John A. Maksem • Stanley J. Robboy
John W. Bishop • Isabelle Meiers

Endometrial Cytology with Tissue Correlations

Essentials in Cytopathology
Series Editor
Dorothy L. Rosenthal

Springer

Endometrial Cytology with Tissue Correlations

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ISBN 978-0-387-89909-1

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ISBN: 978-0-387-89909-1 e-ISBN: 978-0-387-89910-7
DOI: 10.1007/978-0-387-89910-7

Library of Congress Control Number: 2008940340

Printed on acid-free paper

springer.com

The authors wish to dedicate this work to our loved ones and to our beloved mentors, who encouraged us in our pursuit of gynecological pathology and cytopathology, and who inspired us to create this work. Special mention is given to Dr. Liang-Che Tao, whose seminal publications, heartfelt support, and brilliant invention both inspired and encouraged us to gain a further understanding of the art of endometrial cytopathology. Also, especial thanks must be extended to Dr. David G. Bostwick, whose innovative views on the practice of anatomic pathology and cytopathology have helped to move this field forward and to make this effort possible.

Foreword

Although only 2 cm separate the cervical mucosa from the lining of the uterine cavity, the endometrium has not been screened for neoplasms to the same extent as has the uterine cervix. As a result, endometrial cancers are now the most common malignancies of the female reproductive organs in the United States. Two factors have long been blamed as the reason to bypass cytological examination of endometrial tissue in favor of biopsy: inadequate material to reliably categorize the sample, and inadequate experience by cytopathologists to accurately triage the patient for further management.

Maksem and his colleagues provide convincing evidence, both by data and by superb micrographs, that good material can be harvested and experience can be acquired to direct clinicians in managing their patients. In an era in which cost containment is essential, the ability to adequately sample a potentially malignant tissue in an office setting without sedation or anesthesia is a bonus.

The Editorial Board of the EIC Series predicts that this volume will become a seminal work. We look forward to reading about your experience with this long-neglected frontier in cytopathology.

Dorothy L. Rosenthal, M.D., F.I.A.C.

Series Preface

The subspecialty of cytopathology is 60 years old and has become established as a solid and reliable discipline in medicine. As expected, cytopathology literature has expanded in a remarkably short period of time, from a few textbooks prior to the 1980's to a current and substantial library of texts and journals devoted exclusively to cytomorphology. *Essentials in Cytopathology* does not presume to replace any of the distinguished textbooks in cytopathology. Instead, the series will publish generously illustrated and user-friendly guides for both pathologists and clinicians.

Building on the amazing success of *The Bethesda System for Reporting Cervical Cytology,* now in its second edition, the *Series* will utilize a similar format, including minimal text, tabular criteria, and superb illustrations based on real-life specimens. *Essentials in Cytopathology* will, at times, deviate from the classic organization of pathology texts. The logic of decision trees, elimination of unlikely choices, and narrowing of differential diagnosis via a pragmatic approach based on morphologic criteria will be some of the strategies used to illustrate principles and practice in cytopathology.

Most of the authors for *Essentials in Cytopathology* are faculty members in The Johns Hopkins University School of Medicine, Department of Pathology, Division of Cytopathology. They bring to each volume the legacy of John K. Frost and the collective experience of a preeminent cytopathology service. The archives at Hopkins are meticulously catalogued and form the framework for text and illustrations. Authors from other institutions have been selected on the basis of their national reputations, experience. and enthusiasm for cytopathology. They bring to the series complementary

viewpoints and enlarge the scope of materials contained in the photographs.

The editor and authors are indebted to our students, past and future, who challenge and motivate us to become the best that we possibly can be. We share that experience with you through these pages, and hope that you will learn from them as we have from those who have come before us. We would be remiss if we did not pay tribute to our professional colleagues, the cytotechnologists and preparatory technicians who lovingly care for the specimens that our clinical colleagues send to us.

And finally, we cannot emphasize enough throughout these volumes the importance of collaboration with the patient care team. Every specimen comes to us as a question begging an answer. Without input from the clinicians, complete patient history, results of imaging studies and other ancillary tests, we cannot perform optimally. It is our responsibility to educate our clinicians about their role in our interpretation, and for us to integrate as much information as we can gather into our final diagnosis, even if the answer at first seems obvious.

We hope you will find this series useful and welcome your feedback as you place these handbooks by your microscopes, and into your book bags.

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Introduction

Direct cytological sampling and examination of the endometrium is not generally practiced, which is surprising as the endometrium is exceedingly easy to sample. Over the years, as we gained more experience with specimen acquisition, processing, and interpretation, we found endometrial cytology to be an effective method both for ensuring endometrial normalcy and for discovering and diagnosing malignant and premalignant states. In comparing endometrial cytology to biopsy, we found that, among samples obtained by individuals experienced in specimen collection, cytology outperforms outpatient biopsy in terms of tolerance of the procedure by the patient, adequacy of the sample among postmenopausal women, and detection of occult neoplasms.

By using the Tao brush (also known as the Indiana University Medical Center endometrial sampler) and devising a technical strategy to ensure the simultaneous creation of cell blocks and cytological samples from a single collection, we moved our appreciation of endometrial brush collection into an arena whose significance equals other methods of specimen collection and interpretation. Cytology performs equally as well as biopsy in detecting hyperplasia and carcinoma. If nothing else, by reliably identifying benign normal endometrial states, it serves to confidently exclude more than 70% of women from unnecessary follow-up testing.

Because the Tao brush samples only the superficial 2 mm of the endometrium, the method is not designed to detect endome-

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trial polyps, leiomyomas, stromal tumors, and tumors of the uterine wall musculature. However, it is useful for detecting benign estrogen-excess states such as disordered proliferation and various degrees of benign hyperplasia and for separating these from neoplastic states such as endometrial intraepithelial neoplasm (EIN), endometrial gland dysplasia (EmGD), and cancer. Nonetheless, it cannot always subclassify benign hyperplastic states of the endometrium without the aid of cell blocks.

When endometrial brushing is combined with liquid fixation and with other techniques such as immunohistochemistry, cell block examination, hysteroscopy, or sonohysterography, endometrial benignancy can be confidently assured. In a woman with a patent cervix, endometrial brushing successfully collects material, even from late postmenopausal atrophic endometrium. It detects serious low-volume diseases such as endometrial intraepithelial carcinoma (serous surface carcinoma, or EIC) under conditions where suction biopsy may have missed or otherwise obviated the diagnosis.

This monograph focuses on the background, collection technique, and reliability of endometrial cytology. It overviews diagnostic criteria and diagnostic pitfalls encountered in practicing this art. Because endometrial cytology interpretation relies heavily on intuiting tissue patterns from cytology preparations, emphasis is placed on cytohistological correlations with cell block material, and, where effective as part of a diagnostic strategy, on ancillary immunohistochemical staining. The discussion moves from normal states, through otherwise benign changes induced by an altered hormonal milieu or surface irritants, into precancerous and malignant endometrial conditions. Finally, it covers fixative and slide preparation methods for the benefit of those who wish to repeat this work in their own practice.

Suggested Reading

Maksem JA, Knesel E. Liquid fixation of endometrial brush cytology ensures a well-preserved, representative cell sample with frequent tissue correlation. Diagn Cytopathol 1996; 14: 367–73.

Yang GC, Wan LS, Del Priore G. Factors influencing the detection of uterine cancer by suction curettage and endometrial brushing. J Reprod Med 2002; 47: 1005–10.

Kyroudi A, Paefthimiou M, Symiakaki H, Mentzelopoulou P, Voulgaris Z, Karakitsos P. Increasing diagnostic accuracy with a cell block preparation from thin-layer endometrial cytology: a feasibility study. Acta Cytol 2006; 50: 63–9.

Maksem JA, Meiers I, Robboy SJ. A primer of endometrial cytology with histological correlation. Diagn Cytopathol 2007; 35: 817–44.

1
Office-Based Endometrial Sampling

Outpatient biopsy has replaced dilatation and curettage for evaluating most endometrial disorders, including hyperplasia and cancer. The literature is replete with arguments that support its accuracy, convenience to the patient and physician, and cost containment benefits. The most common option for outpatient sampling is the suction biopsy device, best exemplified by the Unimar Pipelle. A meta-analysis of 142 published studies ranked the success of endometrial sampling methods in women with abnormal vaginal bleeding and showed that outpatient endometrial biopsy adequately samples the uterus from 24% to 97% of the time and detects from 67% to nearly 100% of endometrial cancers.

This work presents direct cytological sampling of the uterine cavity as another diagnostic tool available to clinicians and pathologists alike. As with suction biopsy, its purpose is the assurance of endometrial benignity and the detection of endometrial neoplasm. Cytological sampling of the endometrium is a gentle method that is less painful than suction biopsy. Our intention is to show that in experienced hands direct cytological sampling is at least as thorough and as accurate as suction biopsy.

Endometrial sampling is generally performed as a response to abnormal vaginal bleeding of endometrial origin. Abnormal vaginal bleeding may be caused by anything from a physiological event such as dysfunctional uterine bleeding resulting from endometrial atrophy, to a benign tumor such as a polyp or leiomyoma, or

J.A. Maksem et al., *Endometrial Cytology with Tissue Correlations*, Essentials in Cytopathology 7, DOI: 10.1007/978-0-387-89910-7_2,

neoplastic disease such as endometrial intraepithelial neoplasm (EIN) and cancer. Persistent postmenopausal bleeding in the setting of an endometrial stripe in excess of 4-mm thickness imposes a greater than 60-fold-increased risk for endometrial cancer, and some investigators report that combining suction curettage with endometrial cytology is the best strategy for examining outpatients with abnormal uterine bleeding.

Endometrial sampling may be used to screen or monitor selected "at-risk" populations. For example, because of an increase in the use of hormones as adjuvant therapy in both postmenopausal women and women with breast cancer, endometrial sampling has become a mainstay of therapeutic monitoring. It is also useful for monitoring women with premalignant endometrial changes who have been treated with hormones to assess their response to therapy.

Endometrial cytology is a reliable and well-tolerated method of detecting uterine pathology in tamoxifen-treated women. In one study of 687 tamoxifen-treated women, 189 had a double-layer endometrial thickness (i.e., stripe) of more than 8 mm. Of these, 150 underwent cytological endometrial sampling followed by hysteroscopy and curettage. The cytological and histological findings correlated well in 145 cases, leading investigators to recommend the combination of ultrasonography and brush cytology as a monitoring strategy for women treated with this drug.

When coupled to ultrasonography, liquid-based cytology, and cell block examination, endometrial cytology is at least as sensitive and specific as other office-based biopsy methods. The advantage of liquid-based processing is that it affords standardized and reproducible endometrial preparations, which in turn fosters the application of common diagnostic criteria among cytopathologists. For example, in a study of 162 endometrial samplings fixed in CytoLyt and processed with ThinPrep, nearly perfect interobserver diagnostic agreement was achieved.

A study of abnormal uterine bleeding using PreservCyt fixative and ThinPrep recorded a 96% overall specificity for endometrial cytology, a 78% positive predictive value and a 96% negative predictive value for atypical hyperplasia and adenocarcinoma, and a 15% unsatisfactory rate, about half that of endometrial biopsy (26%). Another study evaluated the accuracy of liquid-based endometrial cytology as compared to biopsy in 670 women

scheduled for hysteroscopy because of thickened endometrium. Endometrial biopsy detected pathology in 41 (6%) cases (21 of which were adenocarcinomas). Cytological study found pathology in 62 (9%) cases (19 of which were adenocarcinomas). Two hundred ninety-one biopsies (43%) and 28 (4%) cytologies were inadequate. Cytology provided sufficient diagnostic material more often than biopsy ($P < 0.01$).

The same investigators subsequently evaluated 917 women scheduled for hysteroscopy by sequentially performing hysteroscopy, endometrial cytology, and biopsy sampling. Cytohistological correlations were possible in 519 cases (57%). In 361 (39%) cases the biopsy was inadequate, in 15 (2%) the cytology was inadequate, and in 22 (2%) both were inadequate. Again, cytology provided sufficient material more often than did biopsy ($P < 0.04$). Its sensitivity for atypical hyperplasia and adenocarcinoma was 96%, specificity was 98%, positive predictive value was 86%, and negative predictive value was 99%.

Although unpopular in the United States until recently, cytology has been recognized as an acceptable diagnostic method for endometrial assessment. A Health Technology Assessment report issued to the National Health Service of the United Kingdom compared outpatient methods of endometrial evaluation in terms of performance, patient acceptability, and cost-effectiveness. An endometrial cytology sampling device, the Tao brush, was as effective as the Pipelle device in obtaining tissue from moderate-risk (premenopausal and perimenopausal) women (77% vs. 79% effective, respectively) and far more effective in obtaining tissue from high-risk (postmenopausal) women (72% vs. 49% effective, respectively). Women preferred the Tao brush as it caused less discomfort.

The report concluded that Tao brush cytology should be the method of choice for endometrial sampling of postmenopausal women and that it should be available as backup in premenopausal and perimenopausal women if the Pipelle biopsy fails to obtain tissue. In short, it affirmed that endometrial cytology is diagnostically reproducible, as reliable as biopsy in case-finding, and generally more reliable than biopsy in obtaining sufficient diagnostic material.

Others have reported similar experiences with the Tao brush. By using both the Tao brush and the Pipelle, investigators achieved a

highly effective strategy for both diagnosing and excluding uterine cancer, approaching nearly 100% specificity and sensitivity. The contribution of simultaneously examined cell block material was underscored in one study in which cell block diagnoses from 263 endometrial samplings perfectly matched the diagnoses of control hysterectomy specimens. By adding cell block histology to endometrial cytology, investigators increased the overall diagnostic accuracy of endometrial cytology to 96% and 100% for benign/atrophic endometrium and adenocarcinoma, respectively, and increased the diagnostic accuracy for hyperplasia to more than 95%.

Suggested Reading

Buccoliero AM, Gheri CF, Castiglione F, Garbini F, Fambrini M, Bargelli G, Barbetti A, Pappalardo S, Taddei A, Boddi V, Scarselli GF, Marchionni M, Taddei GL. Liquid-based endometrial cytology in the management of sonographically thickened endometrium. Diagn Cytopathol 2007; 35: 398–402.

Buccoliero AM, Gheri CF, Castiglione F, Garbini F, Barbetti A, Fambrini M, Bargelli G, Pappalardo S, Taddei A, Boddi V, Scarselli GF, Marchionni M, Taddei GL. Liquid-based endometrial cytology: cytohistological correlation in a population of 917 women. Cytopathology 2007; 18: 241–9.

Chambers JT, Chambers SK. Endometrial sampling: when? where? why? with what? Clin Obstet Gynecol 1992; 35: 28–39.

Critchley HO, Warner P, Lee AJ, Brechin S, Guise J, Graham B. Evaluation of abnormal uterine bleeding: comparison of three outpatient procedures within cohorts defined by age and menopausal status. Health Technol Assess 2004; 8(34): iii–iv, 1–139.

Garcia F, Barker B, Davis J, Shelton T, Harrigill K, Schalk N, Meyer J, Hatch K. Thin-layer cytology and histopathology in the evaluation of abnormal uterine bleeding. J Reprod Med 2003; 48: 882–8.

Gull B, Karlsson B, Milsom I, Granberg S. Can ultrasound replace dilation and curettage? A longitudinal evaluation of postmenopausal bleeding and transvaginal sonographic measurement of the endometrium as predictors of endometrial cancer. Am J Obstet Gynecol 2003; 188: 401–8.

Kondo E, Tabata T, Koduka Y, Nishiura K, Tanida K, Okugawa T, Sagawa N. What is the best method of detecting endometrial cancer in outpatients? -endometrial sampling, suction curettage, endometrial cytology. Cytopathology 2008; 19: 28–33.

Kyroudi A, Paefthimiou M, Symiakaki H, Mentzelopoulou P, Voulgaris Z, Karakitsos P. Increasing diagnostic accuracy with a cell block preparation from thin-layer endometrial cytology: a feasibility study. Acta Cytol 2006; 50: 63–9.

Mathelin C, Youssef C, Annane K, Brettes JP, Bellocq JP, Walter P. Endometrial brush cytology in the surveillance of post-menopausal patients under tamoxifen: a prospective longitudinal study. Eur J Obstet Gynecol Reprod Biol 2007; 132(1): 126–8.

Papaefthimiou M, Symiakaki H, Mentzelopoulou P, Tsiveleka A, Kyroudes A, Voulgaris Z, Tzonou A, Karakitsos P. Study on the morphology and reproducibility of the diagnosis of endometrial lesions utilizing liquid-based cytology. Cancer (Phila) 2005; 105: 56–64.

Shapley M, Redman CW. Endometrial sampling and general practice. Br J Gen Pract 1997; 47: 387–91.

Spencer CP, Whitehead MI. Endometrial assessment re-visited. Br J Obstet Gynaecol 1999; 106: 623–32.

Sonoda Y, Barakat RR. Screening and the prevention of gynecologic cancer: endometrial cancer. Best Pract Res Clin Obstet Gynaecol 2006; 20: 363–77.

Yang GC, Wan LS. Endometrial biopsy using the Tao Brush method. A study of 50 women in a general gynecologic practice. J Reprod Med 2000; 45: 109–14.

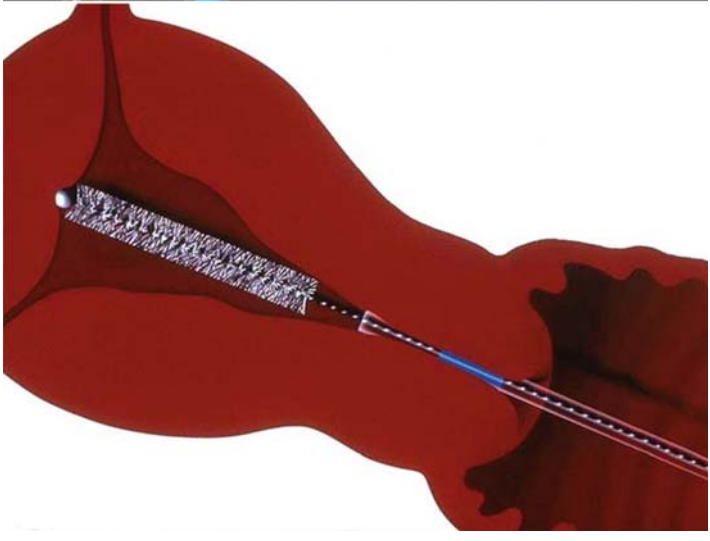
Yang GC, Wan LS, Del Priore G. Factors influencing the detection of uterine cancer by suction curettage and endometrial brushing. J Reprod Med 2002; 47: 1005–10.

2
Tao Brush and Endometrial Cytology

The traditional method of endometrial biopsy uses a specialized catheter to simultaneously suction and slice away a portion of the uterine lining. Tissue is collected through a small orifice present near the catheter tip. In comparison, the Tao brush method uses a small, flexible brush to gently brush the inside of the uterus. Thus, the Tao brush gathers a complete sampling of the uterine lining, removes tissue in a less traumatic fashion, and, as there is no need for continuous movement of the device across the cervical canal, is less painful than suction biopsy. Because the Tao brush method is able to collect samples from a 3-cm or greater length of the midportion of the uterine corpus without significant discomfort to the patient, it is an excellent method for early detection, therapeutic monitoring, and, among selected women, screening.

When cell blocks are added into the diagnostic algorithm, the Tao brush becomes an endometrial cytology and histology collection device whose distinguishing features include a long brush that allows a substantial length (3 cm) and thickness (2 mm) of the endometrium to be sampled without excessive manipulation. The Tao brush has an outer sheath of similar outer diameter to the Pipelle that reduces endocervical and vaginal cell contamination, a smooth, rounded end that minimizes uterine wall injury or inadvertent myometrial perforation, and a flexible wire core that acts as a guidewire and allows the sampling portion of the device to move easily through uterine cavity irregularities. The wire is of proper

J.A. Maksem et al., *Endometrial Cytology with Tissue Correlations*, Essentials in Cytopathology 7, DOI: 10.1007/978-0-387-89910-7_3,



length to enable precise retraction of the outer sheath of the device to the level of the internal os of the uterine cervix (Fig. 2.1).

The brush is an easy-to-employ device designed for office use; however, cervical patency and cleanliness is a necessary precondition for successful uterine sampling. If cervical mucus heavily coats the brush's cannula, then, on insertion of the device into the endometrial cavity, the mucus may displace endometrial material and be substituted in its place.

After cervical patency has been ensured and after excessive mucus has been cleared from the endocervical canal, the sheathed Tao brush is inserted to the level of the uterine fundus. Its overlying sheath is retracted to expose the brush bristles to the uterine cavity. Then, because the brush's bristles are arrayed in a helical pattern, after the fashion of an augur, the brush is rotated several (at least four to six) times in one direction using an action similar to that used in winding a wristwatch. The sheath is replaced over the brush's bristles, and the closed assembly is removed from the uterus. Finally, the brush is then pushed from its cannula, cut off, and, in our approach to the art, placed in an appropriate liquid-based cytology fixative.

Suggested Reading

Del Priore G, Williams R, Harbatkin CB, Wan LS, Mittal K, Yang GC. Endometrial brush biopsy for the diagnosis of endometrial cancer. J Reprod Med 2001; 46: 439–43.

Firat P, Mocan G, Kapucuoglu N. Liquid-based endometrial cytology: endometrial sample collection by using Tao brush. Diagn Cytopathol 2002; 27: 393–4.

FIG. 2.1. The Tao brush (Indiana University Medical Center endometrial sampler manufactured by Cook OB/GYN®) has an outer sheath, of similar outer diameter to other endometrial collection devices, that reduces endocervical and vaginal cell contamination; a smooth, rounded end that minimizes uterine wall injury or inadvertent myometrial perforation; and a flexible wire core that acts as a guidewire and allows the sampling portion of the device to move easily through uterine cavity irregularities. The wire is of proper length to enable retraction of the outer sheath of the device to the level of the internal os of the uterine cervix.

Tao LC. Cytopathology of the endometrium. Direct intrauterine sampling. In: Johnston, WW, ed. ASCP Theory and Practice of Cytopathology, vol 2. ASCP Press, Chicago, IL, 1993.

Tao LC. Direct intrauterine sampling: the IUMC Endometrial Sampler. Diagn Cytopathol 1997; 17: 153–9.

Yang GC, Wan LS. Endometrial biopsy using the Tao Brush method. A study of 50 women in a general gynecologic practice. J Reprod Med 2000; 45: 109–14.

Yang GC, Wan LS, Del Priore G. Factors influencing the detection of uterine cancer by suction curettage and endometrial brushing. J Reprod Med 2002; 47: 1005–10.

3
Cytoarchitecture and Nuclear Atypia as the Bases for the Cytology Risk-Stratification of Endometrial Samplings

Many tend to think of cytology as a nucleus-centered diagnostic method that gives little consideration to tissue structure. However, as the Gynecologic Oncology Group's pathology committee moved its diagnostic benchmarks of endometrial cancer from a purely tissue-pattern approach to grading and included nuclear features as an adjunct to cancer diagnosis, so cytopathologists moved from a nucleus-centered diagnosis of endometrial abnormalities to one that considered definable three-dimensional patterns. Confocal microscopy, an optical imaging technique whose concept was patented by Marvin Minsky in 1957, has been used to reconstruct three-dimensional images of specimens that are thicker than the focal plane. Confocal microscopic "sectioning" of endometrial cytology microbiopsies present on thick smears showed, in addition to mitotic figures and altered chromatin patterns, glandular architecture and the shattered remnants of tissue patterns.

The nucleus also represents an important part of the whole of any endometrial cytology diagnostic algorithm. Atrophic and proliferative endometrial epithelial nuclei may be taken as benchmarks of normal nuclear morphology (Fig. 3.1). Among tumor nuclei, FIGO grade 1 nuclei are uniform, round to oval nuclei, with inconspicuous nucleoli, and occasionally resemble the nuclei of functional endometrium, not greatly exceeding the variability of proliferative endometrial nuclei (Fig. 3.2). Grade 2 nuclei are irregular or oval with moderate-size nucleoli (Fig. 3.3). Grade 3 nuclei are large and pleomorphic and with moderate to large irregular nucleoli (Fig. 3.4).

J.A. Maksem et al., *Endometrial Cytology with Tissue Correlations*, Essentials in Cytopathology 7, DOI: 10.1007/978-0-387-89910-7_4,

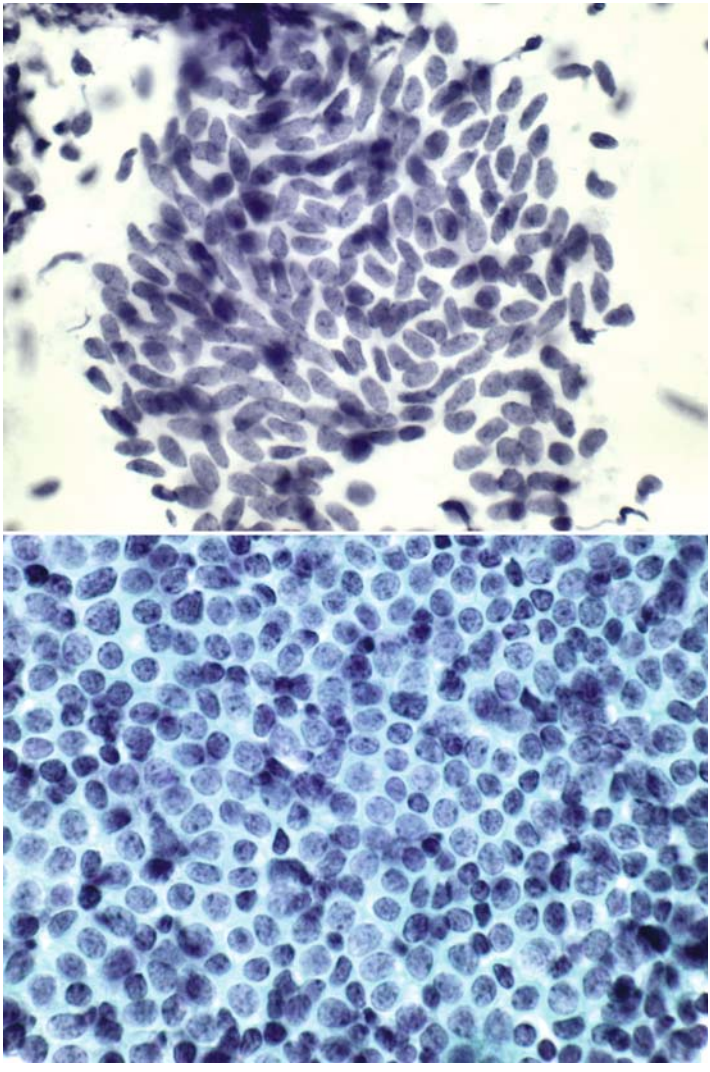


FIG. 3.1. Inactive endometrial epithelial nuclei are shown splayed out (*top*) and en face (*bottom*). Although many authors describe these benchmark nuclei as round, they are actually shaped more like rugby balls. In their ideal form, they are highly regular; but, in practice, they may show moderate variation derived, for example, from foci of variable proliferative activity, senile associated symplastic change, and epithelial metaplasia. These images represent the "ground zero" from which other endometrial epithelial nuclear changes may be judged and graded.

