longitudinal septa will be found to be only partially obstructing and a small opening in the septum can be found during menses with close inspection. Symptoms may vary from irregular and prolonged bleeding to profuse vaginal discharge. Occasionally, the pinpoint opening provides a pathway for organisms to access the obstructed vagina leading to pelvic infection and pyocolpos. Physical examination is unlikely to reveal a tense bulge, but a slight fullness may sometimes be appreciated in the paravaginal area.

26.15.4 Surgical Technique

Accurate delineation of anatomy is a prerequisite for surgical excision of a noncommunicating longitudinal vaginal septum. The first step is to place a needle into the bulging vaginal wall to identify the correct plane of dissection. Once blood extrudes from the needle, the adjacent tissue is incised with electrosurgery to gain access into the obstructed vagina. Allis clamps are placed on the edges of this incision and the cavity is assessed. When removing the medial border of this septum, care must be taken to avoid damaging the urethra. The septum should be removed in its entirety to allow easy access to the second cervix for pap smears. The raw mucosal edges are

approximated with 2–0 absorbable suture. Use of a vaginal mold after surgery is not necessary, since post-resection stenosis is rare. In difficult cases, use of either a resectoscope or hysteroscope to remove longitudinal vaginal septum has been described [92, 93].

The previously hidden cervix and obstructed vaginal canal will often appear abnormal. The cervix is usually flush with the vaginal fornix and often appears erythematous and glandular. Histologically, the obstructed vaginal canal and septum on its obstructed side will have columnar epithelium and glandular crypts [91]. Some patients may complain of profuse vaginal discharge after removal of the septum. Metaplastic transformation of the vaginal mucosa to mature squamous epithelium can take many years.

Simultaneous laparoscopy during removal of a vaginal septum is not recommended unless the diagnosis is unclear on MRI or imaging studies indicate concomitant pelvic masses. As in all cases of obstructive Müllerian anomalies, endometriosis is frequently encountered, even if the septum is only partially obstructing [94, 95]. With the possible exception of endometriomas, excision of the endometriosis is not recommended, since these lesions will regress after removal of the obstruction [94].

The obstetric outcome of such patients is similar to that reported for patients with simple uterine didelphys. Pregnancy rates of 87% and live birth rates of 77% have been reported [91].

26.16 Transverse Vaginal Septum

The incidence of **transverse vaginal septum** appears to be between 1 in 21,000 and 1 in 72,000 [68]. A transverse vaginal septum may be located in the upper (46%), middle (40%), and lower (14%) third of the vagina [96, 97]. A transverse vaginal septum may be complete or incomplete and varies in thickness (Fig. 26.19).

26.17 Presenting Symptoms

Patients with a complete transverse vaginal septum generally present with a complaint of primary amenorrhea in early to mid-puberty. Pelvic pain is a common, but not universal, presenting complaint. Patients with high transverse vaginal septa are most likely to experience pelvic pain, and the pain will manifest earlier than in patients with septa located lower in the vagina. This is believed to be secondary to decreased space for the hematocolpos that ensues after initiation of menses.

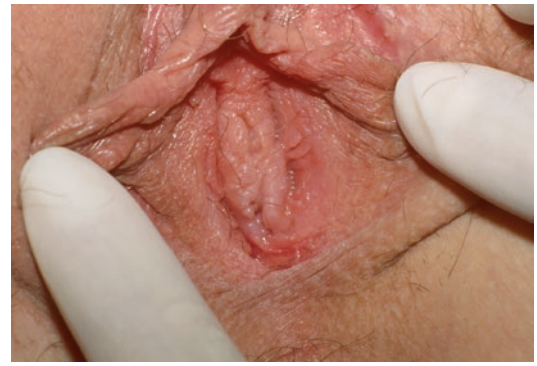

Fig. 26.19 Complete transverse septum. Notice that there is no bulge with a Valsalva maneuver that would be seen with an imperforate hymen (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

Patients with an incomplete transverse vaginal septum may complain of profuse vaginal discharge, dyspareunia, inability to insert tampon, or tear during intercourse with resultant bleeding. If asymptomatic, it may not be discovered until a routine gynecologic examination.