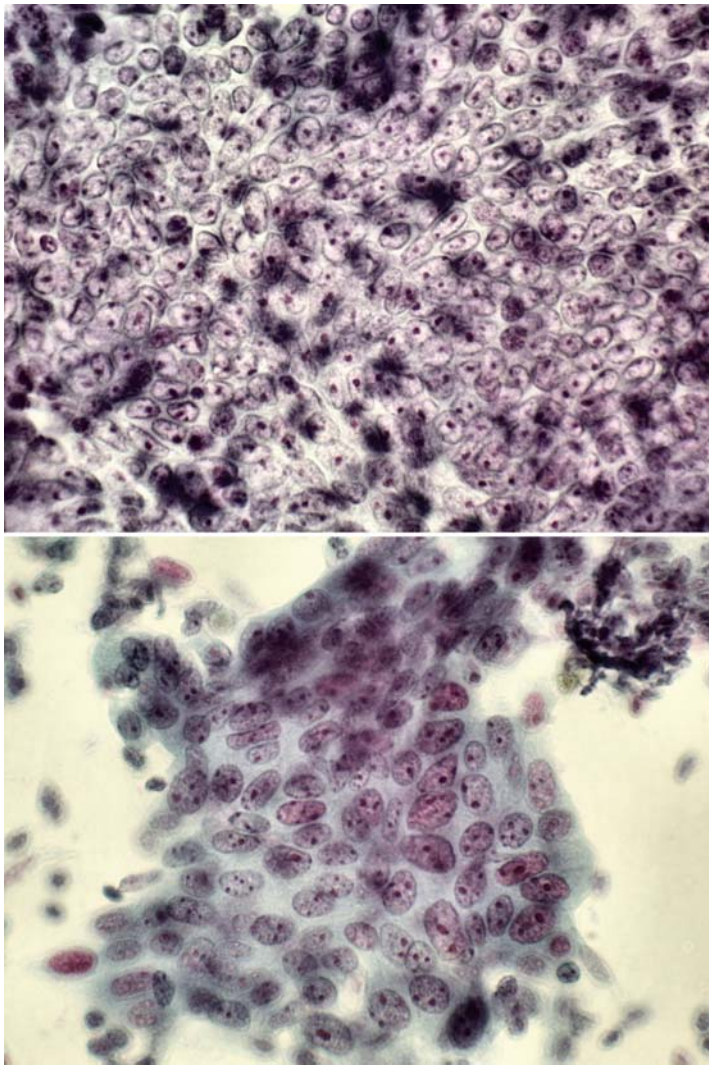


FIG. 3.2. Grade 1 nuclei are uniform, round to oval nuclei, with inconspicuous nucleoli, and occasionally resemble the nuclei of functional endometrium, not greatly exceeding the variability of proliferative endometrial nuclei. Nuclei of this type may be seen with lesions ranging from hyperplasia and endometrial intraepithelial neoplasm (EIN) to well-differentiated type 1 endometrioid endometrial adenocarcinoma. They differ from the nuclei of inactive endometrium, but may be seen in some benign proliferative states.

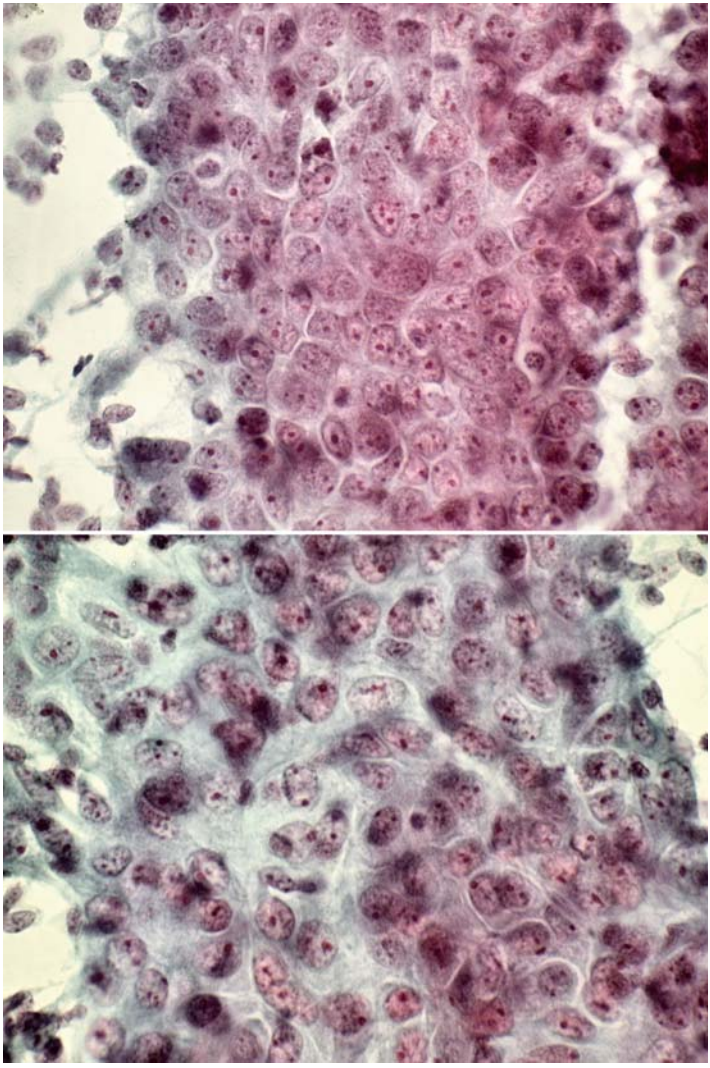


FIG. 3.3. Grade 2 nuclei are irregular or oval with moderate-size nucleoli. Although they stand out as neoplastic appearing and are the type of nuclei seen among many of the endometrioid neoplasms, they may also be seen in activated endometrial epithelium and be associated with, for example, polyps or the pressure irritation caused by submucous fibroid tumors. When nuclei of this sort largely represent the endometrium, consider endometrioid neoplasia; and, when they represent a minor component of the brush sampling, consider EIN or its mimics.

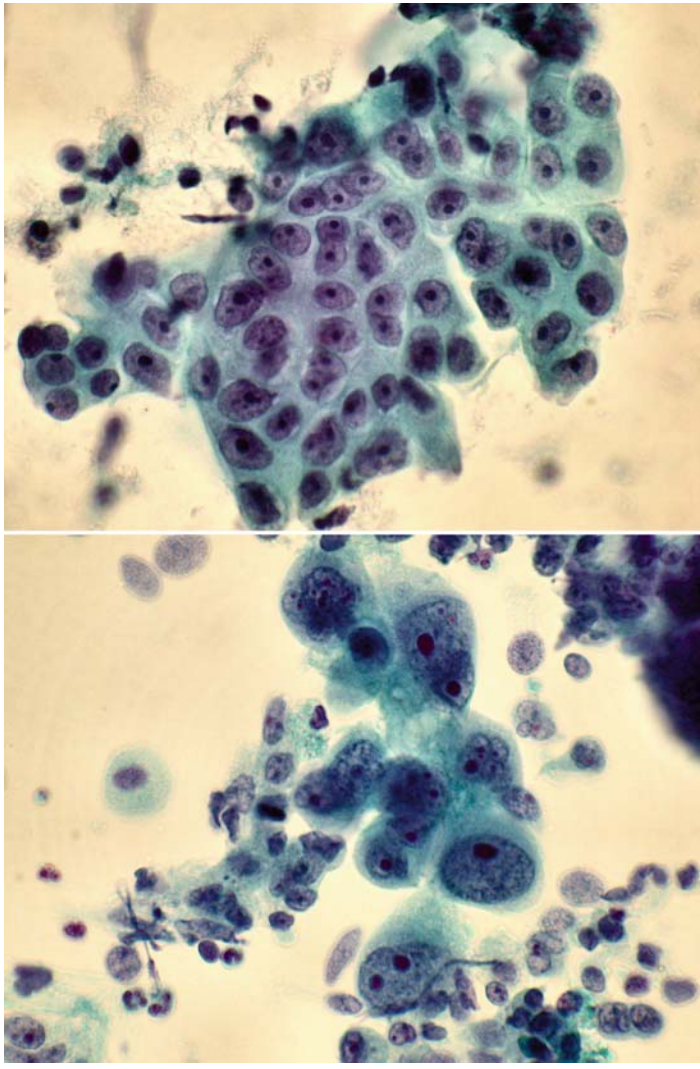


FIG. 3.4. Grade 3 nuclei are large and pleomorphic and have moderate to large irregular nucleoli. Cells with grade 3 nuclei generally derive from neoplasms and may even indicate serous carcinoma or its antecedent lesions such as endometrial intraepithelial carcinoma (EIC) or endometrial gland dysplasia (EmGD). When grade 3 nuclei are seen in any numbers, p53 and Ki-67 immunostaining may be useful to exclude these lesions as their diagnosis may warrant hysterectomy and extended surgical staging, even in the absence of measurable endometrial thickening.

By benchmarking the nuclear atypia of endometrial cells to the nucleus of the inactive or proliferative gland cell, nuclear changes can be designated as progressively atypical for pathological states beyond anovulatory endometrium and simple hyperplasia. The problem with relying on nuclear atypia for triaging cytology cases into "benign" versus "benign abnormal" versus "neoplastic" categories is that about one-quarter of endometrial cancers, specifically, those with FIGO grade 1 nuclei, show pale-staining nuclei without coarse chromatin or prominent nucleoli. In those endometrial adenocarcinomas where dyskaryosis is at a minimum, the tissue diagnosis of "cancer" defaults to pattern recognition. Pattern recognition is feasible in cytology collections, and it is made easier and more accurate when diagnoses are augmented by the examination of cell blocks.

Gland diameter and nuclear characteristics have been used to classify endometrial cytology into normal endometrium, altered endometrial proliferative states, and neoplasia. There is progressive enlargement of proliferative gland diameter through anovulatory endometrium to simple hyperplasia. Variability of gland shape and nuclear enlargement typify endometrial intraepithelial neoplasm (EIN)/endometrioid neoplasia, whereas the loss of gland structure characterizes outspoken adenocarcinoma.

Cell aggregates with tube or sheet patterns are universally present in normal proliferative endometrium. Dilated or irregularly branched patterns are seen in many endometrial hyperplasias without atypia. Cellular aggregates (microbiopsies) with irregular protrusions or tubulopapillary structures occur in many atypical hyperplasias and most low-grade endometrial carcinomas. When the small amounts of cellular material that sticks to endometrial sampling tools are used to create cell blocks and demonstrate endometrial structural abnormalities, the accuracy of endometrial cytology increases.

Investigators have developed criteria for endometrial cytology diagnoses based on nuclear atypia, stromal clustering, and epithelial cell clustering and tested the applicability of these criteria to typical cases of normal endometrium, simple endometrial hyperplasia without atypia, complex endometrial hyperplasia without atypia, and grades 1 and 2 adenocarcinomas. In one study, epithelial clustering was classified as simple, large and regular, large and irregular, and small and irregular. Nuclear atypia was significantly

more frequent in grade 2 adenocarcinoma than in any of the other four categories ($P < 0.001$). Stromal clustering was significantly more frequent in normal endometrium and simple endometrial hyperplasia without atypia than in the other three categories ($P < 0.001$). Adenocarcinoma showed a significantly higher frequency of large and irregular epithelial clusters than the other three categories ($P < 0.001$). Complex endometrial hyperplasia without atypia exhibited a significantly higher frequency of large and regular epithelial clusters than the other four categories ($P < 0.001$).

It is interesting to compare the foregoing observations based, literally, on small fragments of tissue material observable on cytology slides to the three-dimensional reconstructions of normal and abnormal endometrial states based on serially examined tissue sections that were illustrated by Hendrickson and Kempson about 25 years earlier. By doing so, one appreciates how much was already known about endometrial microbiopsy structure and how much could have been anticipated about the translation of this knowledge between histology and cytology.

The sensitivity and specificity of endometrial cytology are enhanced by combining cytology patterns and nuclear features. Building on the pioneering observations of Tao and others, we propose a pattern/dyskaryosis-based risk-ranking strategy for endometrial cytodiagnosis that combines cytoarchitecture and nuclear features to rank the relative risk of a cytology slide for being diagnostic of a cancer or a precancerous state. Our definitions of nuclear atypia follow those proposed by the Gynecologic Oncology Group and are described and illustrated above. Our cytology patterns reflect low-power morphological changes, which can be seen in histology, cell block, and cytology preparations, and which progressively evolve along the spectrum of benign to malignant endometrial states.

Flat epithelial sheets and uniform epithelial tubules define the low-power morphology of benign endometrium, variably modified by its hormonal milieu. Cystic epithelial glands, bulbous irregularities of glands, cup-shaped epithelial sheets, and smoothly contoured multiply outpouched glands are the hallmark changes of endometrium that has resided in an environment of unopposed estrogens, ranging from disordered proliferation and posthyperplastic atrophy to frank hyperplasia. Tufted epithelial cell cluster,

TABLE 3.1. Risk of endometrial abnormality as a function of pattern and nuclear grade.

	FIGO Nuclear Grade		
Microbiopsy pattern	≤N1	N2	N3
Flat epithelial sheets and uniform epithelial tubules	Normal	Benign abnormality	Probable precancer
Cystic epithelial glands, bulbous irregularities of glands, cup-shaped epithelial sheets, and smoothly contoured multiply outpouched glands	Benign abnormality	Benign abnormality	Probable precancer
Tufted epithelial cell cluster, pseudopapillae, and balled-up epithelial cell groups	Benign abnormality	Probable precancer	Probable cancer
Bridge-like connections composed of epithelial cells, cell clusters with intraepithelial soap-bubble-like lumens, distorted acini	Probable precancer	Probable cancer	Probable cancer
Cribriform structures, solid clusters of epithelial cells (excluding morules and metaplastic squamous cells), or dyshesive epithelial cells	Probable cancer	Probable cancer	Probable cancer

pseudopapillae, and balled-up epithelial cell groups may be seen with endometrial breakdown, with polyps, or in atypical hyperplastic and cancerous states of the endometrium. Bridge-like connections composed of epithelial cells or cell clusters with intraepithelial soap-bubble-like lumens with or without acini that have been distorted by tight epithelial packing with little stromal cushioning are seen in low-grade neoplastic endometrial states including low-grade adenocarcinomas. Cribriform structures, solid clusters of epithelial cells (excluding morules and metaplastic squamous cells), or dyshesive epithelial cells generally indicate outspoken endometrial cancer.

Although imperfect, this pattern/dyskaryosis-based approach to cytology risk-ranking allows for a coarse separation of specimens into categories of normal endometrium, benign endometrial abnormalities, and neoplastic endometrium (comprising the spectrum of EIN to outspoken adenocarcinoma) with some degree of certainty. This approach, outlined in Table 3.1 and tested against more than 2,000 hysterectomy controls, allows for the exclusion of numerous women with totally normal-appearing endometrium from further invasive testing.

Suggested Reading

Boon ME, Luzzatto R, Brucker N, Recktenvald MS, Benita EM. Diagnostic efficacy of endometrial cytology with the Abradul cell sampler supplemented by laser scanning confocal microscopy. Acta Cytol 1996; 40: 277–82.

Hendrickson MR, Kempson RL. Surgical pathology of the uterine corpus. In: Bennington JL, ed. Major Problems in Pathology. Saunders, Philadelphia, PA, 1980.

Ishii Y, Fujii M. Criteria for differential diagnosis of complex hyperplasia or beyond in endometrial cytology. Acta Cytol 1997; 41: 1095–102.

Kobayashi H, Otsuki Y, Simizu S, Yamada M, Mukai R, Sawaki Y, Nakayama S, Torii Y. Cytological criteria of endometrial lesions with emphasis on stromal and epithelial cell clusters: result of 8 years of experience with intrauterine sampling. Cytopathology 2008; 19: 19–27.

Maksem JA. Performance characteristics of the Indiana University Medical Center endometrial sampler (Tao Brush) in an outpatient office setting, first year's outcomes: recognizing histological patterns in cytology preparations of endometrial brushings. Diagn Cytopathol 2000; 22: 186–95.

Maksem J, Sager F, Bender R. Endometrial collection and interpretation using the Tao brush and the CytoRich fixative system: a feasibility study. Diagn Cytopathol 1997; 17: 339–46.

Norimatsu Y, Shimizu K, Kobayashi TK, Moriya T, Tsukayama C, Miyake Y, Ohno E. Cellular features of endometrial hyperplasia and well differentiated adenocarcinoma using the Endocyte sampler: diagnostic criteria based on the cytoarchitecture of tissue fragments. Cancer (Phila) 2006; 108: 77–85.

Palermo VG. Interpretation of endometrium obtained by the Endo-pap sampler and a clinical study of its use. Diagn Cytopathol 1985; 1: 5–12.

Papaefthimiou M, Symiakaki H, Mentzelopoulou P, Giahnaki AE, Voulgaris Z, Diakomanolis E, Kyroudes A, Karakitsos P. The role of liquid-based cytology associated with curettage in the investigation of endometrial lesions from postmenopausal women. Cytopathology 2005; 16: 32–9.

Papaefthimiou M, Symiakaki H, Mentzelopoulou P, Tsiveleka A, Kyroudes A, Voulgaris Z, Tzonou A, Karakitsos P. Study on the morphology and reproducibility of the diagnosis of endometrial lesions utilizing liquid-based cytology. Cancer (Phila) 2005b; 105: 56–64.

Skaarland E. New concept in diagnostic endometrial cytology: diagnostic criteria based on composition and architecture of large tissue fragments in smears. J Clin Pathol 1986; 39: 36–43.

Tao LC. Cytopathology of the endometrium. Direct intrauterine sampling. In: Johnston WW, ed. ASCP Theory and Practice of Cytopathology, vol 2. ASCP Press, Chicago, IL, 1993.

Zaino RJ, Kurman RJ, Diana KL, Morrow CP. The utility of the revised International Federation of Gynecology and Obstetrics histologic grading of endometrial adenocarcinoma using a defined nuclear grading system. A Gynecologic Oncology Group study. Cancer (Phila) 1995; 75: 81–6.

4
Performance Characteristics of Endometrial Cytology in a Hysterectomy-Controlled Environment

This section concerns estimating the degree of confidence that can be placed in endometrial cytology diagnoses. It asks, "How does an endometrial cytology diagnosis compare with the actual state of the endometrium?" and, "Does the diagnosis that we render on cytology material alone give the clinician sufficient data upon which to counsel and/or treat a woman, and does it allow the clinician to confidently decide whether ancillary procedures should be performed or not?" Studies designed to answer these questions have generally compared the outcomes of using various sampling devices to some gold standard such as dilatation and curettage or hysterectomy.

In our data set, about 90% of the office-based cytology collections have comprised diagnostic endometrial studies, less than 10% have been cellular studies limited to the endocervix and low uterine segment, and only 0.2% have been hypocellular and nondiagnostic. The difference between the Tao brush and standard tissue biopsy devices is that a failure to collect cytology or histology material (in the form of a cell block) is neither a function of advanced patient age nor of endometrial thickness. The thin endometria of elderly women can still yield adequate and informative cytology material. This characteristic is important when assessing benign endometrial states or thin endometrial lesions such as endometrial intraepithelial cancer or endometrial gland dysplasia (EIC/EmGD).

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Arriving at a diagnosis that is based on the successful sampling of an organ and actually knowing the condition of the organ are two entirely different things. Any diagnosis that we render is limited by what is collected, its quantity, quality, and representative nature, and how the collection is interpreted. Comparing two sample-based biopsy methods does little to determine the actual condition of an organ, whether normal or pathological. This information is probably best achieved by completely examining the extirpated organ, but even this approach is limited by the amount of histological sampling that can be performed. Nonetheless, considering the breadth of diagnoses that can be made by cytological methods, hysterectomy is probably the best benchmark against which to measure biopsy-sampled or cytology-sampled endometrial diagnoses.

We present such a comparison herein by combining our data on Tao brush hysterectomy samplings (that are further considered in subsequent sections of this volume) with material derived from a previously published series of endometrial samplings obtained by brushing 656 hysterectomy specimens with a cytobrush (Tables 4.1, 4.2). Using this comparative series, we can work toward some understanding about the limitations inherent in purely cytology-based

TABLE 4.1. Hysterectomy outcomes of endometrial brushings: source of material.

Diagnosis	Tao Brush	Cytobrush	Total
Proliferative	347	263	610
Interval	25		25
Secretory	310	165	475
Menstrual	47	3	50
Inactive/atrophic	235	100	335
Benign NOS	48		48
Disordered proliferative	150		150
Nonatypical hyperplasia	68	78	146
Noncyclical bleeding	15		15
Polyp	44		44
Carcinoma	93	29	122
EIN (atypical hyperplasia)	54	18	72
EIN (other)	15		15
EIC/EmGD	6		6
Total	1,457	656	2,113

NOS, not otherwise specified; EIN, endometrial intraepithelial neoplasm; EIC/EmGD, endometrial intraepithelial cancer or endometrial gland dysplasia.

TABLE 4.2. Hysterectomy outcomes of endometrial brushings: risk stratification of hysterectomy diagnoses by cytology findings.

Hysterectomy Diagnosis	Normal	Benign Abnormality	Probable Precancer	Probable Cancer
Proliferative	580	30		
Interval	24	1		
Secretory	475			
Menstrual	33	17		
Inactive/atrophic	258	77		
Benign NOS	43	5		
Disordered proliferative	40	109	1	
Nonatypical hyperplasia	24	119	2	1
Noncyclical bleeding	1	7	6	1
Polyp	6	29	8	1
Carcinoma		2	33	87
EIN (atypical hyperplasia)		13	29	30
EIN (other)		10	5	
EIC/EmGD			5	1

endometrial diagnoses and learn something about the predictive value of an endometrial diagnosis that is based solely on examining cytological material.

The analysis presented in this section is limited to cytology–hysterectomy comparisons, which means that it represents the "worst possible scenario" for the cytopathologist, because ancillary cell block material and specimen immunochemistry, both of which are available in many office-based biopsy specimens, are omitted from this analysis.

Histologically Normal Endometria

The endometria of 1,543 (of 2,113 total) hysterectomies were normal by histological examination of hysterectomy specimens. Cytological examination of 91.6% of these cases was simultaneously benign and normal, 8.4% was considered to represent a benign abnormality, and no cases were diagnosed as neoplastic. A stromal component was well represented among all cases of cycling endometrium and a fibrous stromal component was seen with most collections from atrophic endometrium. The details of these data are presented in Table 4.3.

TABLE 4.3. Frequency of cytology microbiopsy pattern and nuclear grade as compared to benign hysterectomy diagnoses.

Diagnosis	Microbiopsy Pattern	Nuclear Grade	Number	Risk Category
Proliferative	Flat epithelial sheets and uniform epithelial tubules	≤N1	580	B
610	Flat epithelial sheets and uniform epithelial tubules	N2	30	BA
Interval	Flat epithelial sheets and uniform epithelial tubules	≤N1	24	B
25	Flat epithelial sheets and uniform epithelial tubules	N2	1	BA
Secretory 475	Flat epithelial sheets and uniform epithelial tubules	≤N1	475	B
Menstrual	Flat epithelial sheets and uniform epithelial tubules	≤N1	33	B
50	Tufted epithelial cell cluster, pseudopapillae, and balled-up epithelial cell groups	≤N1	17	BA
Inactive/atrophic	Flat epithelial sheets and uniform epithelial tubules	≤N1	258	B
335	Flat epithelial sheets and uniform epithelial tubules	N2	6 of 77	BA
	Cystic epithelial glands, bulbous irregularities of glands, cup-shaped epithelial sheets, and smoothly contoured multiply outpouched glands	≤N1	74 of 77	BA
Benign NOS	Flat epithelial sheets and uniform epithelial tubules	≤N1	43	B
48	Flat epithelial sheets and uniform epithelial tubules	N2	3 of 5	BA
	Cystic epithelial glands, bulbous irregularities of glands, cup-shaped epithelial sheets, and smoothly contoured multiply outpouched glands	≤N1	3 of 5	BA

B, benign; BA, benign abnormality; PC, precancer; C, cancer.

Among 610 uteruses with proliferative endometrium, 580 comparative cytology cases showed flat epithelial sheets and uniform epithelial tubules with banal to proliferative-appearing nuclei. In 30 cases, occasional epithelial sheets and/or tubules showed more activated, grade 2, nuclei; 22 of these 30 cases showed uterine distortion by fibroids and/or adenomyosis. In the remaining 8 (1.3%) cases, no explanation for nuclear activation was apparent. A similar finding was seen in 1 of 24 cases of interval endometrium, and this uterus showed concomitant adenomyosis. All the 475 cases of secretory endometrium appeared cytologically benign.

Proportionally, menstrual endometrium was the commonest type of benign endometrium that was misclassified, often mistaken for noncyclical breakdown and bleeding, probably because in the latter stages of menstruation the pars basalis was abraded and the endometrial cytology was interpreted as showing the breakdown of either proliferative or disordered proliferative endometrium.

Following this, inactive or atrophic endometrium, having the propensity to show either cystic glands (22%) or minimally to moderately symplastic nuclei (1.8%), was misclassified as benign but abnormal. It is possible that some cases with symplastic nuclei represented cytological endometrial intraepithelial neoplasm (EIN); however, we did not perform p53/Ki-67 staining on these cases or embed the entire endometrium, and, in the absence of gross neoplasm, a misclassification would not have significantly affected treatment.

The few unclassified benign endometria generally represented physiologically unclassifiable postmenopausal states of the endometrium; and, similarly, those cases that showed either cystic glands (6%) or minimally to moderately symplastic nuclei (6%), were classified as benign but abnormal.

In summary, about 92% of uteruses with histologically normal endometrium afforded totally normal cytological outcomes. None of the cases was classified as neoplastic.

Benign Endometrial Abnormalities

The endometria of 355 hysterectomies were benign, disclosing only abnormalities such as disordered proliferation, benign hyperplasia, noncyclical bleeding, or polyp. Cytological examination

classified 20% as normal, 74% disclosed a benign abnormality, and 6% showed what was cytologically interpreted as the presence of neoplasm, with less than 1% mistakenly thought to represent carcinoma (Tables 4.4, 4.5.)

The most common undercall in cytology material was underestimating hypermature endometrium (either disordered proliferation or hyperplasia) as benign proliferative endometrium; this occurred 22% of the time. Overestimation of the diagnosis occurred in four (1.4%) instances. All cases involved finding moderately to markedly atypical nuclei in isolated epithelial aggregates or cystic epithelial structures. In these instances, the hyperplasia was diagnosed as "atypical." It is possible that some of the cases with symplastic nuclei represented cytological EIN, but we did not perform confirmatory p53/Ki-67 staining. Interestingly, this degree of overestimation is equivalent to the long-term risk of cancer emergence reported for nonatypical hyperplasia. Furthermore, in a recent assessment of the hyperplasia problem, even the intraobserver agreement between nonatypical and atypical hyperplasia often remained problematical.

Only 15 cases of active noncyclical bleeding were diagnosed on hysterectomy material. This finding is more common among office biopsy specimens. One case that showed only mild residual changes of dysfunctional uterine bleeding was cytologically normal appearing. Cytologically, 7 of the 15 cases showed features of underlying pathology, generally hypermature endometrium with residual cystic glands (6 cases), and 2 of these 7 cases also showed marked syncytial change. An additional 6 cases showed the epithelial tufting of syncytial change that was indistinguishable from the epithelial tufting of endometrioid neoplasia. In practice, we would have used ancillary Ki-67 in the workup of such a case. In one case, there was significant neutrophilic emperipolesis among the cellular tufts, and this change was interpreted as suspicious for carcinoma. These cases show that active endometrial bleeding may occasionally have florid syncytial change simulating neoplasm. In about 90% of cases, office samplings of actively bleeding endometria afford ample cell blocks that are helpful in clarifying the situation.

Forty-four hysterectomy specimens contained polyps. The second most common underestimation and perhaps the most common overestimation of risk involved this category. Cytology does not

TABLE 4.4. Frequency of cytology microbiopsy pattern and nuclear grade as compared to disordered proliferation/hyperplasia (hypermature endometrium) hysterectomy diagnoses.

Diagnosis	Microbiopsy Pattern	Nuclear Grade	Number	Category
Disordered proliferative	Flat epithelial sheets and uniform epithelial tubules	≤N1	40	B
150	Cystic epithelial glands, bulbous irregularities of glands, cup-shaped epithelial sheets, and smoothly contoured multiply outpouched glands	≤N1	109	BA
	Tufted epithelial cell cluster, pseudopapillae, and balled-up epithelial cell groups	N2	1	PC
Hyperplasia	Flat epithelial sheets and uniform epithelial tubules	≤N1	24	B
146	Cystic epithelial glands, bulbous irregularities of glands, cup-shaped epithelial sheets, and smoothly contoured multiply outpouched glands	≤N1	119	BA
	Cystic epithelial glands, bulbous irregularities of glands, cup-shaped epithelial sheets, and smoothly contoured multiply outpouched glands	N3	2	PC
	Tufted epithelial cell cluster, pseudopapillae, and balled-up epithelial cell groups	N3	1	C
All hypermature endometrium	Flat epithelial sheets and uniform epithelial tubules	≤N1	64	B
296	Cystic epithelial glands, bulbous irregularities of glands, cup-shaped epithelial sheets, and smoothly contoured multiply outpouched glands	≤N1	228	BA
	Cystic epithelial glands, bulbous irregularities of glands, cup-shaped epithelial sheets, and smoothly contoured multiply outpouched glands	N3	2	PC
	Tufted epithelial cell cluster, pseudopapillae, and balled-up epithelial cell groups	N2	1	PC
	Tufted epithelial cell cluster, pseudopapillae, and balled-up epithelial cell groups	N3	1	C

B, benign; BA, benign abnormality; PC, precancer; C, cancer.

TABLE 4.5. Frequency of cytology microbiopsy pattern and nuclear grade as compared to benign abnormality hysterectomy diagnoses.

Diagnosis	Microbiopsy Pattern	Nuclear Grade	Number	Risk Category
Noncyclical bleeding	Flat epithelial sheets and uniform epithelial tubules	≤N1	1	B
15	Cystic epithelial glands, bulbous irregularities of glands, cup-shaped epithelial sheets, and smoothly contoured multiply outpouched glands	≤N1, N2	6 of 7	BA
	Tufted epithelial cell cluster, pseudopapillae, and balled-up epithelial cell groups	≤N1	2 of 7	BA
	Tufted epithelial cell cluster, pseudopapillae, and balled-up epithelial cell groups	N2	6	PC
	Bridge-like connections composed of epithelial cells, cell clusters with intraepithelial soap-bubble-like lumens, distorted acini	N2	1	C
Polyp	Flat epithelial sheets and uniform epithelial tubules	≤N1	6	B
44	Cystic epithelial glands, bulbous irregularities of glands, cup-shaped epithelial sheets, and smoothly contoured multiply outpouched glands	≤N1, N2	13 of 29	BA
	Flat epithelial sheets and uniform epithelial tubules	N2	26 of 29	BA
	Cystic epithelial glands, bulbous irregularities of glands, cup-shaped epithelial sheets, and smoothly contoured multiply outpouched glands	N3	6 of 8	PC
	Tufted epithelial cell cluster, pseudopapillae, and balled-up epithelial cell groups	N2	3 of 8	PC
	Tufted epithelial cell cluster, pseudopapillae, and balled-up epithelial cell groups	N3	1	C

B, benign; BA, benign abnormality; PC, precancer; C, cancer.

identify polyps, although polyps may be associated with hyperplastic features and may irritate the endometrial surface epithelium or its immediate subsurface stroma to the point that it is mistaken for neoplasm. Nonetheless, some polyps remained undetectable by cytological methods. Polyps and noncyclical bleeding appear to be among the most common reasons for overestimating an endometrial neoplasm, including cancer.

When cytological material is risk stratified as a benign abnormality, additional testing such as immunohistochemistry, sonohysterography, or hysteroscopy helps to determine the type of lesion. When cytological material from histologically benign abnormalities is interpreted as "normal," the abnormalities generally entail benign altered physiological states, dysfunctional bleeding, or benign polyp. Under such circumstances, interpreting these situations as benign cytological outcomes does not harm the patient.

Endometrial Neoplasms

The endometria of 215 hysterectomies disclosed neoplasms ranging from cytological EIN (discussed below) to outspoken endometrial carcinoma. Reassuringly, no case was interpreted as cytologically "normal." Cytologically, 11.6% of endometrial neoplasms were interpreted as a benign abnormality and 88.4% as neoplasms.

All EIC/EmGD lesions discovered by cytology were confirmed by p53/Ki-67 immunohistochemistry of decolorized cytology slides and/or hysterectomy sections. Two (1.6%) of 122 hysterectomy-confirmed endometrial cancers were classified as benign abnormalities. One of these neoplasms was localized to the uterine fundus whereby cytological underestimation may have represented a sampling problem. Thirteen (18%) of 73 atypical hyperplasias were classified as benign abnormalities (either nonatypical hyperplasia or breakdown bleeding). This proportion of variance among hyperplasia cases is within the reported range of intraobserver reproducibility. Only 5 of 15 cytological EIN lesions were recognized as neoplastic on the basis of cytological examination alone. The rest appeared indistinguishable from symplastic changes associated with inactive or atrophic endometrium, causing their misclassification into a benign atypical category (Tables 4.6–4.8).

TABLE 4.6. Frequency of cytology microbiopsy pattern and nuclear grade as compared to EIN (atypical hyperplasia) hysterectomy diagnoses.

Diagnosis	Microbiopsy Pattern	Nuclear Grade	Number	Risk Category
EIN (atypical hyperplasia)	Cystic epithelial glands, bulbous irregularities of glands, cup-shaped epithelial sheets, and smoothly contoured multiply outpouched glands	≤N1, N2	9 of 13	BA
72	Tufted epithelial cell cluster, pseudopapillae, and balled-up epithelial cell groups	≤N1	13 of 13	BA
	Cystic epithelial glands, bulbous irregularities of glands, cup-shaped epithelial sheets, and smoothly contoured multiply outpouched glands	N3	9 of 29	PC
	Tufted epithelial cell cluster, pseudopapillae, and balled-up epithelial cell groups	N2	27 of 29	PC
	Bridge-like connections composed of epithelial cells, cell clusters with intraepithelial soap-bubble-like lumens, distorted acini	≤N1	6 of 29	PC
	Tufted epithelial cell cluster, pseudopapillae, and balled-up epithelial cell groups	N3	28 of 30	C
	Bridge-like connections composed of epithelial cells, cell clusters with intraepithelial soap-bubble-like lumens, distorted acini	N2, N3	8 of 30	C

B, benign; BA, benign abnormality; PC, precancer; C, cancer.

TABLE 4.7. Frequency of cytology microbiopsy pattern and nuclear grade as compared to cytological EIN/EIC/EmGD (microscopic preneoplasia) hysterectomy diagnoses.

Diagnosis	Microbiopsy pattern	Nuclear grade	Number	Risk category
EIN (other)	Flat epithelial sheets and uniform epithelial tubules	N2	8 of 10	BA
15	Cystic epithelial glands, bulbous irregularities of glands, cup-shaped epithelial sheets, and smoothly contoured multiply outpouched glands	N2	3 of 10	BA
	Flat epithelial sheets and uniform epithelial tubules	N3	5	PC
EIC/EmGD	Flat epithelial sheets and uniform epithelial tubules	N3	5 of 5	PC
6	Cystic epithelial glands, bulbous irregularities of glands, cup-shaped epithelial sheets, and smoothly contoured multiply outpouched glands	N3	3 of 5	PC
	Tufted epithelial cell cluster, pseudopapillae, and balled-up epithelial cell groups	N3	1	C

B, benign; BA, benign abnormality; PC, precancer; C, cancer.

TABLE 4.8. Frequency of cytology microbiopsy pattern and nuclear grade as compared to malignant hysterectomy diagnoses.

Diagnosis	Microbiopsy Pattern	Nuclear Grade	Number	Risk Category
Carcinoma	Tufted epithelial cell cluster, pseudopapillae, and balled-up epithelial cell groups	≤N1	2	BA
122	Tufted epithelial cell cluster, pseudopapillae, and balled-up epithelial cell groups	N2	23 of 33	PC
	Bridge-like connections composed of epithelial cells, cell clusters with intraepithelial soap-bubble-like lumens, distorted acini	≤N1	27 of 33	PC
	Tufted epithelial cell cluster, pseudopapillae, and balled-up epithelial cell groups	N3	74 of 87	C
	Bridge-like connections composed of epithelial cells, cell clusters with intraepithelial soap-bubble-like lumens, distorted acini	N2, N3	68 of 87	C
	Cribriform structures, solid clusters of epithelial cells (excluding morules and metaplastic squamous cells), or dyshesive epithelial cells	≤N1, N2, N3	83 of 87	C

B, benign; BA, benign abnormality; PC, precancer; C, cancer.

The lesson learned from this exercise is that cytology alone detects most cancers and EIC lesions, but to find all cancers and EINs, additional testing is necessary. Women with cytological material that is risk stratified as a "benign abnormality" should be tested further (for example, with either sonohysterography or hysteroscopy). These latter two techniques assess the uterine fundus, which generally escapes adequate study by simple ultrasonography. These data also stress the need for confirmatory p53/Ki-67 staining among cases with dichotomous nuclear atypia that presents in sheets, glands, or cystic epithelial structures.

Conclusion

From our data, when a diagnosis is limited to the examination of cytological material, clinical decision making is optimized at the level of normal versus abnormal, with "abnormal" including both "benign abnormality" and "neoplasm." Stratifying diagnoses at this level show that 70% of brushings are normal and require no further workup. Among the remaining 30% of cytologies diagnosed as either abnormal but benign or as frankly neoplastic, 34% proved to be neoplasms on hysterectomy. At the level of benign versus abnormal, endometrial cytology when unaided by cell block examination or immunochemistry has a sensitivity of 88%, specificity of 92%, positive predictive value of 79%, and negative predictive value of 95% (Tables 4.9, 4.10).

What modifies this performance estimate in practice is that about 80% of our office biopsy collections afforded cell blocks for contemporaneous examination. Others have reported that a cell block may be obtained in up to 87% of cases. By adding cell block examination, others report that the overall diagnostic accuracy

TABLE 4.9. Hysterectomy outcomes of endometrial brushings: decision matrix.

Endometrial Cytology	Histology "Abnormal +"	Histology Normal	Totals
Cytology "abnormal +"	499	130	629
Cytology normal	71	1,413	1,484
Totals	570	1,543	2,113

TABLE 4.10. Hysterectomy outcomes of endometrial brushings: decision matrix.

True "abnormal +" cytology	499
True normal cytology	1,413
False "abnormal +" cytology	130
False normal cytology	71
Sensitivity	87.5%
Specificity	91.6%
Positive predictive value	79.3%
Negative predictive value	95.2%

of endometrial cytology increases to 96% and 100% for benign/atrophic endometrium and adenocarcinoma, respectively, and the diagnostic accuracy for hyperplasia increases to more than 95%. We would add to this that with the addition of p53/Ki-67 immunostaining to cases where nuclear atypia appears in only a small proportion of the collected material, the sticky issue of cytological EIN and EIC/EmGD can oftentimes be resolved.

Suggested Reading

Kurman RJ, Kaminski PF, Norris HJ. The behavior of endometrial hyperplasia. A long-term study of "untreated" hyperplasia in 170 patients. Cancer (Phila) 1985; 56: 403–12.

Kyroudi A, Paefthimiou M, Symiakaki H, Mentzelopoulou P, Voulgaris Z, Karakitsos P. Increasing diagnostic accuracy with a cell block preparation from thin-layer endometrial cytology: a feasibility study. Acta Cytol 2006; 50: 63–9.

Maksem JA, Knesel E. Liquid fixation of endometrial brush cytology ensures a well-preserved, representative cell sample with frequent tissue correlation. Diagn Cytopathol 1996; 14: 367–73.

Papaefthimiou M, Symiakaki H, Mentzelopoulou P, Giahnaki AE, Voulgaris Z, Diakomanolis E, Kyroudes A, Karakitsos P. The role of liquid-based cytology associated with curettage in the investigation of endometrial lesions from postmenopausal women. Cytopathology 2005; 16: 32–9.

Maksem JA, Meiers I, Robboy SJ. A primer of endometrial cytology with histological correlation. Diagn Cytopathol 2007; 35: 817–44.

Sherman ME, Ronnett BM, Ioffe OB, Richesson DA, Rush BB, Glass AG, Chatterjee N, Duggan MA, Lacey JV Jr. Reproducibility of biopsy diagnoses of endometrial hyperplasia: evidence supporting a simplified classification. Int J Gynecol Pathol 2008; 27: 318–25.

5
Normal Endometrium

During the reproductive years, normal endometrium comprises glands, stroma, and vascular elements that synchronously proliferate, differentiate, and then disintegrate at roughly 28-day intervals. During a menstrual cycle, the epithelium lining the glands, stroma, and vasculature of the functional layer of the endometrium undergoes well-defined morphological changes. At the same time, the glands and stroma of the basal portion of the corpus endometrium and the lower uterine segment endometrium show no significant morphological changes. Our descriptions of normal endometrium are based on 1,012 contemporaneously gathered hysterectomy-controlled cases and 1,690 office-based samplings (Table 5.1).

Proliferative Endometrium

During its proliferative phase, the endometrium responds to increasing estrogen levels by the synchronous proliferation of glands, stroma, and blood vessels. Based on an average 28-day menstrual cycle, proliferative endometrial changes may be divided into early (days 4–7), mid (days 8–10), and late (days 11–13) intervals. Because of persisting estrogens, perimenopausal endometrium is generally indistinguishable from proliferative endometrium. During the early proliferative phase, the growth of all three endometrial components is coordinated. Later, both gland and blood vessel

J.A. Maksem et al., *Endometrial Cytology with Tissue Correlations*, Essentials in Cytopathology 7, DOI: 10.1007/978-0-387-89910-7_6,

TABLE 5.1. Normal endometrium.

Diagnosis	Hysterectomy	Average Age (years)	Office Biopsy (% with cell block)	Average Age (years)
Proliferative	347	46	500 (90%)	42
Interval	25	41	61 (97%)	41
Secretory	310	42	481 (97%)	42
Menstrual	47	41	76 (97%)	41
Mixed function			29 (87%)	48
Inactive/atrophic	235	55	433 (51%)	58
Benign NOS	48	45	110 (71%)	45
Total	1,012		1,690	

NOS, not otherwise specified.

development outpace the development of the stroma, and, as a result, glands and blood vessels become coiled.

The epithelium of early proliferative endometrium typically shows narrow, straight glands (Fig. 5.1) and cohesive, flat sheets (Fig. 5.2) that exhibit mild nuclear crowding and overlapping which reflects nuclear pseudostratification. Although the epithelial cell layer of early proliferative endometrium is only one cell thick, its pseudostratification results from and reflects resting nuclei occupying a basal position within the cell's apical-to-base axis and actively dividing nuclei occupying an apical position (Fig. 5.3). Epithelial cell nuclei are oval to cigar shaped, with smooth contours, evenly dispersed chromatin, and small, conspicuous nucleoli. The nuclear-to-cytoplasmic ratio is relatively high. Mitotic figures, although present, are less conspicuous in early than in mid and late proliferative periods. The background of cytology preparations is clean. Because of persisting endometrial breakdown and remodeling, some stromal/epithelial exodus-like structures, similar to the stromal–epithelial aggregates seen in contemporaneously collected cervicovaginal smears, are seen. This finding does not indicate noncyclical endometrial breakdown and bleeding.

Mid and late proliferative endometrium resembles early proliferative endometrium except that glandular cells show slightly more prominent small nucleoli and more frequent mitoses. The nuclear size of dividing cells varies (anisonucleosis) because dividing cells have 2N or 4N chromatin in addition to mitotic figures. The cells' nuclei may resemble those of a benign hyperplasia and, rarely,

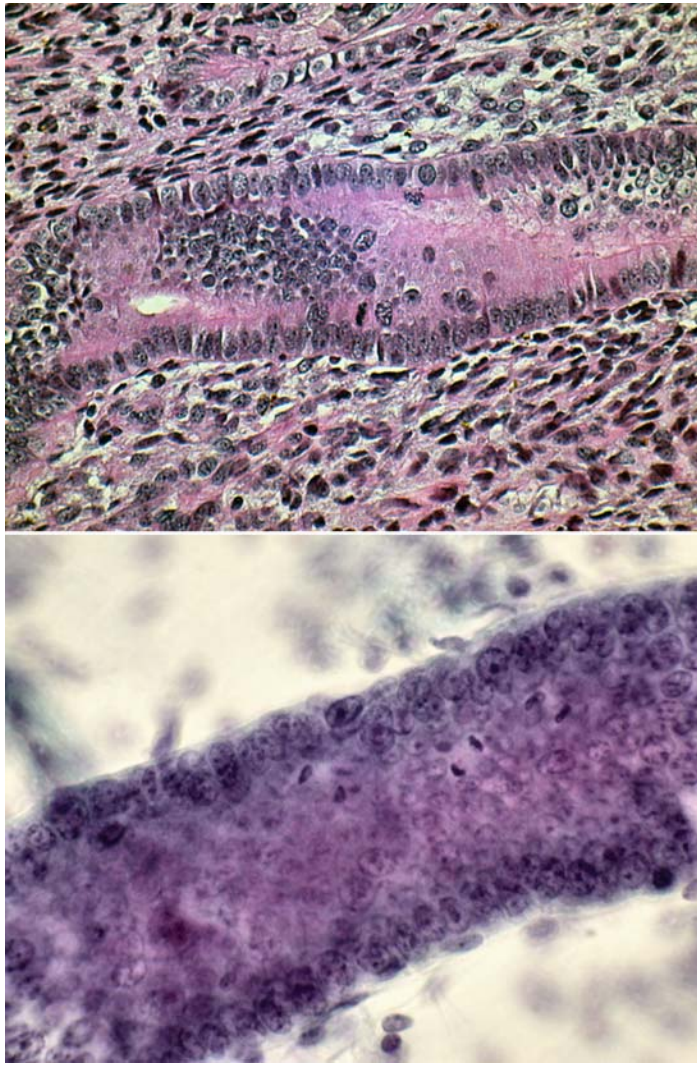

FIG. 5.1. A cell block (*top*) and brush cytology preparation (*bottom*) show the organization of the proliferative endometrial gland. The nuclei are tightly packed and pseudostratified. Apical mitoses are seen. The glands of proliferative endometrium replicate one another in size and in proliferative activity in that they are regularly regular. One of the subtlest keys to the cytological recognition of disordered proliferative states is up to threefold size variability between otherwise architecturally preserved glands.

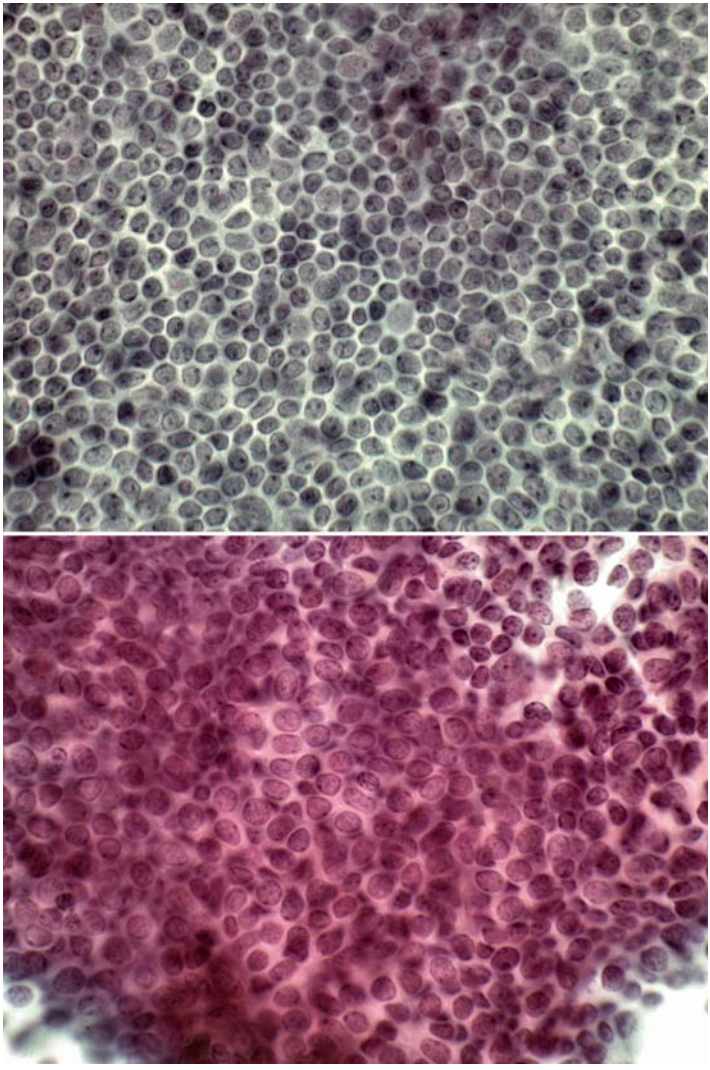

FIG. 5.2. An en face view of surface proliferative endometrium is not unlike that seen in surface epithelium of the early postmenopausal years. Nuclei appear crowded because of pseudostratification. The *top image* shows occasional interspersed ciliated cells that appear larger with more voluminous nucleoplasm. The *bottom image* shows how cell crowding with pseudostratification causes nuclei to appear as if they are mounted one over another. Pseudostratification should not be misinterpreted as loss of polarity; rather, it reflects the functional status of the epithelial sheet.

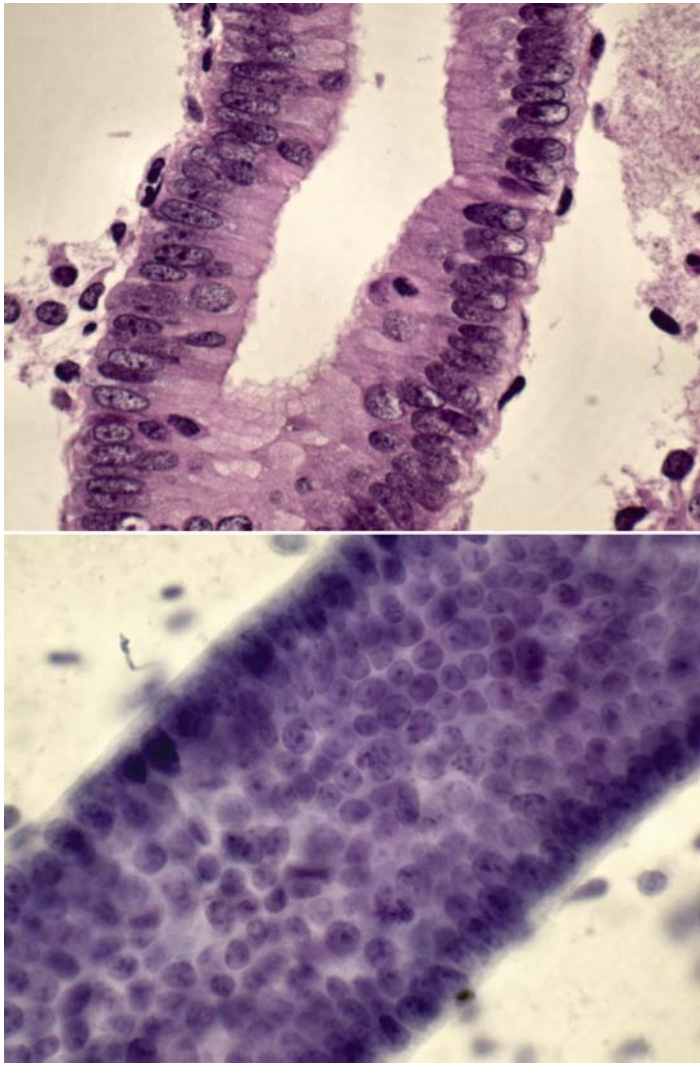

FIG. 5.3. A cell block (*top*) and brush cytology preparation (*bottom*) show the organization of the proliferative endometrial gland. The nuclei are tightly packed and pseudostratified. Apical mitoses are seen. Pseudostratification results from and reflects resting nuclei occupying the basal position within the cell's apical-to-base axis and actively dividing nuclei occupying the apical position. The rather tight but orderly nuclear packing of proliferative endometrium reflects the functional nuclear-dominant metabolism enjoyed by these cells. More mitotic figures may be seen among the glands of proliferative endometrium than in endometrial cancer.

even those of FIGO (International Federation of Gynecology and Obstetrics) grade 1 adenocarcinoma. Mitoses are localized to the apical part of the epithelial cell, effecting even more prominent stratification as nuclei migrate from their basal location to the cell apex in the course of epithelial cell division. The S-phase fraction, mitotic index, and number of surface ciliated cells reach their highest values during the mid to late proliferative phase.

Intact proliferative glands are tubular in both cytology and histology preparations. After about day 7, the glands broaden and begin to gently zigzag or coil. As do perimenopausal or lower uterine segment endometrial glands, early proliferative glands show little evidence of estrogen stimulation, whereas mid and late proliferative glands show increasing degrees of nuclear crowding and overlapping, nuclear enlargement, nucleolar prominence, and mitotic activity.

Because of cell crowding, it becomes difficult to focus through these tubular glands in Papanicolaou-stained cytology preparations, although gland interiors may be seen by rinsing the cytology slide with 10% acetic acid and then staining it with hematoxylin alone, omitting counterstains. We find this "acid-hematoxylin" staining methodology useful for defining the three-dimensional arrangement of nuclei in epithelial aggregates and determining their three-dimensional structure. We apply this technique, in addition to the Papanicolaou stain, to all our cases, which is reflected by the several illustrations presented in this monograph that show only a blue color, without interfering cytoplasmic staining.

In late proliferative and in interval endometria, occasional cells, while maintaining their proliferative activity, exhibit perinuclear and subnuclear clearing that corresponds to small subnuclear vacuoles seen on histological sections (Fig. 5.4). This appearance results from accumulated glycogen that may be confirmed using periodic acid–Schiff (PAS) staining with and without diastase digestion. Ciliated cells are present in endometrium under estrogenic control and, during the late proliferative phase, about 25% of endometrial surface cells are ciliated. Ciliated cells are exquisitely sensitive to estrogens. When estrogen levels drop, cells lose their cilia.

When seen en face, ciliated cells stand out, displaying large, round nuclei with clear cytoplasm, and have a "fried egg" appearance as they intercalate with smaller nonciliated endometrial cells

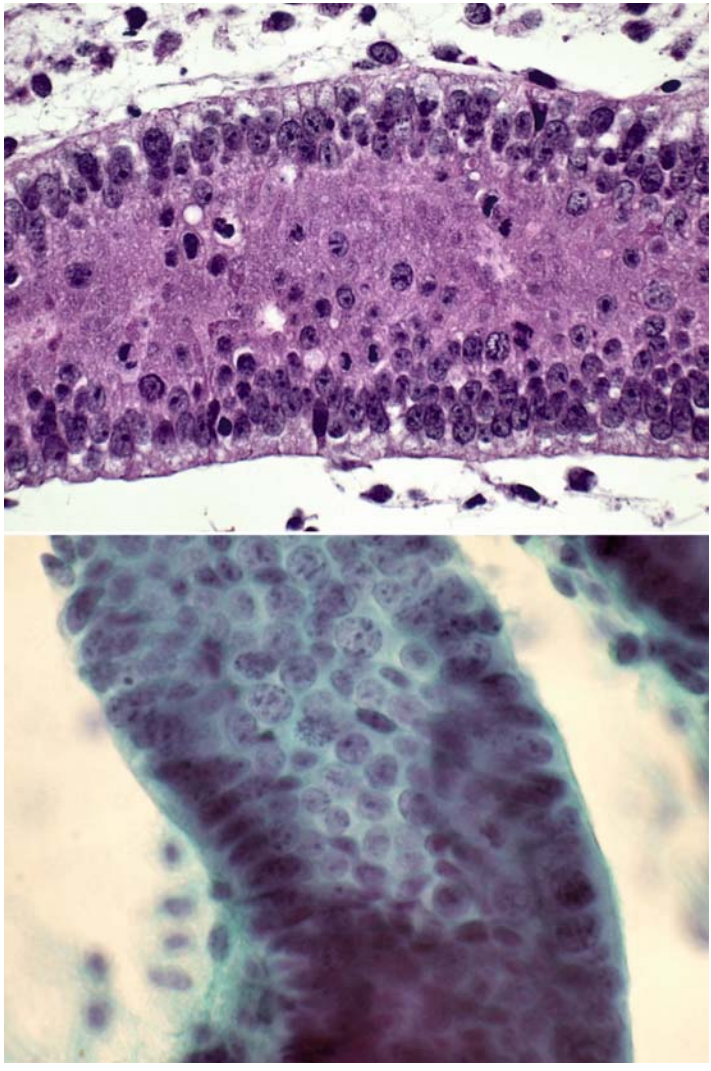

FIG. 5.4. Because of an accumulation of cytoplasmic glycogen in late proliferative and in interval endometrium, occasional cells show perinuclear and subnuclear clearing that corresponds to small subnuclear vacuoles seen on histological sections while some cells maintain their proliferative activity. This point is well demonstrated in the cell block section (*top*) and the in the corresponding brush cytology preparation (*bottom*), where subnuclear clearing and mitotic figures are seen. These changes are not to be taken as indicative of ovulation; indeed, they may be seen in some estrogen-excess states.

in cytology preparations of epithelial sheets. Thus, the presence of cilia in proliferative endometrium is physiological and does not constitute a form of metaplasia. Persisting unopposed estrogen effect is seen in the perimenopause, with endometrial hyperplasia, or with some well-differentiated endometrioid neoplasms, and may be associated with increased numbers of ciliated cells as well as with their accumulation within glands. In contrast to their presence in other organ sites, the presence of ciliated cells does not exclude the diagnosis of endometrial adenocarcinoma.