Very rarely a transverse vaginal septum may be detected in an infant or young child. In such instances, a mucocolpos can present as an abdominal mass [68]. If large enough, this mass may cause ureteral obstruction with secondary hydronephrosis. Compression of the vena cava and cardiopulmonary failure have also been reported.

26.17.1 Diagnosis

Manual and speculum examinations provide the most important information for diagnosis of a transverse vaginal septum. If the septum is very low, a vaginal opening may not be appreciated on evaluation of the external genitalia. A low transverse vaginal septum can usually be differentiated from an imperforate hymen by visual inspection. By increasing the intra-abdominal pressure and increasing the bulge of the imperforate hymen, the Valsalva maneuver may further assist in this differentiation.

If an opening to the vagina is noted, a manual or speculum exam may reveal a higher location of

the septum. A rectal exam is very helpful in detecting a hematocolpos, since the bulge is readily palpable.

Transperineal and transabdominal ultrasonography can sometimes diagnose and determine the thickness of a transverse vaginal septum. However, in most cases an MRI of the pelvis will be required to differentiate a transverse vaginal septum from other Müllerian anomalies such as cervical agenesis.

These patients should also be evaluated for associated anomalies, including aortic coarctation, atrial septal defects, urinary tract anomalies, and malformations of the lumbar spine [96].

26.17.2 Surgical Technique

Surgical removal of a transverse vaginal septum is recommended as soon as practical after diagnosis to avoid continued retrograde menstruation. Endometriosis is common in patients with a transverse vaginal septum. However, removal of endometriosis lesions is not recommended, since relief of the obstruction leads to their spontaneous resolution.

Delay in detection or treatment of a transverse vaginal septum may lead to impaired fertility secondary to irreversible pelvic adhesions, hematosalpinges, and endometriosis. In one long-term follow-up study of 19 patients with transverse septa, 47% became pregnant [96]. However, a small study in Finland showed a significantly higher live birth rate in women who had undergone very early diagnosis and management of their transverse vaginal septa [98].

The unfortunate consequence of very early surgical management is an increased rate of subsequent vaginal stenosis. This is most likely due to inconsistent use of vaginal dilators by young adolescents, which are a necessary part of treatment of a thick vaginal septum (see below).

An alternative to early surgery for very young patients is medical termination of monthly endometrial shedding using depo-medroxyprogesterone to postpone surgery [99]. Later, when she is emotionally ready for the surgery, the patient can be instructed to dilate the distal vagina to stretch the distal vaginal mucosa, potentially decreasing the need for a graft, and to prepare her for postoperative use of the dilator [86].

The thickness and location of the septum will determine the best approach to surgery. Thin, low transverse vaginal septa are much easier to repair than thick, usually high, septa.

26.17.3 Surgical Technique: Thin Transverse Vaginal Septum

Transverse septa that are thin and low in the vagina can usually be excised without difficulty. If visually a slight bulge cannot be appreciated on examination, an angiocath needle is placed through the septum (Fig. 26.20). With return of thick blood through the angiocath, the plane of dissection becomes clear. Access is gained into the upper vaginal cavity by perforating the transverse septum with unipolar electrosurgery or scissors.

The septum is excised in its entirety and the upper vaginal mucosa is re-approximated to the lower vaginal mucosa using 2–0 absorbable suture (Fig. 26.21). In most instances, to prevent stenosis of the vagina, continual use of a mold is recommended for several weeks after surgery.