Stromal cells show no substantial changes throughout the proliferative phase. Periglandular stromal cell condensation is not seen. Stroma varies from dense and spindly to edematous with nuclei varying from fusiform to oval. Oval nuclei are more common in the mid and late proliferative phase. In liquid-fixed preparations, the fusiform nature of stromal cells is best seen among the cells that align themselves in apposition to thin-walled vessels (Fig. 5.5).

Weakly proliferative endometrium may be seen immediately following menstruation, in women with anovulatory cycles, in perimenopausal or postmenopausal women whose endometria are weakly supported by low levels of endogenous or exogenous estrogen, or in women using oral contraceptives. Weakly proliferative endometrium shows short, stubby, straight proliferative glands with rare mitotic figures that are admixed with a tattered fibrous stroma. Nuclear pseudostratification is reduced over that seen in the mid to late stages of cyclical proliferation. Occasionally, small cystic glands and misshapen bifid glands may be seen. In cell block preparations, the stroma appears compact and collagenous, often resembling the stroma of pars basalis endometrium; this is especially common with endometrial hypermaturity because of a weak estrogenic effect that is unopposed by progesterone. Under this circumstance, thin-walled venules may dilate and may appear larger than their accompanying glands.

Secretory Endometrium

The endometrial lining undergoes cyclic regeneration. Humans and the great apes have a menstrual cycle, whereas most other mammals have an estrous cycle. In both cases, the endometrium

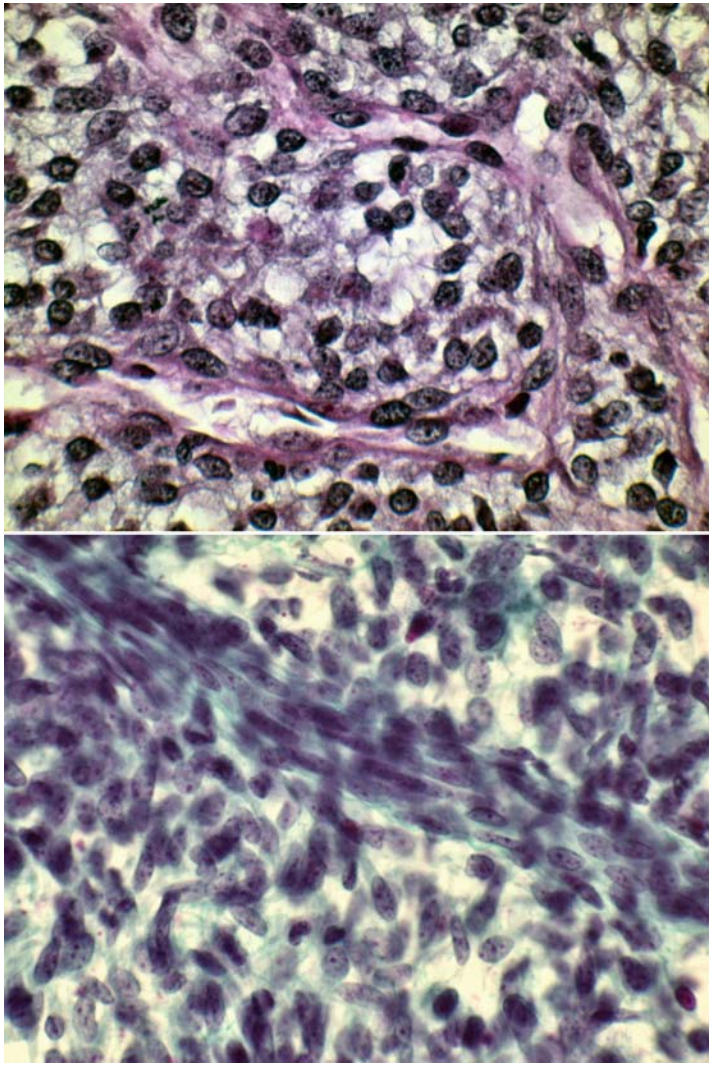

FIG. 5.5. The fusiform nature of proliferative phase stromal cells is best seen among the stromal cells that align themselves in apposition to thin-walled stromal vessels. If there is persistent unopposed estrogen effect, many of these vessels dilate; and, with injury, undergo thrombosis. Very little is generally said about endometrial stroma in most articles that deal with endometrial cytopathology other than it is present or absent; however, the stroma carries within its structure clues as to whether the endometrium is affected by estrogen, progesterone, or an excess of these hormones.

initially proliferates under the influence of estrogen. However, once ovulation occurs, in addition to estrogen, the ovary also produces progesterone. This changes the proliferative pattern of the endometrium to a secretory pattern. Eventually, the secretory lining provides a hospitable environment for one or more blastocysts.

During an average 28-day menstrual cycle, secretory changes are divided into ovulatory or interval (days 14 and 15, the interval phase), early (days 16–19, the vacuole phase), mid (days 20–22, the secretory phase), and late (days 23 and 24 and days 25–28, the early predecidual and late predecidual/exhaustive phase, respectively) phases. In cytology preparations, cyclical secretory endometrium is fully differentiated. Even though there may be slight variation in nuclear size, the cells show no nuclear anaplasia.

Glandular and stromal cells of the immediate ovulatory or interval period resemble those of the late proliferative phase. Rare mitoses are seen in both glands and stroma. Glandular cells become slightly less crowded and show incomplete perinuclear and subnuclear clearing that becomes especially prominent and generalized on postovulatory day 3 (Figs. 5.6, 5.7). Specific morphological changes that indicate ovulation lag for 36–48 h following ovulation. Before 36–48 h, no morphological endometrial features establish whether ovulation has actually occurred.

During the secretory phase, the endometrial epithelial cell moves from a state of "nuclear dominance" to one of "cytoplasmic dominance." The replication of cells is replaced by the amplification of gland size, the production of secretory product, and the reengineering of stromal architecture. As part of its antagonism to estrogen, progesterone causes ciliary lysis, although the retention of ciliated cells in the face of weak stromal/epithelial secretory changes may occur with an inadequate ovulatory effect or as a consequence of combined estrogen/progestin medicaments. In the immediate postovulatory period, ciliated cells become nondescript eosinophilic cells, some of which display apical snouts and then seem to disappear. Perhaps the most characteristic feature of established ovulatory effect is the emergence of a honeycomb-surface epithelium with uniform, rounded-up nuclei (Fig. 5.8).

Early secretory endometrium resembles late proliferative endometrium but with a lower nuclear-to-cytoplasmic ratio, small nucleoli, decreased mitoses, and greater internuclear distance. During

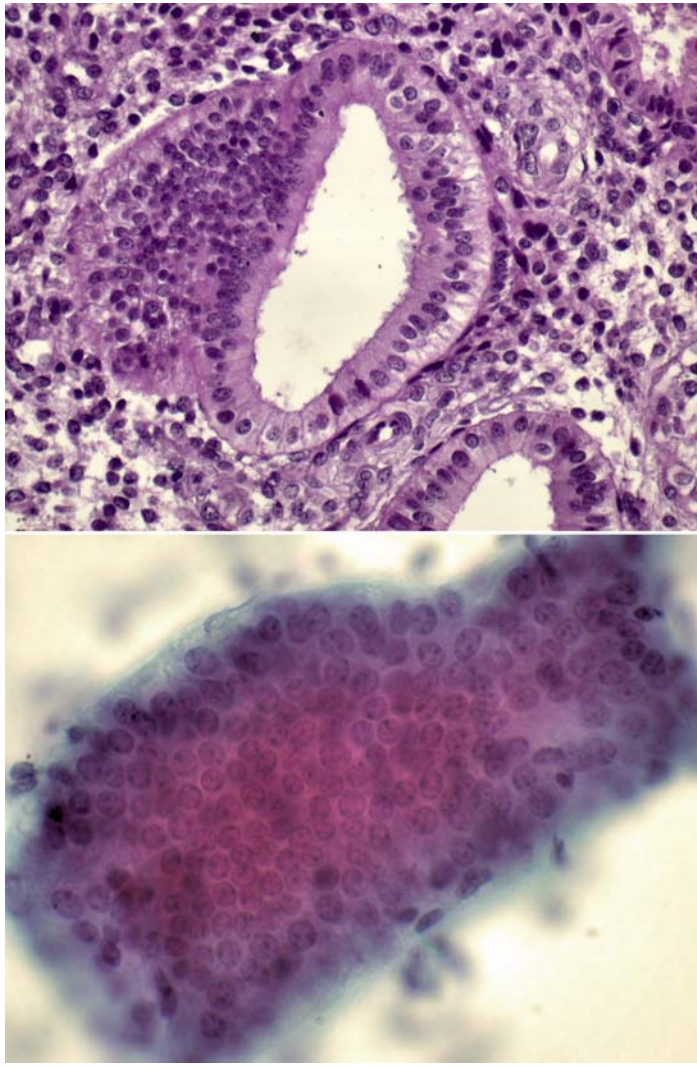

FIG. 5.6. In secretory endometrium, glandular cells become slightly less crowded and show incomplete perinuclear and subnuclear clearing that becomes fully developed and complete on postovulatory day 3. Note also that in these images the nuclei appear round and uniform as opposed to compressed. The secretory phase of the endometrium is one of cytoplasmic functional dominance that comes about in preparation for implantation of the blastocyst.

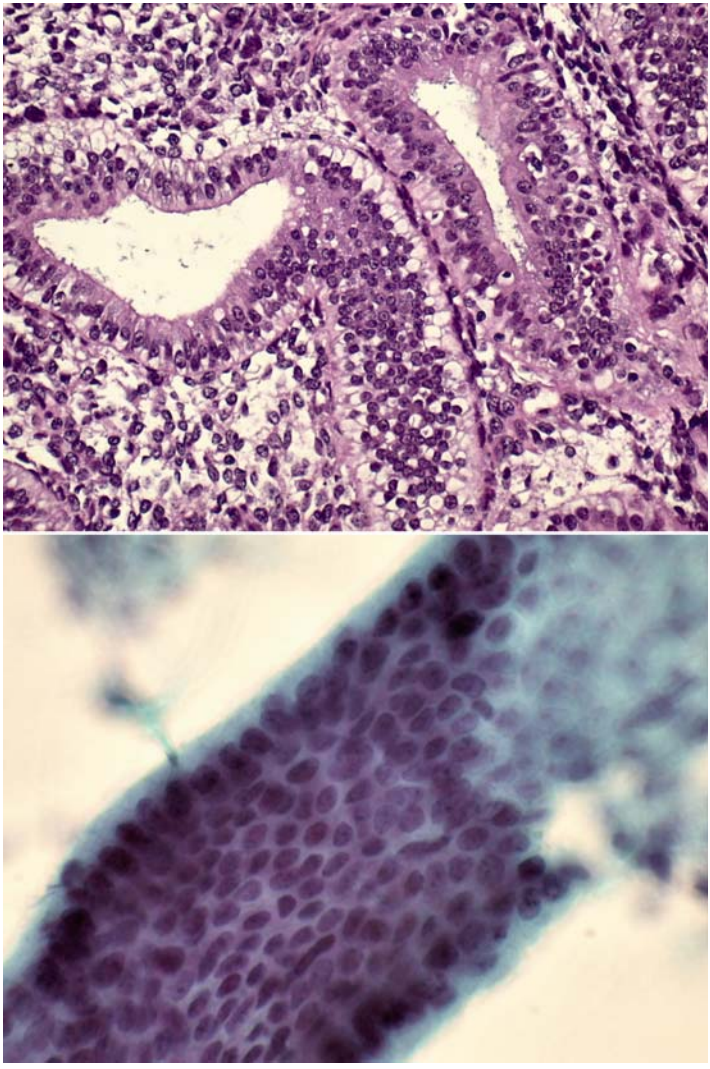

FIG. 5.7. In this image it is clear that both the cell block preparation and the brush cytology preparation show distinct subnuclear clearing. Glycogen has not been extracted in the cytology preparation (*bottom*); hence, the bubble-like accumulation of subnuclear glycogen is not seen in the cytology preparation as it is in the histology preparation (*top*). As this change represents the bulk of the sampled endometrium, the early vacuolar interval of the secretory phase can be firmly established on morphological grounds.

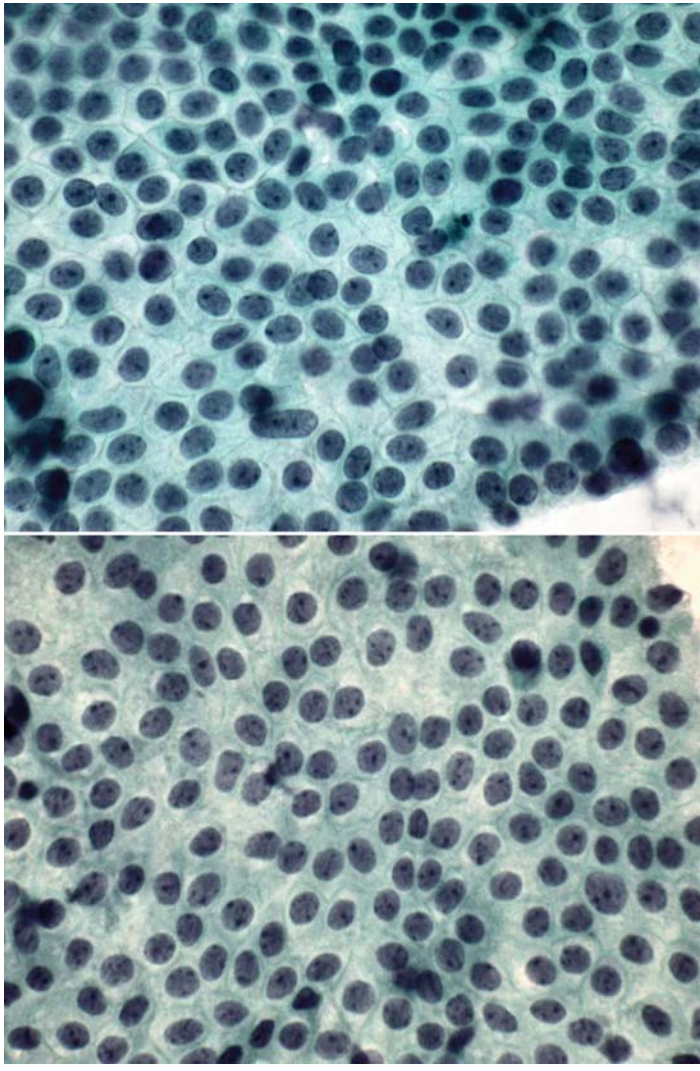

FIG. 5.8. Perhaps the most characteristic cytological feature of established ovulatory or gestagenic effect is the emergence of a honeycomb-surface epithelium with uniform, rounded-up nuclei. This appearance of surface endometrium persists rather steadily throughout the secretory phase from about postovulatory day 4 (*top*) to about postovulatory day 12 (*bottom*); this means that surface sheets of endometrial epithelium are less informative about where in the secretory cycle the endometrium lies than is the appearance of the glands of the functional endometrial compartment and of the stroma.

the early secretory phase, glands enlarge and display moderate amounts of well-defined, clear cytoplasm. Cell sheets and cells within glands begin to assume a honeycomb pattern (Fig. 5.9). Cells are larger because their cytoplasmic machinery is amplified and geared to produce secretory substances, which in cell blocks and in direct smear preparations begin to appear at about day 16 but generally are not appreciated in liquid-fixed cytology preparations. The stroma of early secretory endometrium resembles that of late proliferative phase stroma.

Mid-secretory glands become more dilated, making them appear more coiled as cell sheets take on a wavy topography. Nuclei are more uniform than those of proliferative endometrium. The nuclei appear rounded and vesicular with fine chromatin. Nucleoli are small and inconspicuous. Nuclei are more widely separated from one another, reflecting increased cell cytoplasm (Fig. 5.10). Stromal cells begin to display oval to plump nuclei, the nucleoplasm of which is vesicular. Despite nuclear changes, the stroma, with the exception of immediate periarteriolar and subsurface stroma, resembles that of the proliferative phase.

In the late secretory phase, glands exhaust, cease their secretory activity, and collapse, thus appearing smaller than the glands of the mid-secretory phase. The nuclei come to approximate one another. There is no significant variability of nuclear size, and in contrast to proliferative endometrium, there is no nuclear pseudostratification (Fig. 5.11, top). In paucicellular collections, sheets of both late secretory phase epithelium and inactive/weakly proliferative endometrial epithelium appear to resemble each other. During this period of glandular exhaustion and epithelial collapse, some of the glands and epithelial sheets may tear apart and their cytoarchitectural features may become distorted.

Just before the onset of menstruation, lymphocytes (including recognizable granulated NK cells), polymorphonuclear leukocytes, and the apoptotic dust of these inflammatory cells and of endometrial cells may be seen in endometrial gland walls and lumens, a change not to be misinterpreted as endometritis. Human endometrial epithelial cells undergo apoptosis immediately before the menstrual period, and apoptosis plays a critical role in maintaining tissue homeostasis, representing a normal function that eliminates excess or dysfunctional cells. Apoptosis helps to maintain cellular

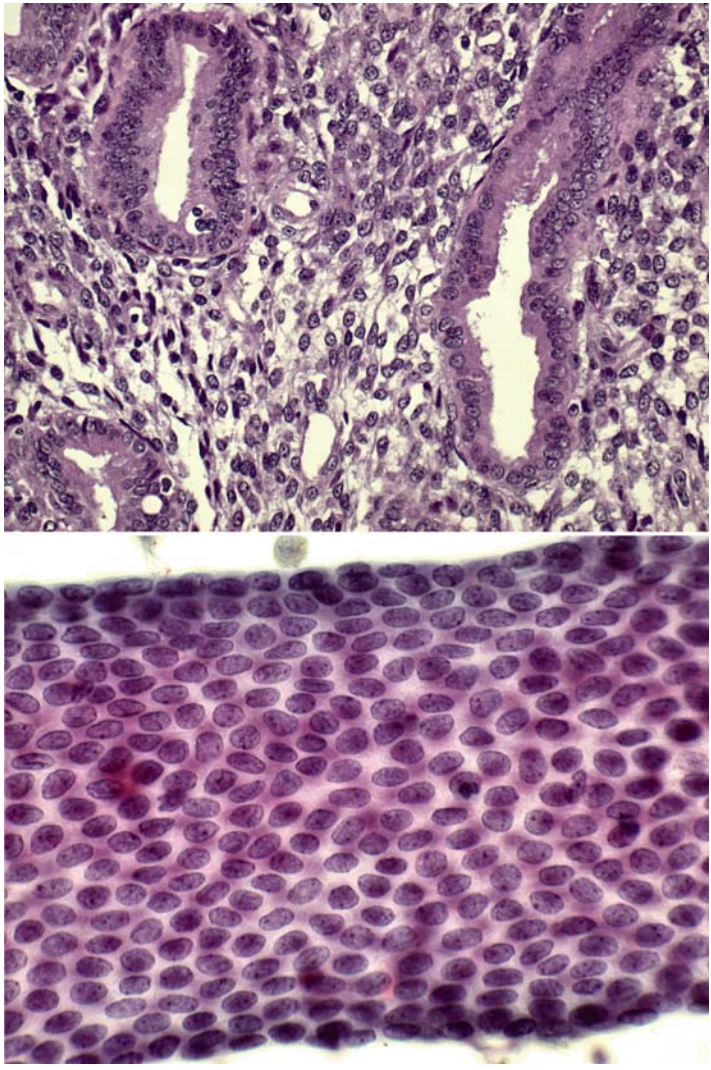

FIG. 5.9. During the early secretory phase, glands enlarge and display a moderate amount of well-defined, clear cytoplasm by which cell sheets and cells within glands begin to assume a honeycomb pattern. Also note that the nuclei appear rounder than those of proliferative endometrium. In the early portion of the mid-secretory phase, the extreme curvilinear nature of the gland is not yet well seen. Liquid-based cytology preparations do not show intraglandular secretory substance, but they reflect the cytoplasmic dominance that characterizes the normal mid-secretory phase.

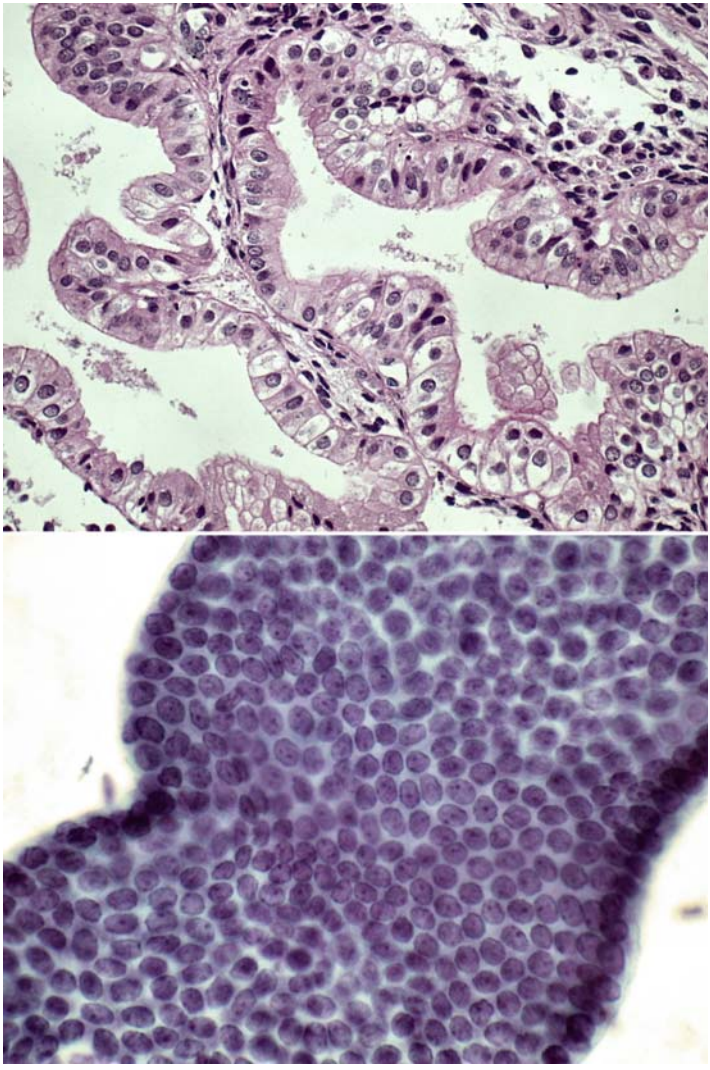

FIG. 5.10. Mid-secretory glands eventually become coiled, as can be seen by the frequent "indentations" that are present in both the cell block (*top*) and brush cytology (*bottom*) preparations; cell sheets take on a wavy topography. Nuclei are more uniform than those of proliferative endometrium. They appear rounded and vesicular with fine chromatin. Nucleoli are small and inconspicuous. Nuclei are more widely separated from one another than they are in the proliferative phase, reflecting increased cell cytoplasm.

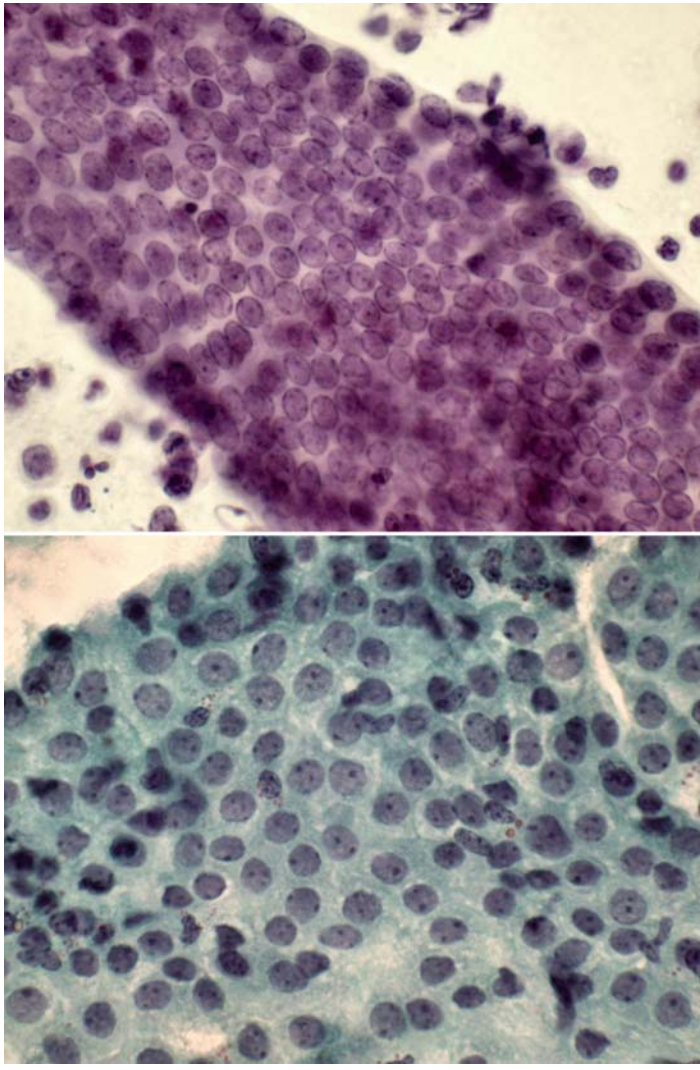

FIG. 5.11. In the late secretory phase, on around postovulatory day 12 to 13, glands exhaust, cease their secretory activity, and collapse, appearing smaller than the glands of the mid-secretory phase. Their nuclei come to approximate one another. There is no significant variability in nuclear size, and in contrast to proliferative endometrium, there is no nuclear pseudostratification (*top*). In collections of surface epithelial sheets, occasional granulated cells with features of granulated NK lymphocytes can be recognized (*bottom*) and can be seen percolating through both the stroma and epithelium.

homeostasis during the menstrual cycle by eliminating senescent cells from the functional layer of the uterine endometrium during the late secretory and menstrual phases of the cycle. The endometrium undergoes continuous cyclic changes of cell death and proliferation. Apoptosis is seen in the stromal cells throughout the menstrual cycle, but is scarce in the glands of proliferating endometrium, becoming maximal only in the glands of menstruating endometrium.

Morphologically, some glandular cells degenerate into cells with small, round, or oval nuclei with homogeneous, dense chromatin and a moderate amount of well-defined, dense cytoplasm that we interpret as apoptosis. Cells with apoptotic nuclei may intermix with glandular cells similar to those of mid-secretory endometrium. Endometrial glandular apoptosis is present in most collections of normal endometrium, but it appears both increased in amount and earlier in some cases of dysfunctional uterine bleeding. The significance of this finding is not known, but increased apoptosis may serve as a morphological marker of abnormal endometrial development in otherwise normal endometrial collections. On the other hand, downregulated apoptosis has been implicated in endometriosis and apoptosis indices in the eutopic endometrium of women with endometriosis have been reported as lower than apoptosis indices of women without endometriosis.

NK cells, the "granulocytes" of the endometrium, reside in various female reproductive tract tissues, and their phenotype and regulation are largely dependent upon their geographic location. They are admixed with endometrial epithelium and stroma in brushings of late secretory endometrium (Fig. 5.11, bottom). The number of NK cells varies with the menstrual cycle in the endometrium (but not in the cervix), suggesting that unique characteristics of the tissues may account for specific localization of different NK cell subsets. Uterine NK cells rapidly increase in number after ovulation and come to represent a major population of leukocytes in the endometrium, playing a role in uterus-specific events such as pregnancy and menstruation. Decreased cytotoxic capability of decidual NK cells from spontaneous aborters has been taken to indicate that a functional deficiency of these cells may be associated with early pregnancy loss. Evidence indicates that these cells may be involved in the reorganization of blood vessels during pregnancy.

Before the fertilized ovum reaches the uterus, the mucous membrane of the uterine body undergoes important changes and is then known as the decidua. Stromal changes characterize the late secretory phase. In its broadest sense, decidualization could be viewed as endometrial remodeling in preparation for pregnancy, which includes secretory transformation of the uterine glands, influx of specialized uterine NK cells, and vascular remodeling (Figs. 5.12, 5.13). With a more restricted definition, reprogramming of the endometrial stromal compartment has the decidualized stromal cells acquire the unique ability to regulate trophoblast invasion, resist inflammatory and oxidative insults, and dampen local maternal immune responses.

In humans, the stromal compartment decidualizes in the midluteal phase of the menstrual cycle and is independent of pregnancy. Stromal cells in the functional endometrial compartment of the upper endometrium undergo predecidual change and acquire abundant well-defined, dense cytoplasm and centrally located round or oval, vesicular nuclei (Fig. 5.13). The immediate subepithelial stroma can be dislodged as sheets of cells with epithelioid features, showing ample, granular cytoplasm and large, clear, uniform nuclei with prominent nucleoli. These stromal cell sheets should not be taken for immature metaplastic or neoplastic squamous epithelium. Similar sheets of deciduoid cells may be seen with endometrial irritation caused, for example, by polyps or leiomyomas. In such cases nuclear changes can be severe, leading to misinterpretation of these cell sheets as neoplastic.

Implantation of a blastocyst is associated with resurgence of glandular secretion and stromal decidualization. The presence of decidual cells is important for the cytological identification of pregnancy-related cells (Figs. 5.14, 5.15). In a retrospective analysis of pregnancy-related cells, 12 of 4,429 endometrial smear samples disclosed eight spontaneous abortions, three placental site nodules, and one partial hydatidiform mole. Decidual cells were observed in all cases, trophoblasts in 5, syncytiotrophoblasts in 1, and nonsyncytiotrophoblasts in 5 of the 12 cases. These cells were prospectively identified in 7 cases and retrospectively discovered in the remaining 5 cases. Pregnancy-related cells may be overlooked because they are difficult to identify or may be surrounded by clusters of endometrial cells.

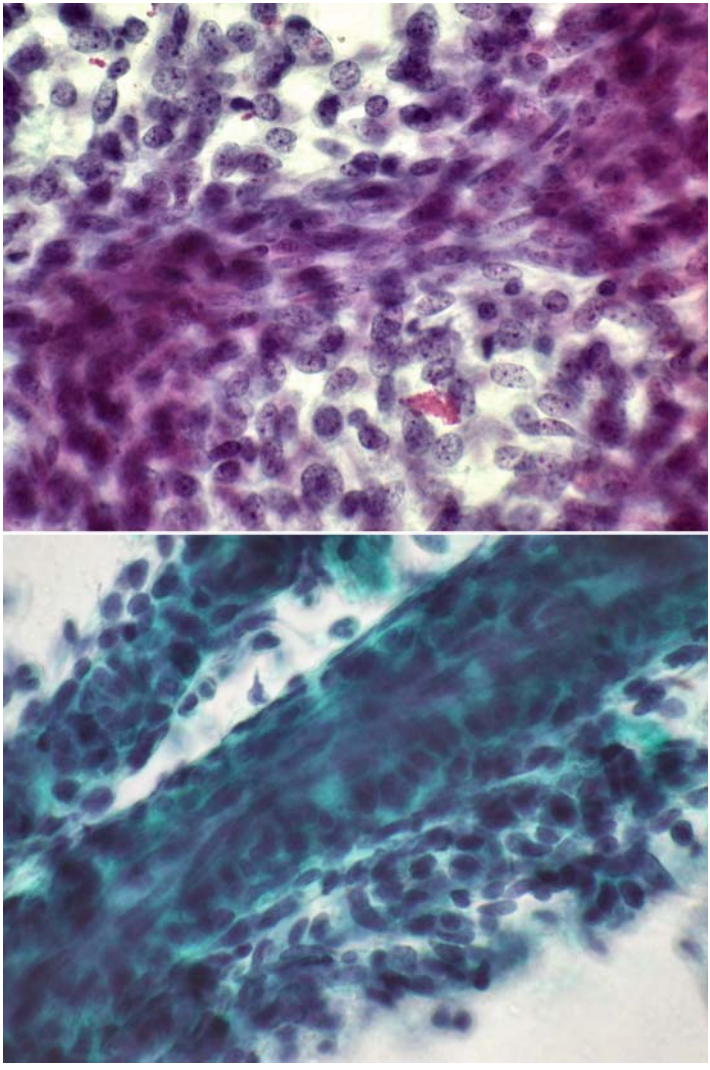

FIG. 5.12. One of the earliest stromal changes induced by progestins is perivascular stromal compaction with condensation and vascular thickening. Both stromal venules (*top*) and arterioles (*bottom*) are decorated by plump stromal cells. These images should be compared to those of Fig. 5.5. Stromal compartmentalization may be studied immunohistochemically, but just observing the cycle-dependent changes of stromal compaction and the variable geographic emergence of deciduoid stromal cells seems sufficient to convince anyone about the complexity of the functional stromal compartment.

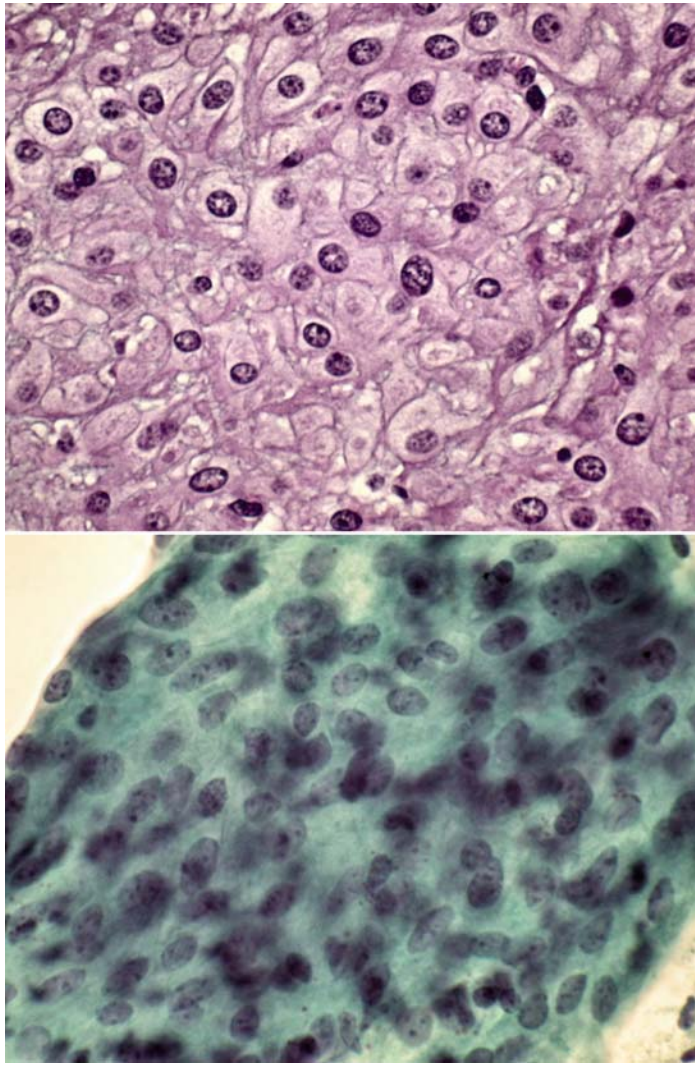

FIG. 5.13. In the late secretory phase, stromal cells in the functional endometrial compartment of the upper endometrium undergo uniform predecidual change and acquire abundant well-defined, dense cytoplasm and centrally located round or oval, vesicular nuclei. In this figure, a cell block section (*top*) is compared to a cytology microbiopsy of deciduoid stroma (*bottom*). Deciduoid cells have epithelial characteristics, including immunostaining with some epithelial markers. They undergo sheet-like organization along the epithelial surface and may occasionally be collected as sheets of epithelioid cells.

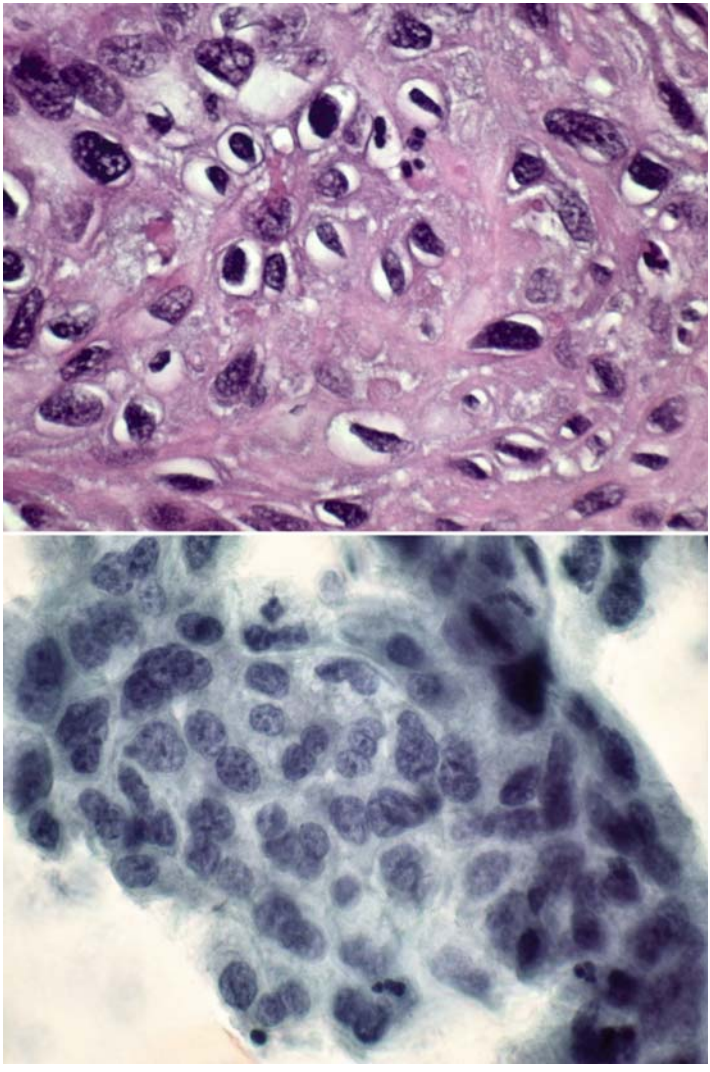

FIG. 5.14. The presence of decidual cells is an important clue to look for the cytological identification of other pregnancy-related cells. The decidual cells of pregnancy are very plump and may display a degree of nuclear heterogeneity. As with their epithelial counterparts, 4N and higher forms may be seen. During pregnancy, especially in the region of stromal vessels, bizarre epithelioid cells may be seen; these represent extravillous (intermediate) trophoblasts. As opposed to decidual cells, extravillous trophoblasts stain for pankeratin (AE 1/3).

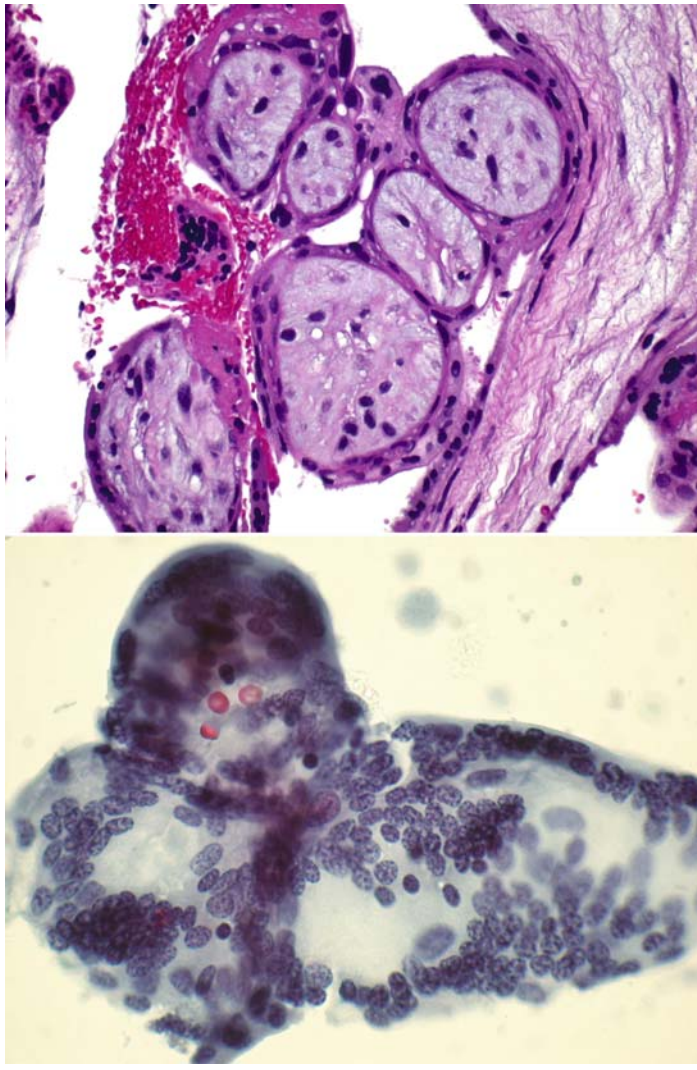

FIG. 5.15. These images show chorionic villi from a case of spontaneous abortion. Although heavy uterine bleeding generally is taken to herald early fetal loss among young women, pregnancy in the near-perimenopause may be clinically overlooked, mistaken for anovulatory cycling and dysfunctional uterine bleeding. In this case, relatively avascular chorionic villi are diagnostic of intrauterine implantation and subsequent spontaneous abortion.

An Arias–Stella reaction (focally altered endometrial epithelium displaying intraluminal budding, nuclear enlargement, and hyperchromatism with cytoplasmic swelling and vacuolation) is found in 80% of intrauterine abortions in which a secretory/hypersecretory pattern appears. A similar pattern is seen rarely in women evaluated for infertility or on hormonal therapy with oral progestational agents for the management of dysfunctional uterine bleeding. As a cautionary note, the Arias–Stella reaction can be mistaken for malignancy, including secretory and clear cell carcinoma of the endometrium (Fig. 5.16). Because the Arias–Stella change occurs in fully differentiated cells, no increased proliferative activity is seen in its epithelium.

The heterogeneity of stromal cells is not readily exemplified by their morphology during the proliferative phase, but it becomes apparent throughout the secretory phase. Human endometrial stroma initially exhibits rather uniform morphology. Predecidua initially develops around vessels and subsequently around glands and under surface epithelium, demonstrating that regional differences exist among stromal cells.

Immunoreactivity of the stromal cells likewise varies during the differing phases of the menstrual cycle. Phenotypically distinct subsets of stromal cells, including cells expressing epithelial markers, are confined to unique microenvironments throughout the menstrual cycle. All stromal cells strongly express vimentin and weakly express cytokeratin. Ber-EP4-positive stromal cells are confined around glands and to the subluminal regions of the surface epithelium. HLA-DR-positive stromal cells are characteristically present around glands and under surface epithelium, around blood vessels, and around HLA-DR-positive lymphoid cells. Stromal cells in the proliferative and early secretory phases are VLA-1 (integrin alpha 1, beta 1) negative. VLA-1 reactivity appears initially in the HLA-DR-positive cells around vessels and then in HLA-DR/Ber-EP4-positive cells around glands and under surface epithelium. In the late part of the secretory phase, all stromal cells in the upper functionalis express VLA-1.

In cases in which the secretory phase has been altered, for example, by advancing age, luteal phase defect, or as a result of exogenous medicaments, the endometrial stroma may appear either uncharacteristically immature and fibrous with well-developed

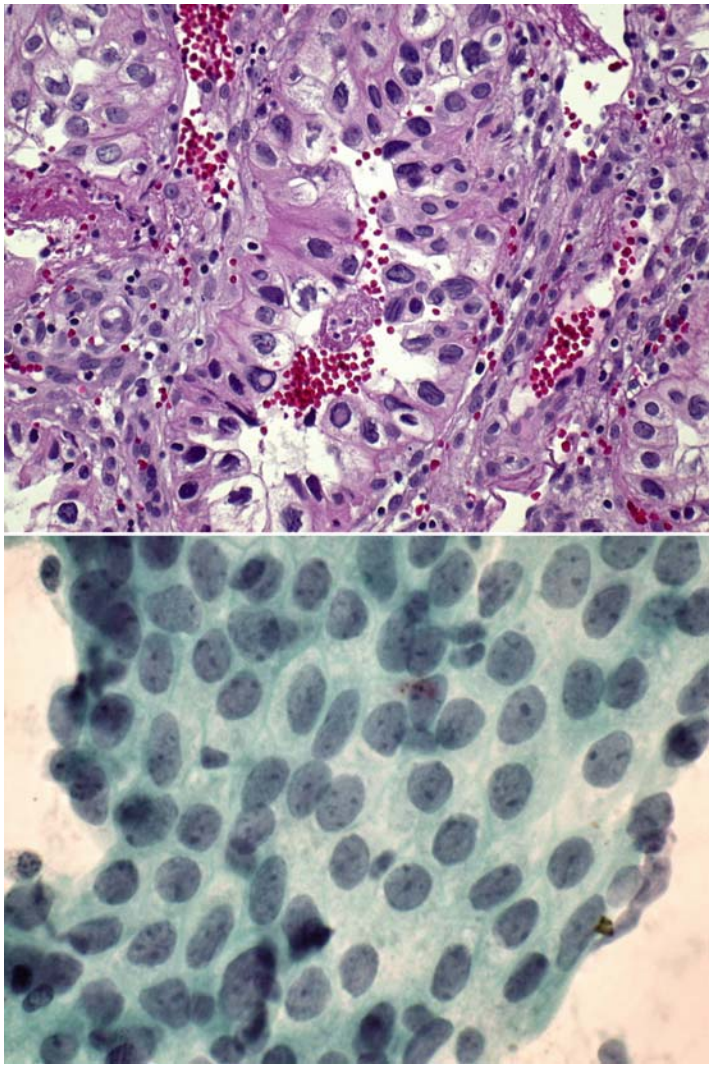

FIG. 5.16. Changes of the Arias–Stella reaction in endometrial glands may be observed in up to 80% of intrauterine abortions in which a hypersecretory pattern appears. The Arias–Stella reaction can be mistaken for malignancy, including secretory and clear cell carcinomas. As opposed to their small, round contour, the epithelial cells of the Arias-Stella reaction are hyperdiploid and, even when the nuclei are enveloped by secretory cytoplasm, they may appear bizarre enough to raise the specter of malignancy.

secretory gland changes or, in the case of progestins, deciduoid with atrophic glands (Fig. 5.17) and thick-walled vessels (Fig. 5.18) whose diameters equal or exceed those of the endometrial glands.

Menstrual Endometrium

Overt menstruation is found primarily in humans and close evolutionary relatives such as chimpanzees. In contrast, the females of other species of placental mammals have estrous cycles in which the endometrium is reabsorbed by the animal at the end of its reproductive cycle. Some speculate that the energy savings of not having to continuously maintain the uterine lining offsets the energy cost of having to rebuild the lining in the next fertility cycle. Menstruation may represent a form of endometrial economy that is of ancient evolutionary origin.

The endometrial microvasculature is designed to provide the blood supply to the endometrium and the placenta, and external bleeding appears to be a side effect of endometrial regression that arises when there is too much blood and other tissue for complete reabsorption. The relatively copious bleeding of humans and chimpanzees relates to the large uterine size relative to adult female body size and to the design of the uterine microvasculature.

As the late secretory phase ends and menstruation begins, predecidual cells of the upper endometrium separate from each other and are shed, in large numbers, into the endometrial cavity. Solitary predecidual cells have rounded, ovoid, or bean-shaped nuclei and variable amounts of relatively well defined, opaque, foamy, or finely granular cytoplasm. The plump nature of predecidual cells is best appreciated when they are in apposition to stromal arterioles or when they are loosely balled up.

As related earlier, the late secretory phase features increasing numbers of NK lymphocytes that contain relaxin, a naturally occurring hormone known to modulate connective tissue remodeling in the uterine corpus and cervix. This hormone may dissolve the reticulum fibers surrounding the stromal cells, fostering stromal cell dissociation and cyclical shedding of the endometrium.

Menstrual endometrium shows the greatest structural polymorphism that is seen with any of the defined phases of cycling

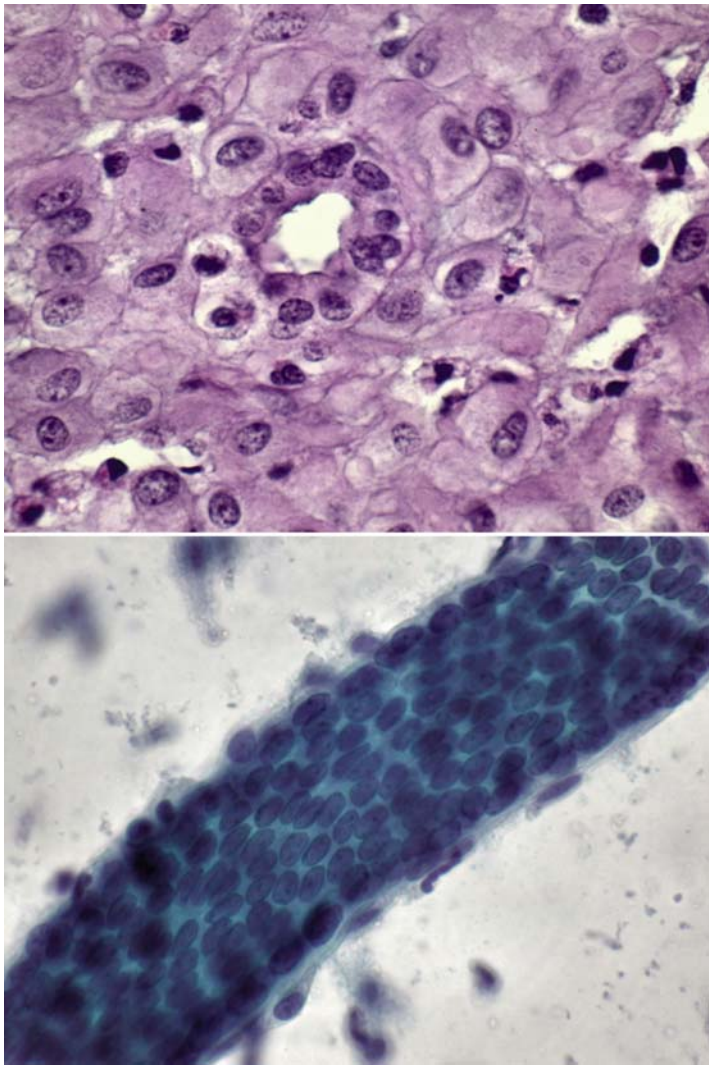

FIG. 5.17. An endometrium with progestin effect shows deciduoid endometrial stroma and collapsed, small glands with inactive-appearing nuclei. This is a classical example of the "pill effect." With the popular use of endometrial ablation for the control of uterine bleeding, many practitioners are modulating the endometrium with gestagens as an intermediate step to control bleeding and to thin the endometrium for more effective thermal injury and removal. The result comprises the triad of deciduoid stroma, thin glands, and prominent vessels.

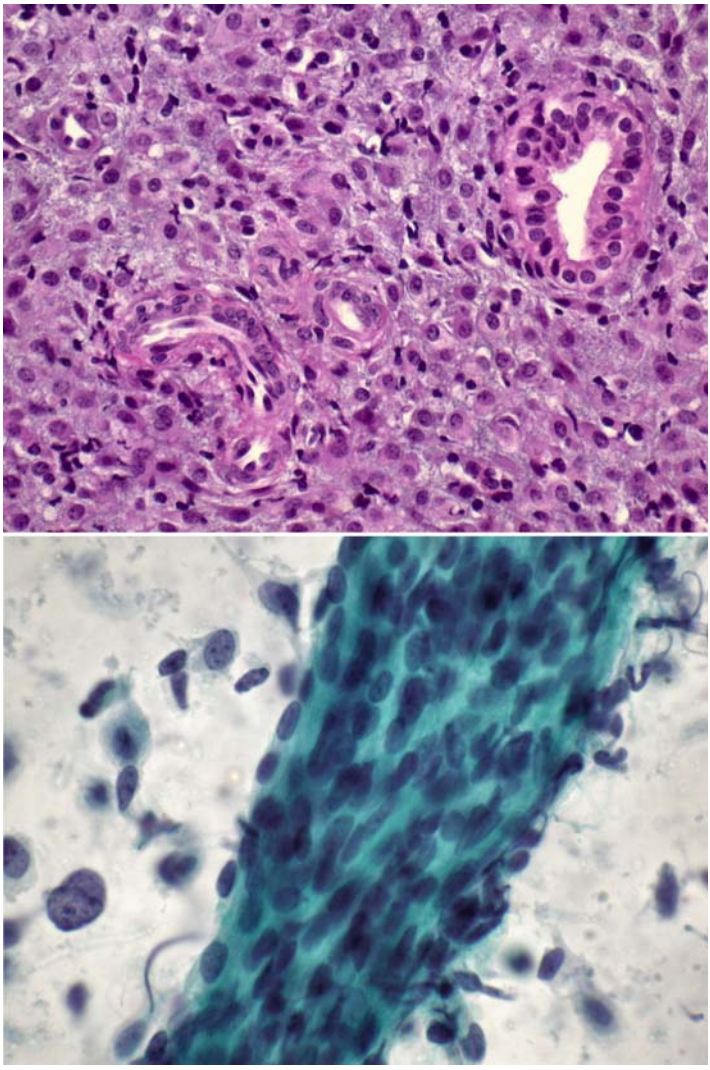

FIG. 5.18. An endometrium with progestin effect shows deciduoid endometrial stroma and prominent vessels whose diameters equal those of small inactive-appearing glands. In these images, the stroma is more fibro-deciduoid than the stroma of Fig. 5.17. Stromal vascular networks that appear in the absence of prominent secretory glands are a signature change of "pill effect." Again, the result of progestational agents comprises the triad of deciduoid stroma, thin glands, and prominent vessels.

endometrium, meaning that it unwittingly may be overdiagnosed as endometrial cancer. As a corollary, some endometrial cancers may be underdiagnosed as menstrual endometrium. The absence of stroma between the glandular elements of menstrual endometrium may give the impression of confluent glands or, in some cases, of solid epithelial growth. Piled-up dyshesive epithelium may lose nuclear polarity and acquire prominent nucleoli, but menstrual endometrium lacks the coarse chromatin and nuclear pleomorphism of malignancy, and both lack a genuine cribriform pattern and invasion. Cytoplasmic vacuoles may be present in some of the predecidual cells of menstrual endometrium and may mimic the lumens of signet ring cell adenocarcinoma, including lobular breast cancer and gastric cancer, both of which have a propensity to metastasize to the endometrium. Not uncommonly, intact neutrophils are found within the vacuoles of these predecidual cells, a change mimicking the neutrophilic emperipolesis of endometrial adenocarcinoma. Encouragingly, correlative cell blocks were available for examination in 97% of office samplings that we diagnosed as menstrual endometrium and in 91% of cases that we diagnosed as carcinoma.