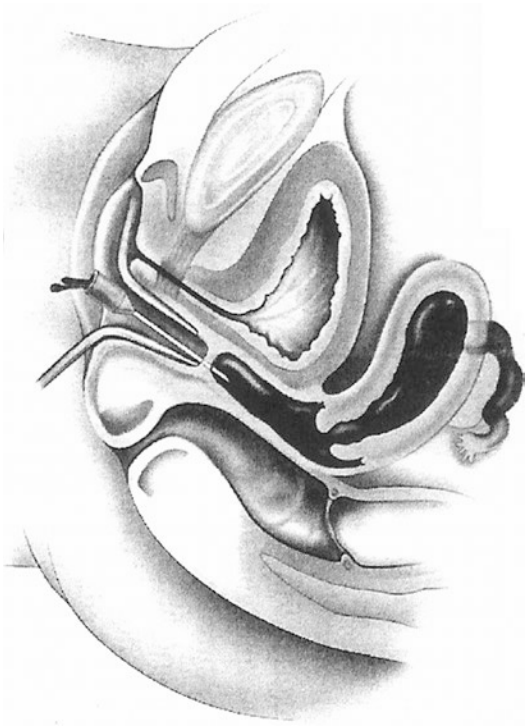

Fig. 26.20 Placement of angiocath into the transverse septum (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

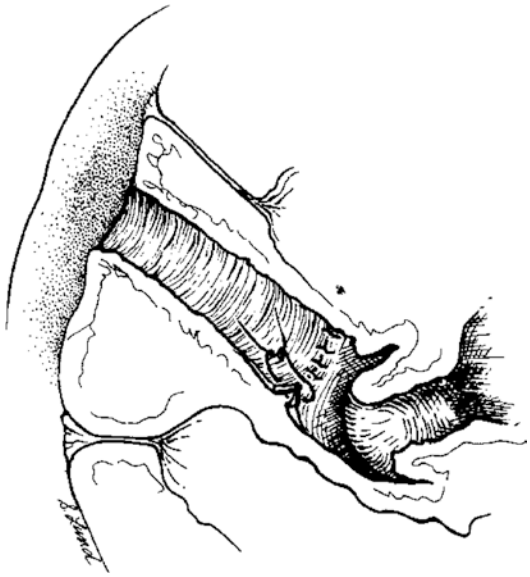

Fig. 26.21 Re-approximation of distal and proximal vagina mucosa after excision of the septum (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

26.17.4 Surgical Technique: Thick Transverse Vaginal Septum

Managing thick transverse septa can be quite challenging. Before surgery, the patient must be prepared for prolonged use of mold and possible split-thickness skin graft to line the vagina. The main concern is potential bowel injury; therefore, a bowel preparation is recommended.

During surgery, a bulge will not be seen in the presence of a thick transverse septum. The correct angle of dissection can be determined by inserting an angiocath needle into the hematocolpos under ultrasound guidance. In difficult cases, the septum can be approached transfundally via the uterus using laparoscopy and laparotomy [68].

The dissection is carried out, taking care to protect the bladder and rectum. A Foley catheter is placed into the bladder. As the loose areolar tissue is being dissected, the rectum is frequently examined to ensure appropriate angle of dissection. If there is inadvertent entry into the bladder or the rectum, the procedure should be stopped and completed at a future date. After the cervix is visualized, the goal is to re-approximate the upper mucosal tissue to the lower vaginal mucosa.

26.18 Z-Plasty Technique

If a thick septum is completely incised, the distance between the vaginal mucosal of the proximal and distal portions of the vagina may be so great that the edges cannot be re-approximated without tension. For this reason, a z-plasty technique, as first described by Garcia and colleagues, should be considered for the correction of thick transverse vaginal septa or when the vagina is short [100].

For this technique, four lower mucosal flaps are created by making oblique crossed incisions through the vaginal tissue on the perineal side of the transverse septum, taking great care to avoid injuring either the bladder or rectum. Four upper mucosal flaps are created by making oblique crossed incisions through the vaginal tissue on the hematocolpos side of the transverse septum. The upper and lower mucosal flaps are separated by sharp and blunt dissection and sutured together at their free edges to form of a continuous z-plasty.

Excellent results have been noted on 13 patients who underwent this procedure [101]. A vaginal mold must be used for 5–8 weeks after the procedure to avoid vaginal stenosis. If the girl is not sexually active, a dilator should be used at night for 6–8 additional months. The patient should be instructed in self-examination and should return if she notices any signs of early stenosis.

In cases of a thick septum where a z-plasty technique is not used, a skin graft may be required. The technique utilized is similar to that described for the McIndoe procedure. Prolonged use of a mold is required postoperatively.

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