The distinction between cyclical menstrual endometrium and endometrium with noncyclical glandular and stromal breakdown is difficult. Unless the pars basalis is sampled in late menstruation, menstrual endometrium lacks mitotic activity. The glands of menstrual endometrium regularly exhibit some residual secretory change, and a careful search will identify typical areas of stromal fragmentation as opposed to areas of coagulative necrosis that are seen with noncyclical endometrial breakdown bleeding. Furthermore, a predecidual component is not part of irregular shedding resulting from noncyclical endometrial breakdown.

Slides show collapsed, benign glands with intraepithelial polymorphonuclear neutrophils, nuclear dust, fibrin strands, and "dissolved" or "ripped-apart" stromal aggregates (Figs. 5.19–5.23). There are many ball-like tissue fragments containing degenerating glandular and predecidual cells, small nonpredecidualized stromal cells from the basal layer of the endometrium, and predecidual cells admixed with leukocytes (Figs. 5.24–5.26).

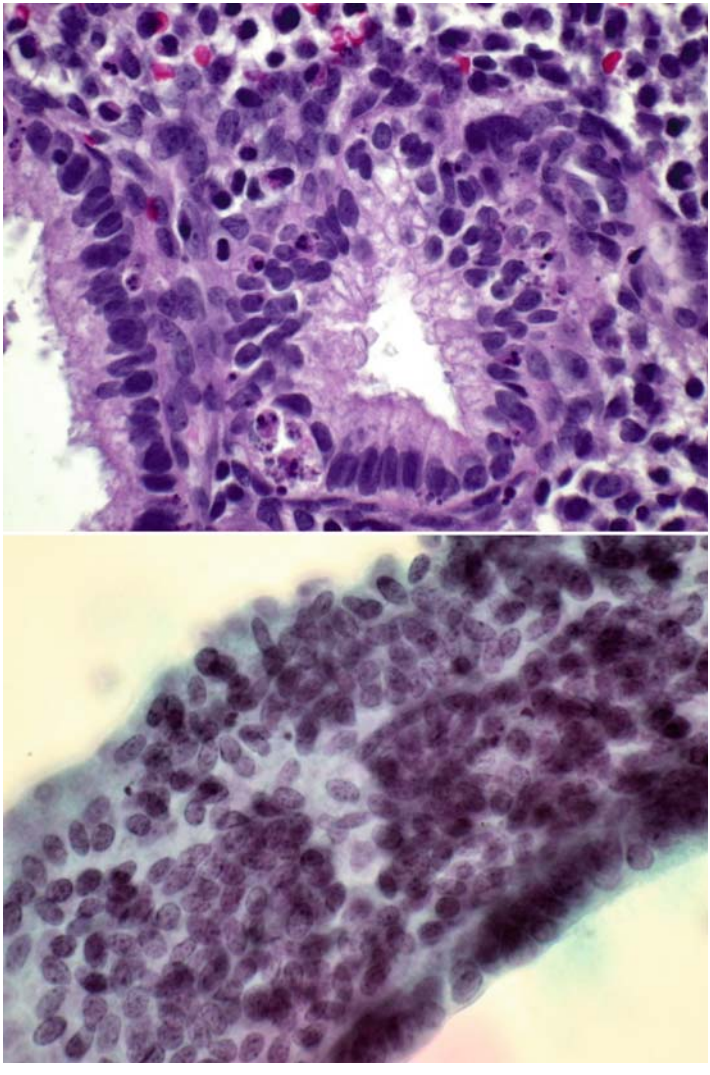

FIG. 5.19. Among the earliest changes seen with menstruation is the influx of polymorphonuclear leukocytes (PMNs) along the basal aspect of the endometrial glands (*top*). The collapsed glands appear to be structurally destabilized, and frequent gland ruptures and tears appear in brush cytology preparations of early menstrual endometrium (*bottom*).

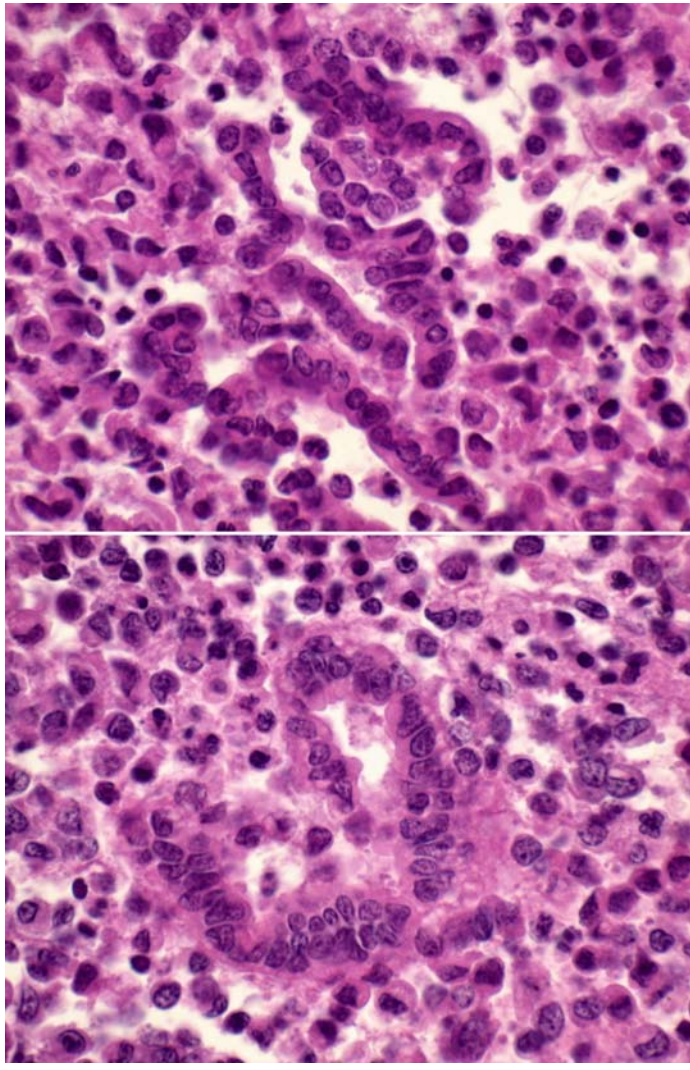

FIG. 5.20. As the stromal–epithelial destabilization of the endometrium increases, both the gland and stromal integrity are compromised and glands begin to shatter, involute, fold over themselves, and take on bizarre shapes. This change is obvious in cell block preparations. Sometimes, unless one takes into account patient age and history of last menstrual period, these complex disorganizational changes along with the bleeding, fibrin, and cellular dyshesion that accompanies them may lead one into a false diagnosis of malignancy. Every endometrial study should be interpreted with knowledge of menstrual history.

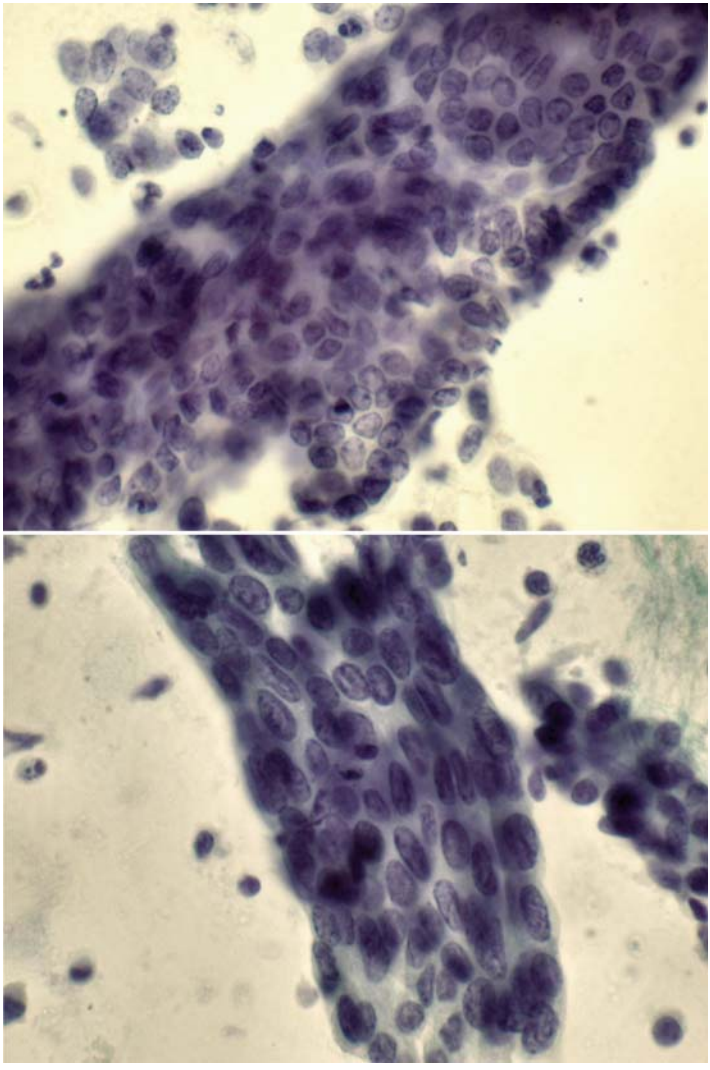

FIG. 5.21. The involution and fracturing of glands are seen in brush cytology preparations as well. Even though gland collapse is obvious, the regular polarity of the epithelial cell nuclei within the collapsed glands begins fading, and it becomes possible to misinterpret these changes as an expression of dedifferentiation. Menstrual endometrium is an attribute of cycling endometrium, but it may also accompany erratic ovulation. The difference from anovulatory bleeding is that the latter generally lacks residual vessels decorated by plump deciduoid stromal cells.

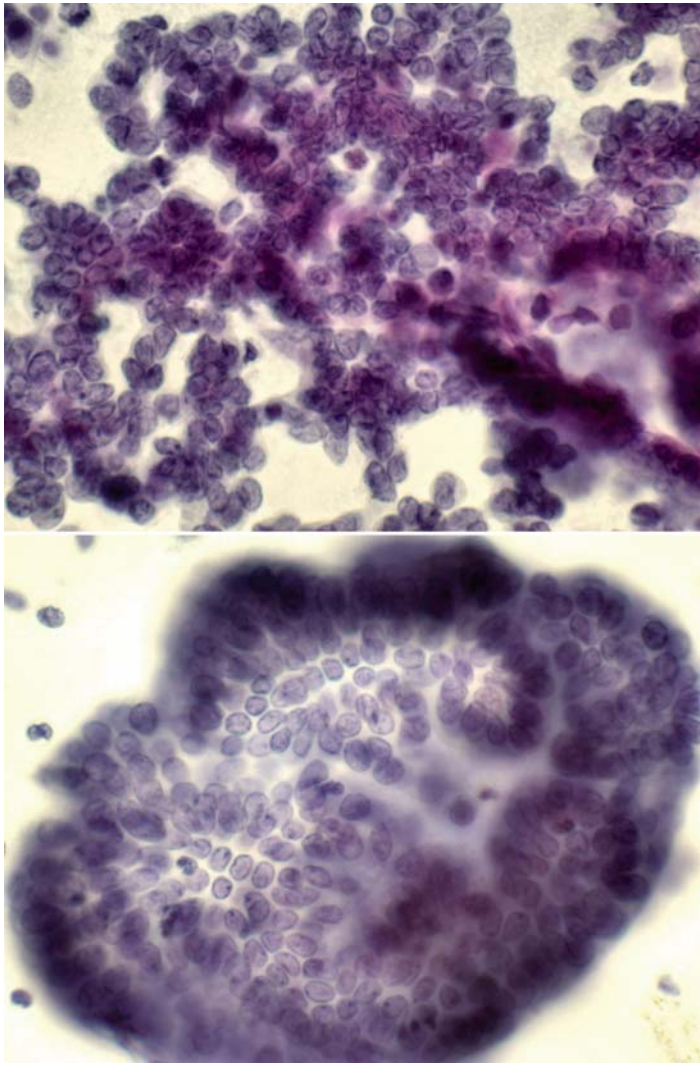

FIG. 5.22. As epithelial sheets and their accompanying stroma tear apart, pseudocribriform arrangements may emerge (*top*). Some fragments of endometrial glands complexly fold over upon themselves and begin to take on odd shapes that may also be seen with endometrioid neoplasms, mimicking irregular acinar structures (*bottom*). Menstrual collapse is not an indication of dedifferentiation or of fractal complexity within preformed epithelial structures; rather, it is the result of a programmed cyclical destructive implosion of previously normal structures.

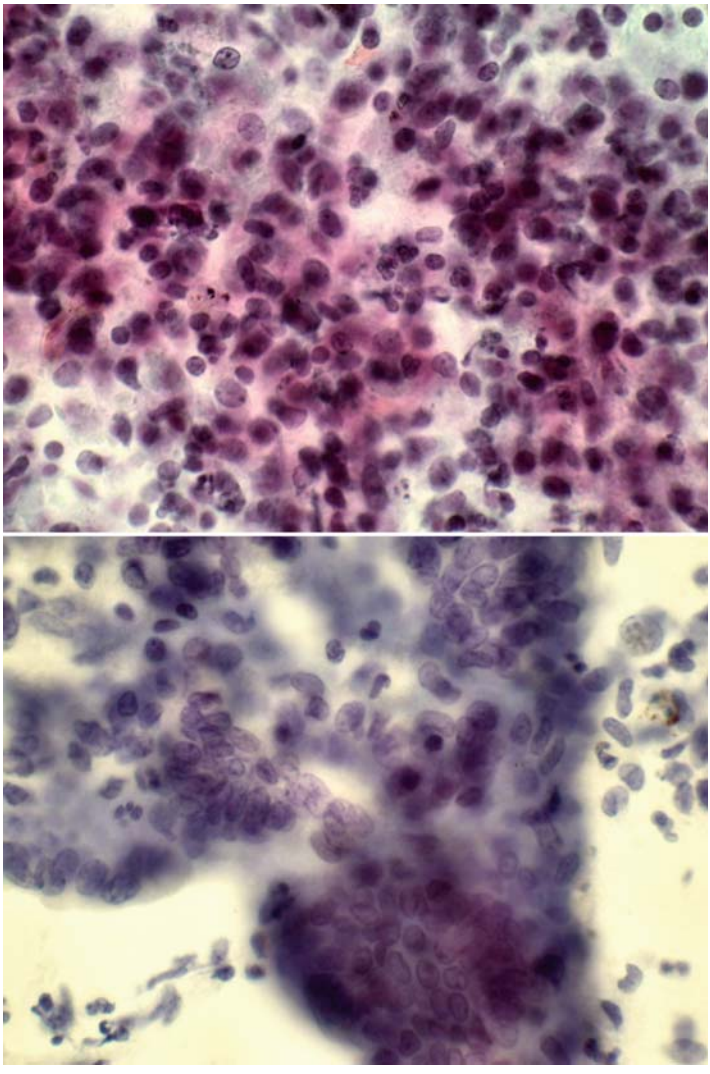

FIG. 5.23. As menstrual stroma and glands separate, dyshesive stromal fragments mimic changes that may be seen with dyshesive cells of outspoken carcinomas that have a solid pattern (*top*); and, when this is accompanied by epithelial aggregates that show what appear to be rings upon rings of epithelial cells in acinus-like arrangements, mimicking cribriform architecture, a false suspicion for carcinoma may come about (*bottom*). Luckily, most collections of menstrual endometrium are copious enough that correlative cell blocks are available for review.

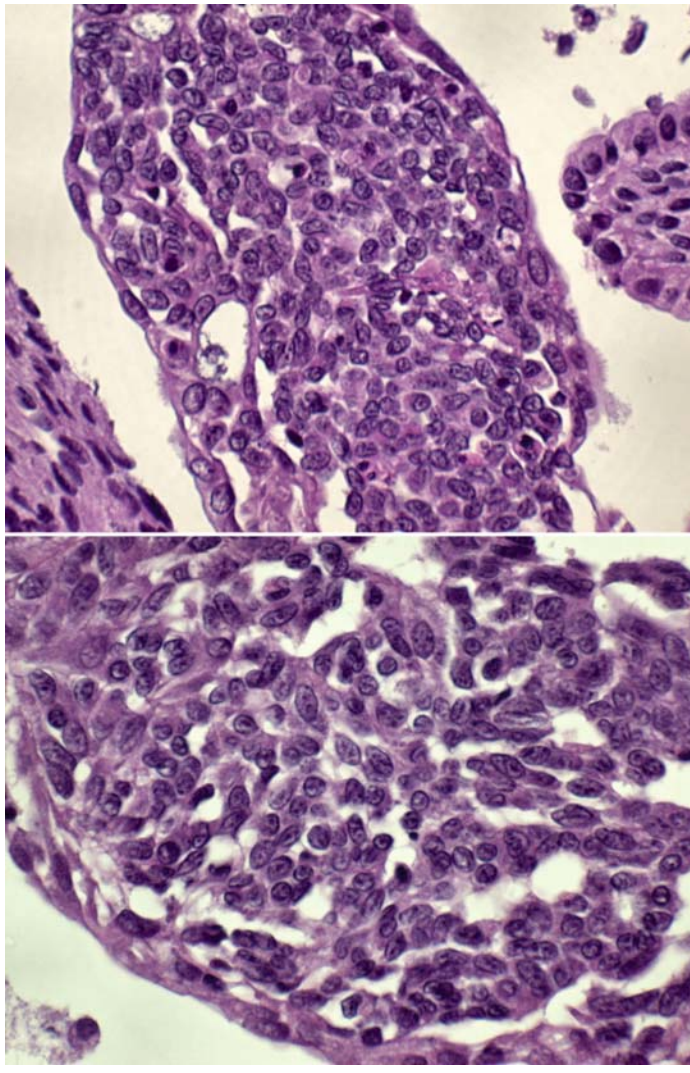

FIG. 5.24. Menstrual brushings show ball-like tissue fragments containing degenerating glandular and predecidual cells, small nonpredecidualized stromal cells from the basal layer of the endometrium, and predecidual cells admixed with leukocytes. What is witnessed in these images is the birth of exodus balls. Because stromal-glandular modeling persists beyond the period of endometrial shedding, exodus balls persist for several days beyond the typical menstrual period. Similar structures are seen with non-cyclical bleeding as well.

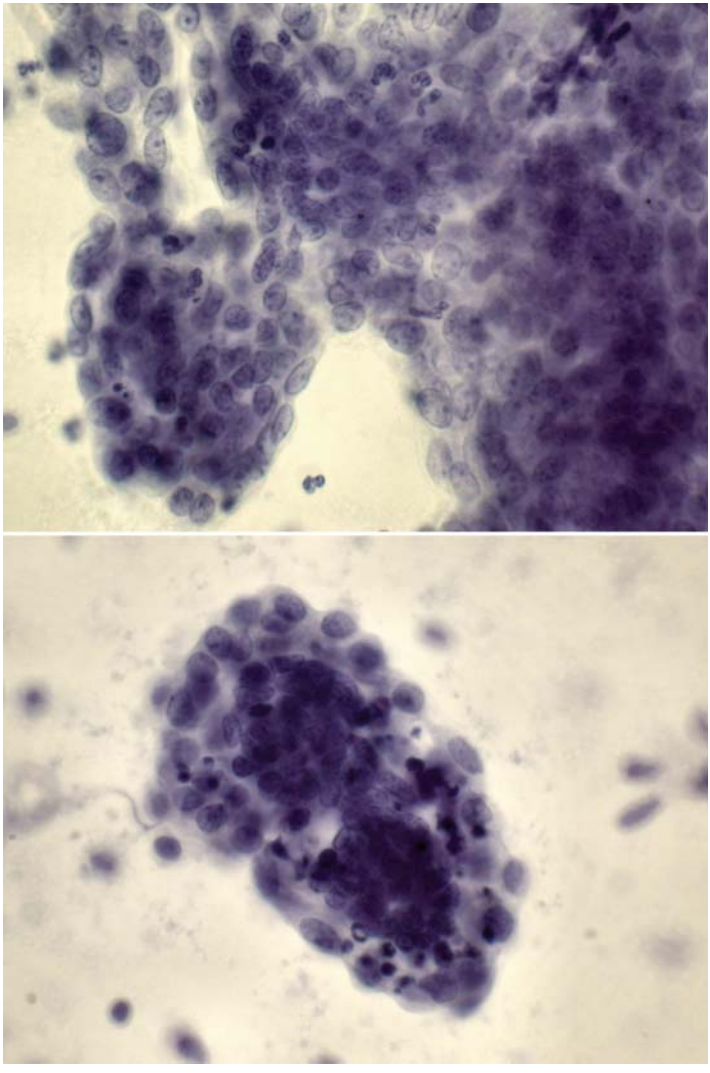

FIG. 5.25. Tissue microfragments seen in endometrial brush cytology specimens of menstrual endometrium show stromal-epithelial cell clusters, either budding off of larger microbiopsies (*top*), or floating freely, having been shed as exodus balls (*bottom*).

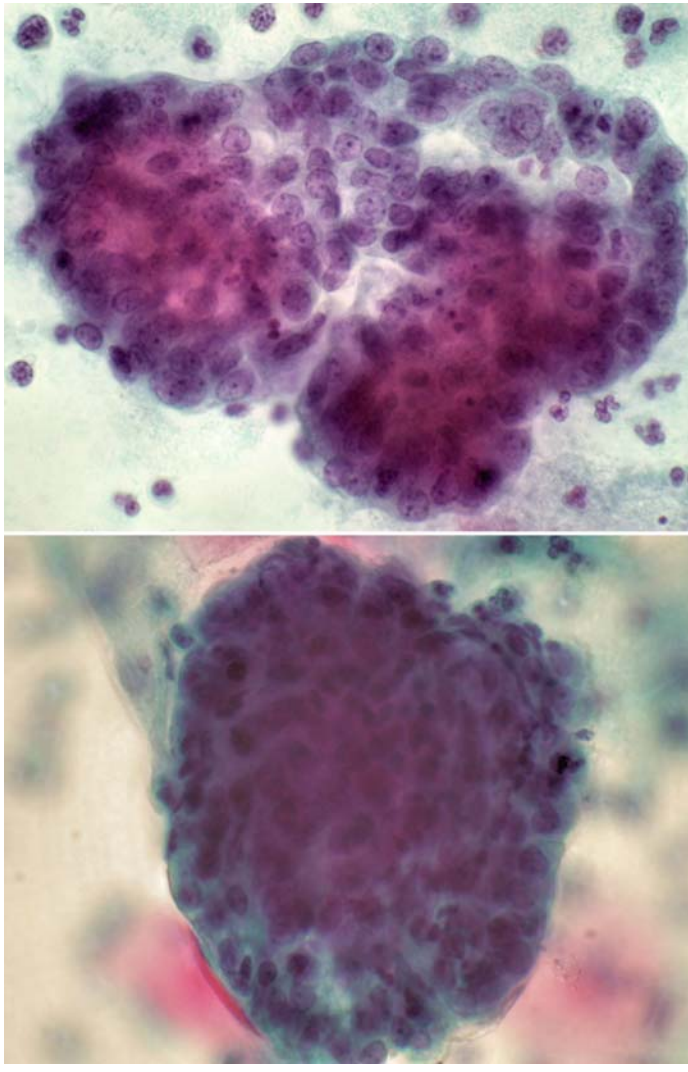

FIG. 5.26. Exodus balls of endometrial stroma and epithelium are similar in appearance to those found in cervicovaginal cytology preparations and are also similar to those seen in cases of noncyclical endometrial breakdown and bleeding; but, in the latter situation, deciduoid stromal cells are generally not seen and the mechanism of bleeding is different than that of menstrual shedding. It is sometimes difficult to distinguish these products from the epithelial surface tufts of syncytial metaplasia; and, oftentimes, both structures are admixed with each other.

Endometrial Atrophy

An age-related morphological continuum extends from weak proliferation in the perimenopause to atrophy in the late postmenopause. This transformation takes years, which is why weak proliferative features persist after the menopause. Inactive or atrophic endometrium may resemble the early phase of cycling proliferation but with less epithelial pseudostratification, shorter apical–base epithelial cell axes, and greatly reduced to absent mitoses. The close nuclear packing of inactive endometrium may resemble that seen in epithelial sheets derived from late secretory endometrium, following secretory exhaustion (Figs. 5.27–5.31).

In more than half of specimens, classification into well-defined functional categories is impossible as epithelial cell sheets show such variable admixtures of pseudostratification and honeycombing, comprising a mixed-function appearance. For example, in one study of 142 adequate endometrial biopsies taken from perimenopausal women by suction curettage and assessed by light microscopy, 82 (58%) specimens defied classification into the well-defined categories of the fertile period because of mixed histological patterns; 59 (42%) showed abnormal secretory endometrium, 3 (2%) had disordered proliferative endometrium, and 20 (14%) showed a mixture of nonsecretory and secretory endometrium.

The stromal cells of atrophic endometrium are often fibroblast like, similar to the fibrous stroma of the lower uterine segment (Fig. 5.32). Fibrous stroma does not readily give up its glands, which is why epithelial sheets outnumber tubular glands in brush preparations of atrophic endometrium and the epithelial/stromal ratio favors flat sheets of surface epithelium.

Because the stroma is more fibrous than that of cycling endometrium, it is also less abundant on brush preparations. Stromal cells have oval, fusiform, or pyknotic nuclei and scant, poorly defined cytoplasm. These cells may occur in loose groupings and occasionally support a myxoid interstitial substance. Crushed, blue stromal debris sometimes litters the background of the slide and appears “torn up” or “ratty.” Pipes or columns of collagenous material may be seen (Fig. 5.33).

Inactive or atrophic endometrial glands comprise a single layer of flattened to cuboidal cells. Some glands are small and cystic

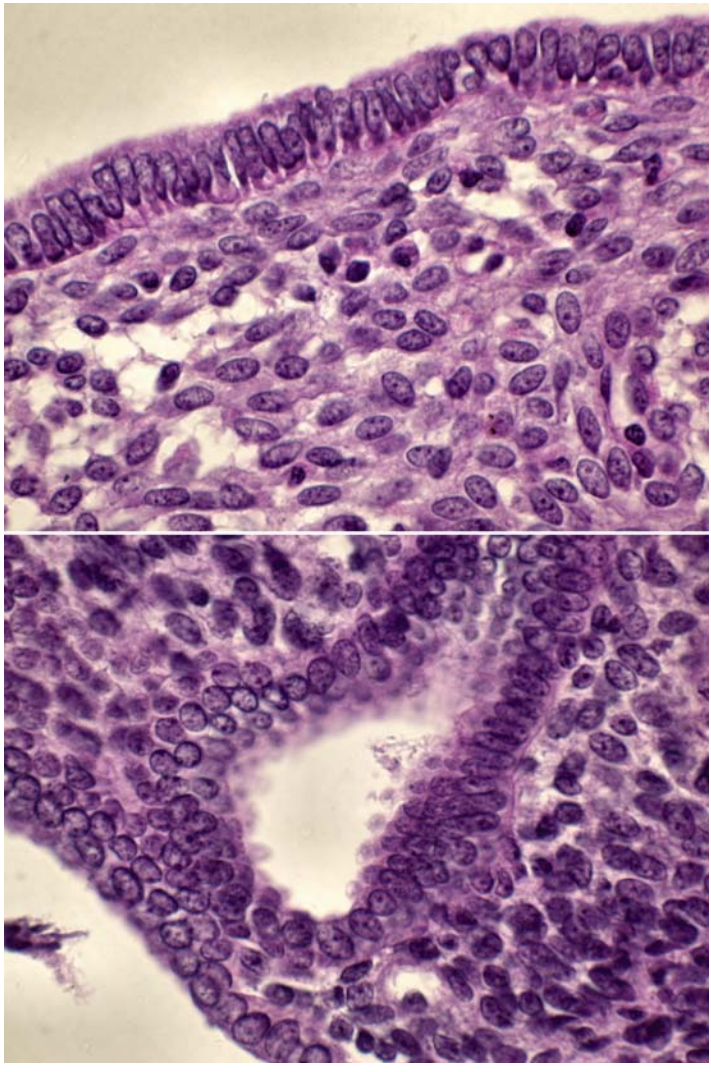

FIG. 5.27. Inactive or atrophic endometrium may resemble the early phase of cycling proliferation but with less epithelial pseudostratification, shorter apical-to-base epithelial cell axes, and greatly reduced to absent mitoses. Endometrial inactivity is not always age qualified. For example, women using birth control pills or those with early ovarian failure, caused for example by pelvic irradiation or chemotherapy, may show changes of endometrial atrophy.

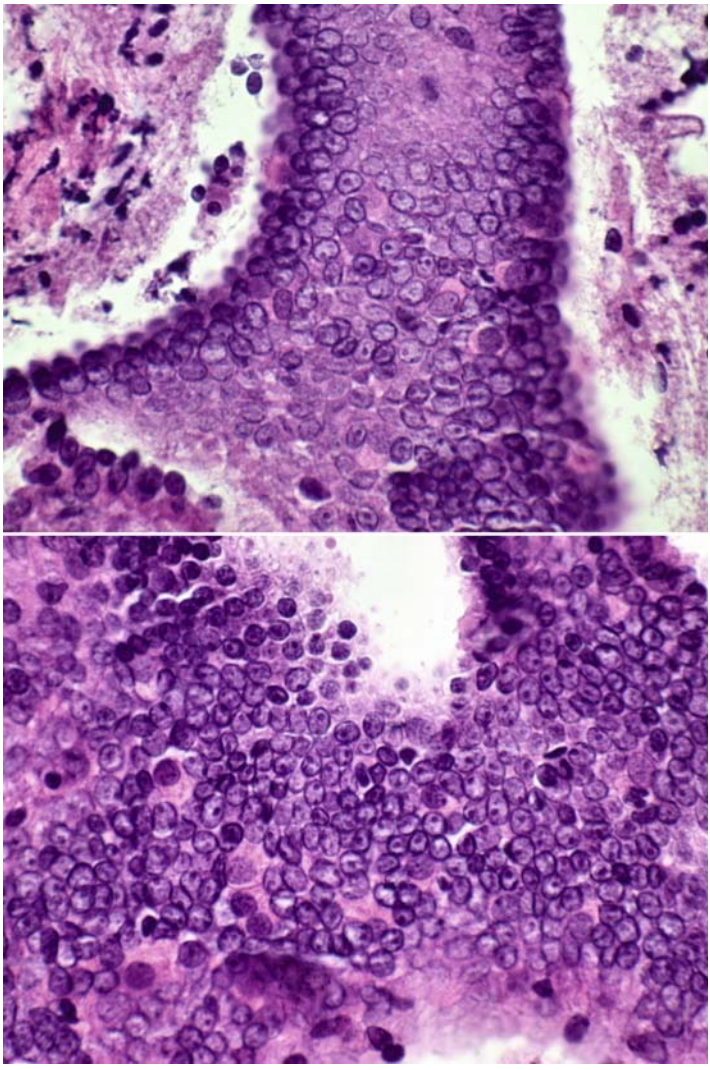

FIG. 5.28. As is shown in these cell block preparations, the glands of atrophic endometrium are short, stubby, and show tightly packed nuclei, generally lacking the pseudostratification that characterizes actively proliferating or early postmenopausal endometrium. Nuclei are packed, not in the nuclear-dominant fashion of proliferative endometrium; rather, there is neither nuclear or cytoplasmic functional dominance, just small resting cells resembling those typically found in the lower uterine segment.

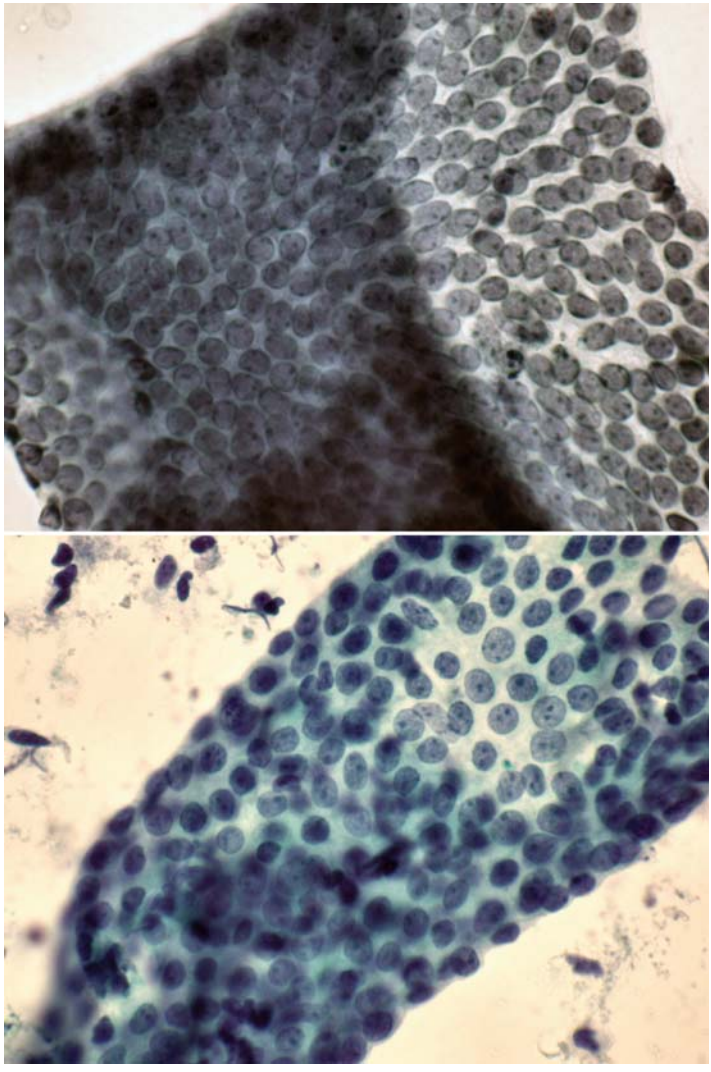

FIG. 5.29. Glands appearing similar to those of Fig. 5.27 are also seen in cytology brush preparations (*top*). Sometimes these glands may mimic the collapsed glands of the late secretory phase (*bottom*). Gland morphology should not be interpreted without an eye on stromal and vascular tissue. In atrophy, the stroma is fibrous; but, in the late secretory phase, the stroma is deciduoid and the vessels are plump.

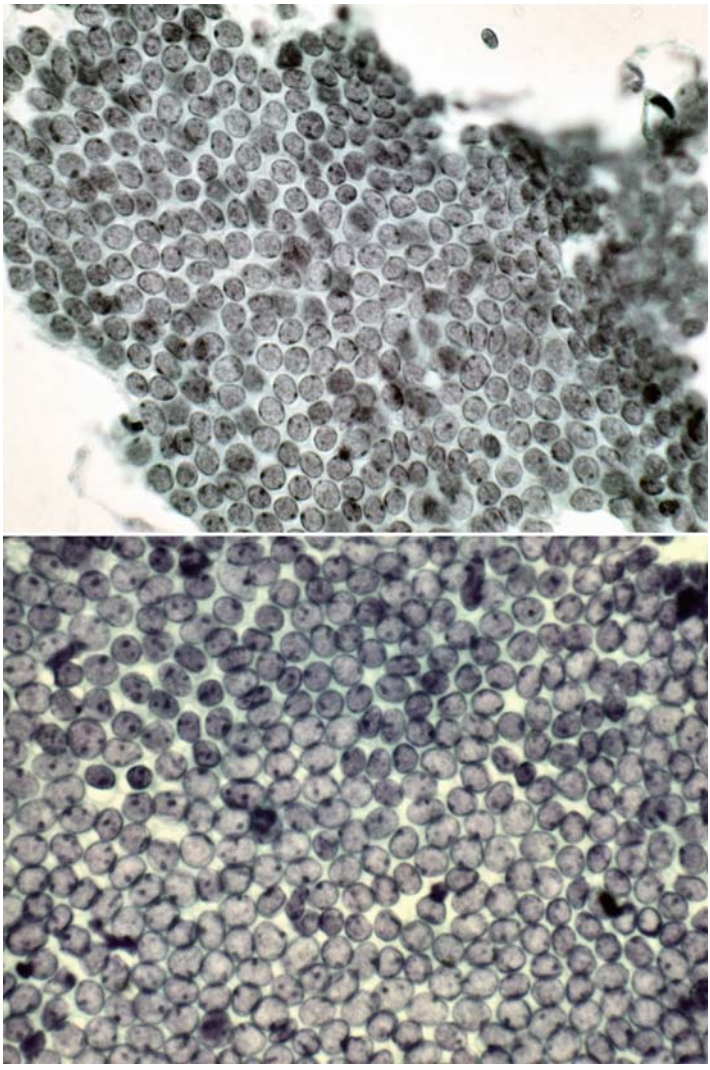

FIG. 5.30. The close nuclear packing of inactive endometrium may resemble that seen in glands derived from late secretory endometrium, following secretory exhaustion. In secretory endometrium, surface epithelium retains its honeycomb appearance; but, in atrophy, the surface epithelium packs tightly, following the example of its accompanying glands. Therefore, the functional role typically assigned to glands over surface epithelium in the cycling endometrium is not seen in atrophic endometrium.

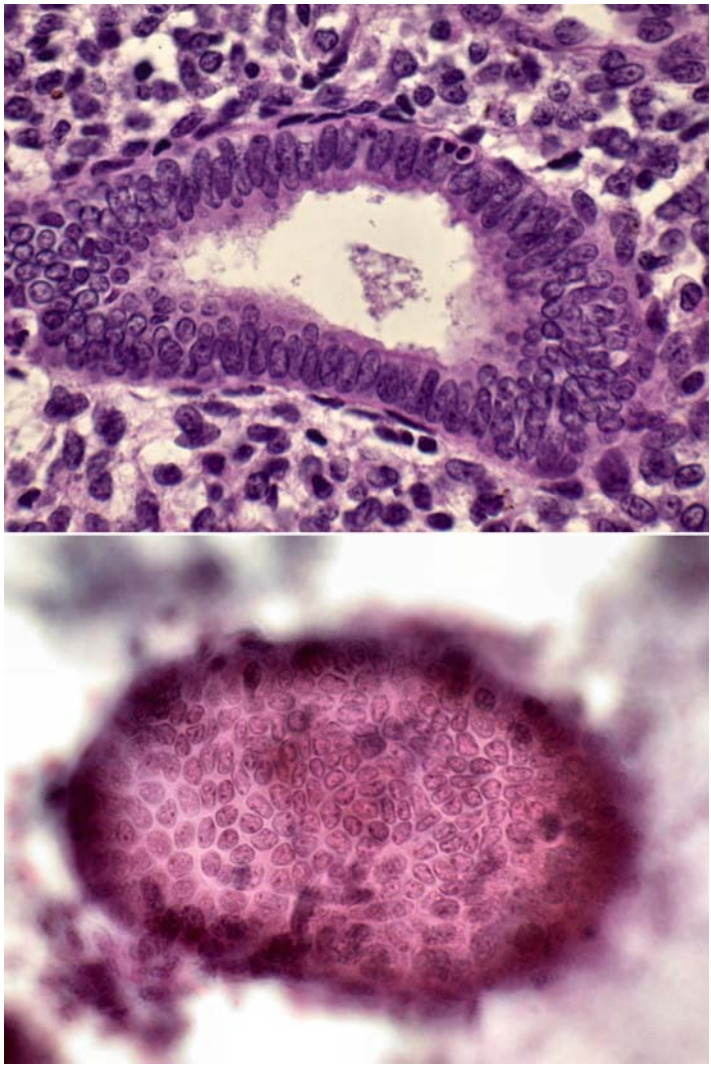

FIG. 5.31. In addition to truncated glands, small cystic glands may be seen in both histology (*top*) and cytology brush preparations (*bottom*) of inactive endometrium. Note that the nuclei of these glands appear similar to the nuclei of the surface epithelium. These glands are small, cystic, and smoothly contoured and vary, for example, with the small irregularly contoured glands found in endometrioid neoplasia. Stroma may be poorly represented in brush cytology preparations because it is now fibroblastic and collagenous.

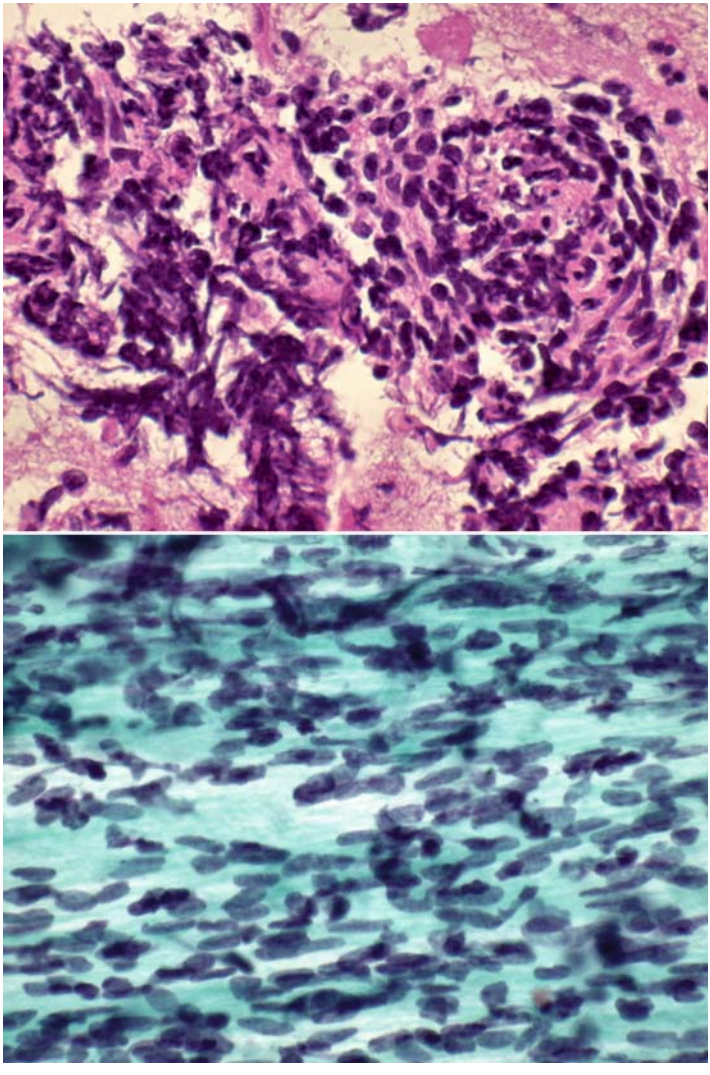

FIG. 5.32. The stromal cells of atrophic endometrium are often fibroblast like, similar to the fibrous stroma of the lower uterine segment. Stromal cells have oval, fusiform, or pyknotic nuclei and scant, poorly defined cytoplasm. They may occur in loose groupings, occasionally supporting a myxoid interstitial substance.

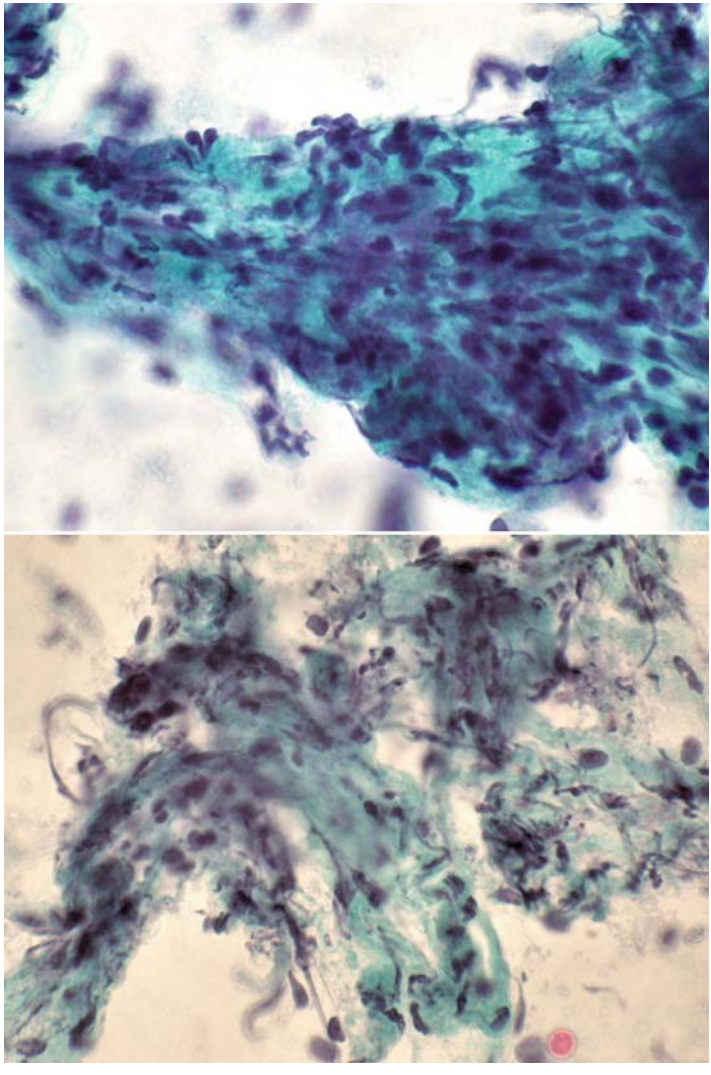

FIG. 5.33. Crushed, blue stromal debris of atrophic endometrium sometimes litters the background of the slide and appears "torn up" or "ratty." Pipes or columns of collagenous material may be seen.

(Fig. 5.34). Clustered large epithelial cysts lined by low cuboidal epithelium embedded in a fibrous stroma are interpreted to represent posthyperplastic ("Swiss cheese") atrophy (Figs. 5.35, 5.36). Giant histiocytes are often admixed with the benign endometrial epithelium of atrophic endometrium, and sometimes they are more numerous when there is endometrial epithelial activation, caused, for example, by sessile or pedunculated polyps or submucous fibroids (Fig. 5.37). Condensed spheroids of proteinaceous material resembling prostatic corpora amylacea may also occupy the cavitary spaces of otherwise inactive epithelial cysts and serve as a clue to their presence (Fig. 5.38).

Epithelial cell nuclei of atrophic endometrium are of variable size, but they are regularly distributed within epithelial sheets, generally as small islands of similar appearing nuclei. Nuclei vary from round, to wrinkled, to proliferative like, but they lack pseudostratification or increased mitoses (Fig. 5.39). The nuclear/cytoplasmic ratio is increased over secretory endometrium because the overall cell size is smaller. Nuclear size variability, especially over small domains within an epithelial sheet, may occasionally be substantial and afford a differential diagnosis that includes cytological EIN, endometrial gland dysplasia (EmGD), and, rarely, endometrial intraepithelial carcinoma (EIC) (Fig. 5.40). Because changes of this type reside in oftentimes senile, symplastic, nonproliferating epithelium, weak focal p53 nuclear decoration may be seen, but not concomitantly substantial proliferative reactivity, as demonstrable by Ki-67 labeling (Fig. 5.41). We speculate that changes of this sort may signify nothing, or they may represent a nonproliferative form of endometrial preneoplasia wherein the accumulation of further mutations might lead to the emergence of an estrogen-independent clone of epithelial cells of the type generally identified as EmGD/EIC.

In cytology preparations, atrophic endometrium is fully differentiated about 70% of the time. Endometrial glandular and stromal breakdown is the most common reason that atrophic endometrium is biopsied, and it shows significant numbers of stromal cells condensed and formed into compact nests with hyperchromatic nuclei and little or no cytoplasm. These structures are often associated with fragmented clusters of endometrial glands. This cytological pattern comprises a benign functional abnormality of the

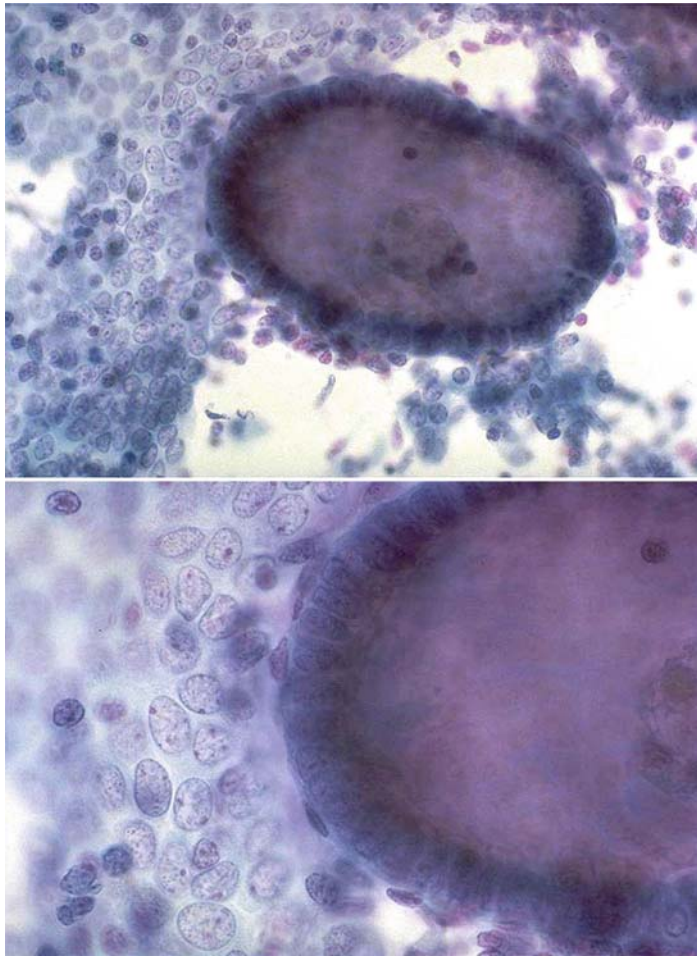

FIG. 5.34. This small inactive cystic gland shows tightly packed banal nuclei that are similar to the nuclei of the surface epithelium that is seen at high magnification in the left upper corner of the bottom image. The stromal component that is seen in the *right lower corner* of the top image appears sparse and breaks apart as small fibrous aggregates.

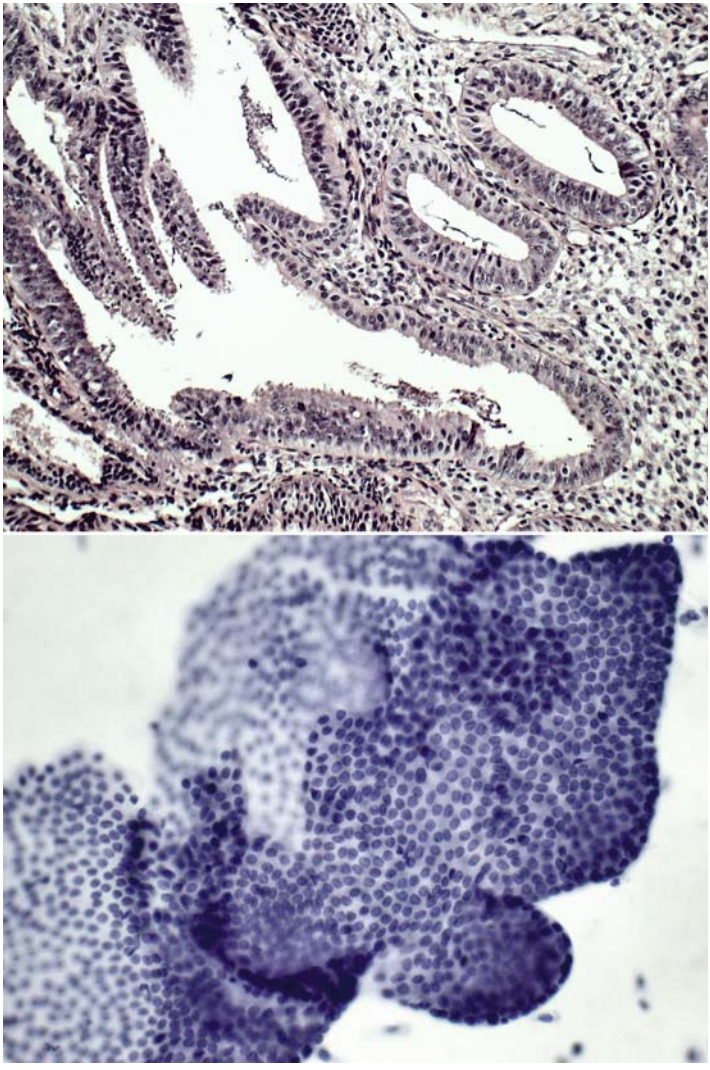

FIG. 5.35. Clustered large epithelial cysts lined by low cuboidal epithelium embedded in a fibrous stroma are interpreted to represent posthyperplastic ("Swiss cheese") atrophy. When these are admixed with smaller cystic glands, the endometrium, as with benign hyperplasia, takes on a "regularly irregular" appearance. The actual origin of Swiss cheese or cystic atrophy is uncertain, but, given the appearance of its cystic glands, it is not a far stretch to imagine that these glands represent the ghosts of benign hyperplasia that has finally "given up its estrogen."

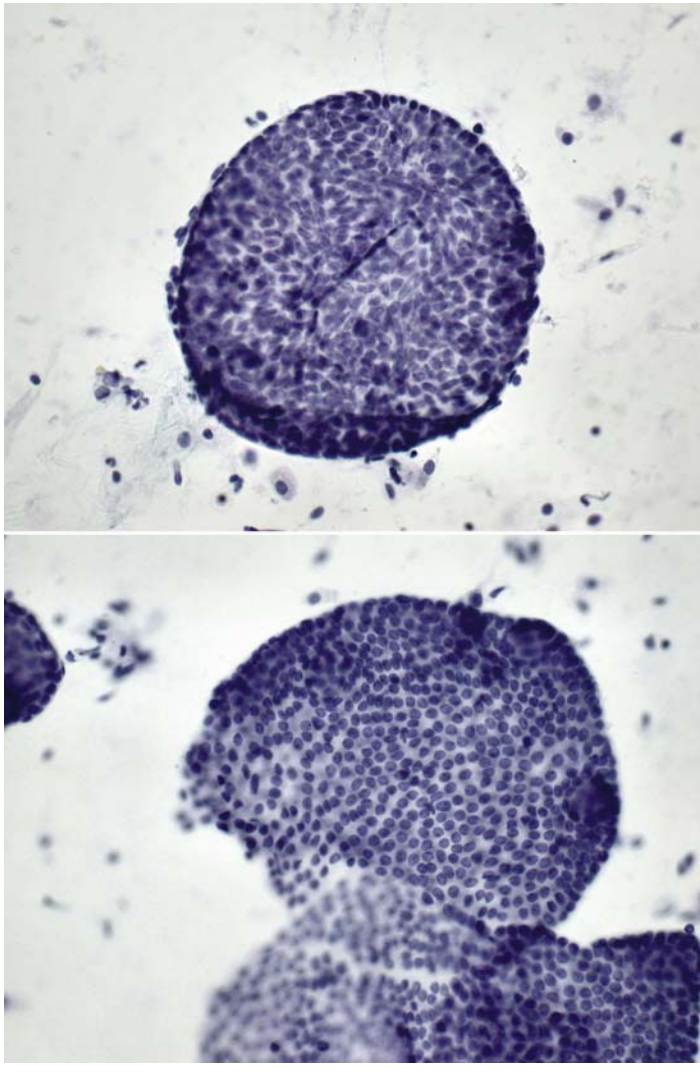

FIG. 5.36. Intermediate- (*top*) and large- (*bottom*) sized and irregularly outpouched cystic structures lined by inactive, atrophic-appearing epithelial cells are illustrated in these images of a woman with "Swiss cheese" endometrium. These findings do not carry with them any increased cancer risk, and, to avoid unnecessary follow-up studies, if there is no epithelial atypia, their presence should not be misinterpreted as benign hyperplasia. Similar changes, often with epithelial activation, can be seen with polyps, a situation seen in some women who have been treated with tamoxifen.

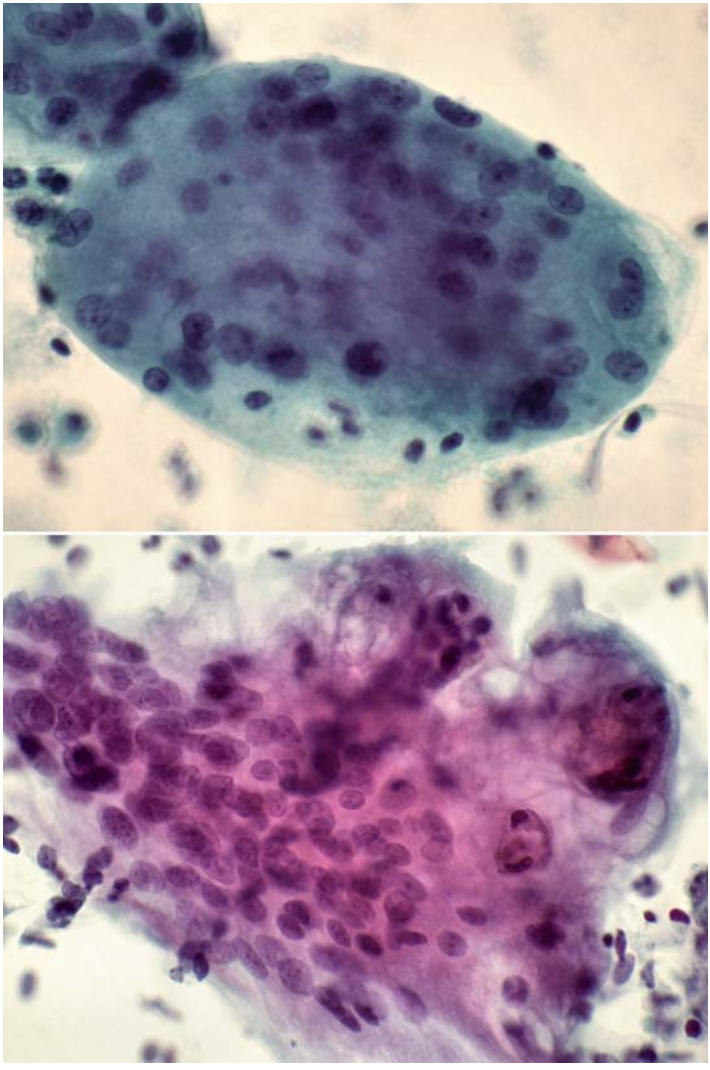

FIG. 5.37. Giant histiocytes are often admixed with the benign endometrial epithelium of atrophic endometrium; sometimes they are more numerous when there is endometrial epithelial activation, caused by, for example, sessile or pedunculated polyps, endometritis, or submucous fibroids. These giant histiocytes must live somewhere, and at times corresponding tissue sections shows them within the glands of atrophic endometrium. We do not take this finding as diagnostic of endometritis or as an indication to recommend further studies to search for malignancy.

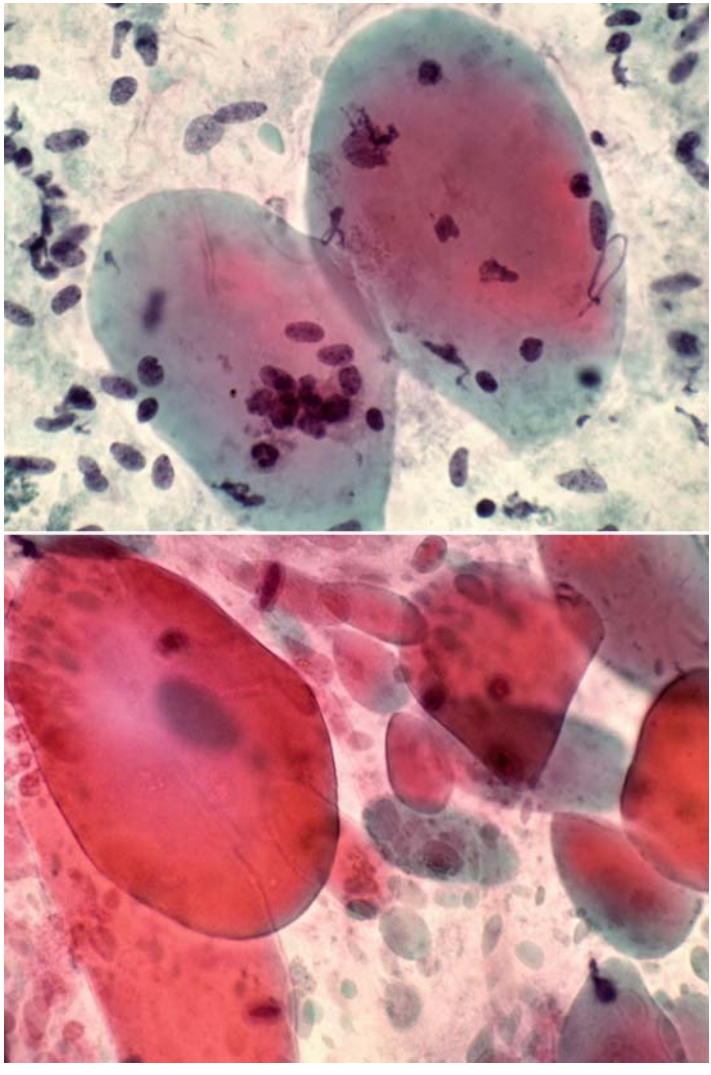

FIG. 5.38. Along with foamy and fused giant histiocytic cells, condensed spheroids of proteinaceous material, resembling prostatic corpora amylacea, may also occupy the cavitary spaces of otherwise inactive epithelial cysts and serve as a clue to the presence of these cysts.

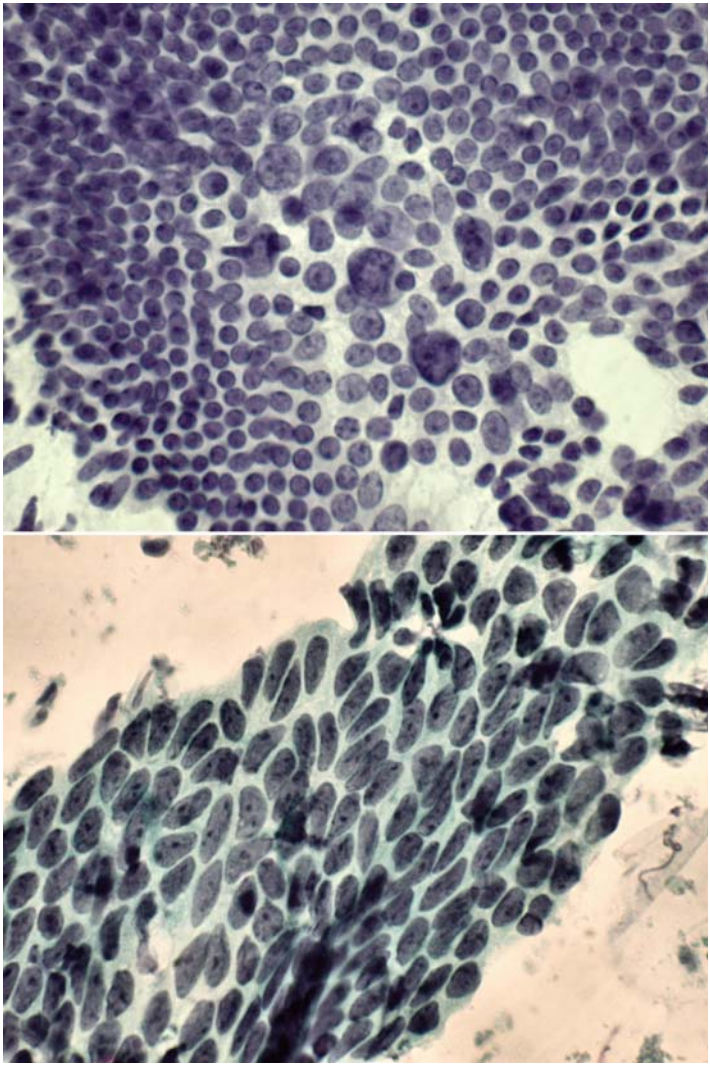

FIG. 5.39. Epithelial cell nuclei of atrophic endometrium may sometimes be of variable size, but they are regularly distributed within epithelial sheets, generally as small islands of similar-appearing nuclei. Nuclei vary from round, to wrinkled, to proliferative like, but, even though they appear odd, they lack pseudostratification or increased mitoses. In cases like this, we stain additional slides with anti-p53 and anti-Ki67 so as to not overlook the possibility that we are dealing with cytological endometrial intraepithelial neoplasm (EIN) or EIC/EmGD, both of which represent preneoplastic epithelial changes.

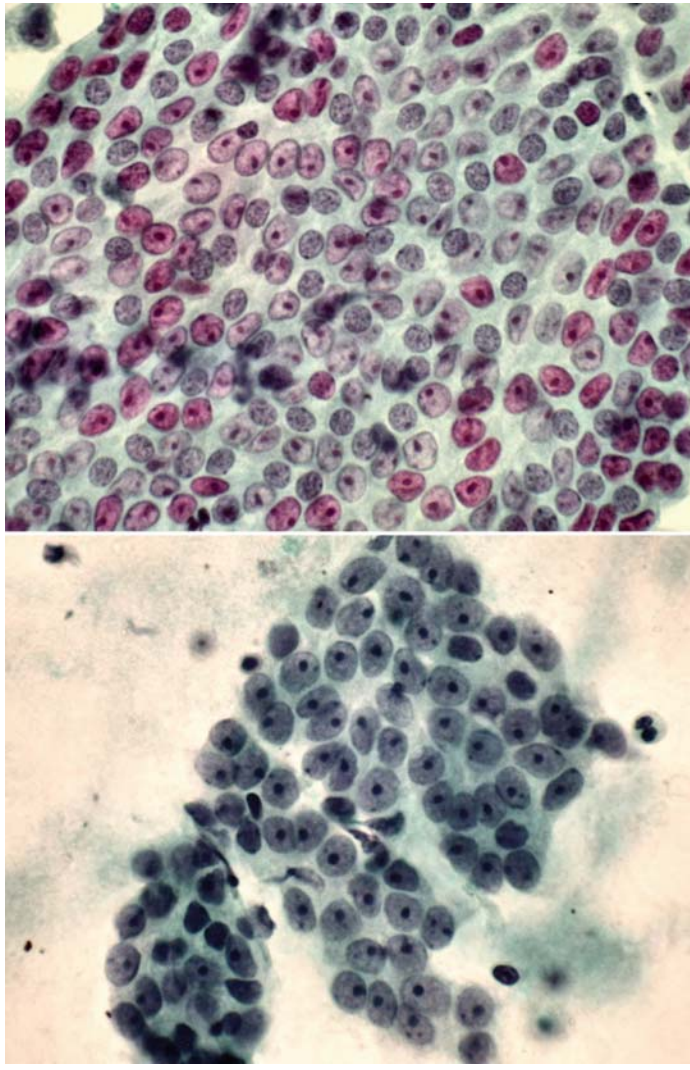

FIG. 5.40. Nuclear size variability and atypia, especially over small domains within an epithelial sheet, may occasionally be substantial and afford a differential diagnosis that includes cytological EIN, endometrial gland dysplasia (EmGD), and, rarely, endometrial intraepithelial carcinoma (EIC). Under such circumstances, Ki-67 and p53 immunostaining may be necessary to sort out the diagnosis and to suggest appropriate clinical responses to this finding. We believe that EIN may be responsive to progestin-containing intrauterine devices (IUD), but a diagnosis of EIC/EmGD indicates pre- or surface-serous carcinoma, and we advise that hysterectomy be considered as a therapeutic option.

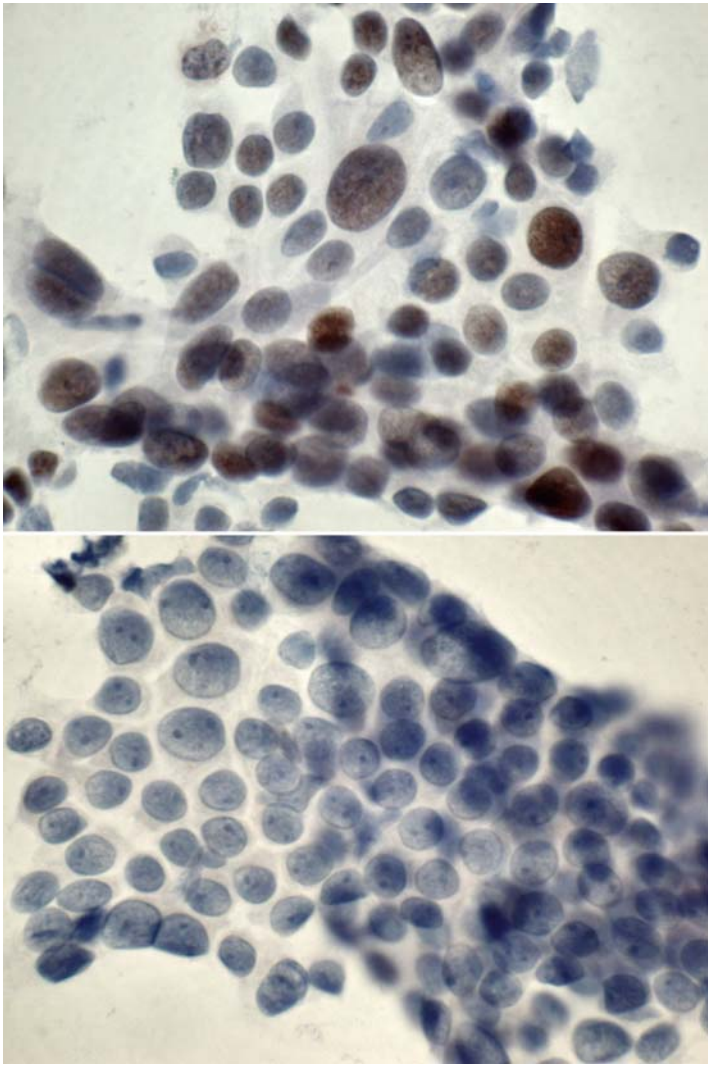

FIG. 5.41. Senile, symplastic, nonproliferating epithelium may show p53 nuclear decoration (*top*), and this may be disconcerting; however, concomitant proliferative activity is not demonstrated with Ki-67 staining (*bottom*); thus, a diagnosis of EIC/EmGD cannot be rendered. Whether this represents a benign oddity or a nonproliferative form of preneoplasia is unknown, but this phenomenon would certainly present a topic for further research into endometrial preneoplasia. For example, if cumulative defects of the p53 gene accumulate in such an epithelium, one could posit the eventual emergence of EIC in this setting.

endometrium. Sometimes, atrophic endometrium is mildly dysdifferentiated, showing epithelial cystic structures. Rarely, it may show unexplained nuclear anaplasia (symplastic nuclei) without evidence of upregulated epithelial proliferation (low to absent Ki-67 labeling index) but, now and again, with spotty weak to moderate p53 nuclear decoration.

Associated endometrial metaplasia is commonly encountered in patients with endometrial atrophy and dysfunctional uterine bleeding (DUB), and occasionally it may be difficult to distinguish these cases from cases of atypical endometrial hyperplasia or carcinoma. On occasion, associated metaplastic tissue shows overexpression of p53. For example, in one study, weak and heterogeneous p53 immunoreactivity was present in 10 of 22 (45%) papillary metaplasias, four of five (80%) tubal metaplasias, and four of seven (57%) eosinophilic metaplasias. The presence of weak and heterogeneous p53 immunoreactivity in metaplastic endometrium might be a consequence of DNA damage, but, in contrast with EIN and EmGD/EIN, it is unaccompanied by upregulated Ki-67.

In a study of 237 uteri in which we performed complete embedding and sectioning of the endometrium, 27 atypical cytology cases led to the discovery of 22 abnormal histology cases that comprised 6 cases of EIC/EmGD, 12 cases of "adenomatous change" comprising small foci architecturally resembling EIN, and 4 endometrioid microcancers, all measuring less than 2 mm. In 5 cases, nuclear anaplasia remained unexplained but lacked strong, confluent p53 nuclear decoration, thus excluding EIC/EmGD. "Cancer-like" nuclei can also occur when the specimen includes symplastic low uterine segment epithelium with enlarged, sometimes bizarre nuclei or when there is surface endometrial irritation as caused, for example, by polyp or leiomyoma.

Various changes accompany the administration of hormone replacements in the perimenopause and postmenopause, as shown by a cytological and histological study of 164 women receiving hormonal therapy, comprising 138 benign cases and 26 abnormal cases (24 cases with disordered proliferative phase and 2 cases with simple endometrial hyperplasia). Among benign cases, single hormone replacement showed atrophy or proliferative phase changes with the co-presence of ciliated cell metaplasia. Cyclical hormone replacement showed a biphasic response. The first half of

the administration term showed proliferative phase changes, and in the latter half secretory phase changes with curvilinear glands and subnuclear vacuolization occurred. Atrophy developed with continuous hormone replacement.

Endometrial atrophy may also occur in young women taking oral contraceptives or progestational agents for extended periods of time. With progestational agents, decidualized stroma accompanies glandular atrophy. This combination of findings defines the consequence of sustained progestin effect and comprises a unique form of glandular and stromal dissociation with atrophic glands and deciduoid to fibro-deciduoid stroma, often with numerous, prominent, thickened vessels whose diameter exceeds that of accompanying atrophic glands (see Figs. 5.17, 5.18).

Suggested Reading

Boon J, van de Putte SC, Scholten PC, Heintz AP. Histological patterns in endometrial samples from perimenopausal women. Maturitas 1999; 32: 155–9.

Dhingra N, Punia RS, Radotra A, Mohan H. Arias-Stella reaction in upper genital tract in pregnant and non-pregnant women: a study of 120 randomly selected cases. Arch Gynecol Obstet 2007; 276: 47–52.

Gellersen B, Brosens IA, Brosens JJ. Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. Semin Reprod Med 2007; 25: 445–53.

Harada T, Taniguchi F, Izawa M, Ohama Y, Takenaka Y, Tagashira Y, Ikeda A, Watanabe A, Iwabe T, Terakawa N. Apoptosis and endometriosis. Front Biosci 2007; 12: 3140–51.

Kaba S, Aoki T, Fukatsu T. Endometrial cytology in postmenopausal hormone replacement therapy. Diagn Cytopathol 1998; 19: 161–7.

Kashimura Y, Fukuda J, Toki N, Doki W, Kashimura M. Evaluation of pregnancy-related cells in endometrial cytology. Acta Cytol 2007; 51: 407–11.

Kitaya K, Yamaguchi T, Yasuo T, Okubo T, Honjo H. Post-ovulatory rise of endometrial CD16(–) natural killer cells: in situ proliferation of residual cells or selective recruitment from circulating peripheral blood? J Reprod Immunol 2007; 76: 45–53.

Maksem JA. Ciliated cell adenocarcinoma of the endometrium diagnosed by endometrial brush cytology and confirmed by hysterectomy: a case report detailing a highly efficient cytology collection and processing technique. Diagn Cytopathol 1997; 16: 78–82.

Mselle TF, Meadows SK, Eriksson M, Smith JM, Shen L, Wira CR, Sentman CL. Unique characteristics of NK cells throughout the human female reproductive tract. Clin Immunol 2007; 124: 69–76.

Norimatsu Y, Shimizu K, Kobayashi TK, Moriya T, Kaku T, Tsukayama C, Miyake Y, Ohno E. Endometrial glandular and stromal breakdown, part 2: cytomorphology of papillary metaplastic changes. Diagn Cytopathol 2006; 34: 665–9.

Quddus MR, Sung CJ, Zheng W, Lauchlan SC. p53 immunoreactivity in endometrial metaplasia with dysfunctional uterine bleeding. Histopathology (Oxf) 1999; 35: 44–9.

Sentman CL, Wira CR, Eriksson M. NK cell function in the human female reproductive tract. Am J Reprod Immunol 2007; 57: 108–15.

Shimizu K, Norimatsu Y, Kobayashi TK, Ogura S, Miyake Y, Ohno E, Sakurai T, Moriya T, Sakurai M. Endometrial glandular and stromal breakdown, part 1: cytomorphological appearance. Diagn Cytopathol 2006; 34: 609–13.

Stewart CJ, Campbell-Brown M, Critchley HO, Farquharson MA. Endometrial apoptosis in patients with dysfunctional uterine bleeding. Histopathology (Oxf) 1999; 34: 99–105.

Strassmann BI. The evolution of endometrial cycles and menstruation. Q Rev Biol 1996; 71: 181–220.

Strassmann BI. Menstrual cycling and breast cancer: an evolutionary perspective. J Womens Health 1999; 8: 193–202.

Szymanowski K. Apoptosis pattern in human endometrium in women with pelvic endometriosis. Eur J Obstet Gynecol Reprod Biol 2007; 132: 107–10.

Tabibzadeh S. Distinct subsets of stromal cells confined to unique microenvironments in human endometrium throughout the menstrual cycle. Am J Reprod Immunol 1991; 26: 5–10.

Tao LC. Cytopathology of the endometrium. Direct intrauterine sampling. In: Johnston WW, ed. ASCP Theory and Practice of Cytopathology, vol 2. ASCP Press, Chicago, IL, 1993.

Tao LC. Direct intrauterine sampling: the IUMC endometrial sampler. Diagn Cytopathol 1997; 17: 153–9.

Vassiliadou N, Bulmer JN. Functional studies of human decidua in spontaneous early pregnancy loss: effect of soluble factors and purified CD56+ lymphocytes on killing of natural killer and lymphokine-activated killer-sensitive targets. Biol Reprod 1998; 58: 982–7.

6
Benign Endometrial Abnormalities

Benign endometrial abnormalities entail those conditions in which the endometrium is neither physiologically cycling nor atrophic, but where changes in hormonal milieu, infection, or structural abnormalities of the stroma or myometrium lead to secondary morphological changes of the endometrium. Benign endometrial abnormalities imply those alterations of endometrial structure and function that have no intrinsic malignant potential. Our descriptions of benign endometrial abnormalities rest on 277 contemporaneously gathered hysterectomy-controlled cases and 535 office-based samplings (Table 6.1).

Hypermature Endometrium Comprising Disordered Proliferation and Nonatypical Hyperplasia

Benign endometrial hyperplasia, also referred to as nonatypical hyperplasia, comprises a functional class of diffuse estrogen-induced lesions exhibiting irregular remodeling of glands that is variably accompanied by vascular thrombi, stromal breakdown, and randomly scattered cytological changes. It is preceded by a phase of disordered proliferative endometrium, an intermediate step between normal proliferative and benign hyperplastic endometrium. Benign endometrial hyperplasia is most frequently encountered around the time of the menopause or postmenopause,

J.A. Maksem et al., *Endometrial Cytology with Tissue Correlations*, Essentials in Cytopathology 7, DOI: 10.1007/978-0-387-89910-7_7,

TABLE 6.1. Benign endometrial abnormality.

Diagnosis	Hysterectomy	Average Age (years)	Office Biopsy (% with cell block)	Average Age (years)
Disordered proliferative	150	45	144 (89%)	46
Disordered secretory			10 (80%)	45
Simple hyperplasia	60	52	45 (82%)	50
Complex hyperplasia	8	50	7 (86%)	49
Proliferative cystic			36 (47%)	52
Inactive cystic			132 (35%)	58
Noncyclical bleeding	15	47	143 (88%)	47
Polyp	44	48	3 (100%)	40
Endometritis			10 (30%)	54
Pyometra			4 (100%)	64
Hematometra			1 (0%)	80
Total	277		535	

when the normal cycle of sequentially regulated estrogen and progesterone is perturbed. It can also occur in young women and teenagers with anovulatory cycles. In a woman of childbearing age, there is often prolonged or excessive bleeding.

In an anovulatory cycle, the failure of ovulation leads to excessive and prolonged estrogen stimulation, with absence of the postovulatory surge in progesterone levels. This event results in a disordered proliferative endometrium. The spiral arterioles do not develop properly in the absence of progesterone. When estrogen levels decline, there is loss of stromal fluid causing collapse of the poorly developed spiral arterioles, vascular stasis, and thrombosis, and breakthrough bleeding occurs. In some cases, there is no drop in estrogen levels, and bleeding occurs because adequate blood supply to the proliferating endometrium cannot be maintained.

As the endometrial bulk increases through proliferation, the bleeding may become more frequent and almost continuous. Dilated and delicate superficial blood vessels undergo thrombosis, and this contributes to intermittent bleeding, for as the condition depends upon estrogen stimulation for its development, the fluctuating estrogenic support results in irregular episodic extensive apoptotic endometrial stromal cell death and tissue breakdown.

Disordered proliferative endometrium is an exaggeration of the normal proliferative phase; and, as such, much of the tissue is similar to that seen in normal proliferative endometrium. The pathognomonic

feature of persistent estrogen stimulation is architectural changes of individual glands distributed randomly throughout the entire hormonally responsive region of the endometrium (Fig. 6.1). The overall glandular: stromal ratio is not significantly increased from that of a normal proliferative phase. The changes involve the entire functional endometrial compartment, and are evident at low magnification as sac-like dilations and irregular glandular branching and reduplication randomly scattered amongst tubular glands (Fig. 6.2). The mitotically active glands resemble proliferative phase glands, composed of a pseudostratified epithelium. Nuclear shape and stromal characteristics resemble those of proliferative endometrium (Fig. 6.3), and gland density is low. Ciliated cell change of endometrial glandular cells is common and involves the glands and glandular saccules as well as the endometrial surface, reflecting the pivotal role of estrogen in the process. Characteristically, glands affected by ciliated cell differentiation are randomly interspersed among proliferative glands, and these glands may also demonstrate tubular, branching, or cystic architecture.

As with proliferative endometrium, nuclear pseudostratification and mitotic activity are present. In cytology preparations correlated to hysterectomy specimens, disordered endometrium appeared fully differentiated about 30% of the time, meaning that in about one-third of cases it was diagnosed as benign proliferative endometrium on the basis of cytology examination alone. Disordered endometrium is mildly dysdifferentiated and shows an accumulation of either completely or partly disrupted enlarged cystic or multipart, smoothly contoured, branched glands on the cytology slide (Fig. 6.2). Even straight proliferative glands may show up to threefold variation in gland diameter and demonstrate variability in diameter along the length of the gland. The correct diagnosis of disordered proliferation is generally augmented by evaluation of cell block preparations, which may be obtained in about 90% of cases.

Benign endometrial hyperplasia develops from disordered proliferative endometrium under the continued influence of unopposed estrogens. The entire endometrial compartment contains variable gland densities caused by remodeling of stroma and glands. It is the variability in gland density that, in histological preparations, sets off benign hyperplasia from disordered proliferative endometrium (Fig. 6.4). This feature is generally not appreciated in cytology preparations in the absence of cell blocks, which may be obtained in about 85% of cases.

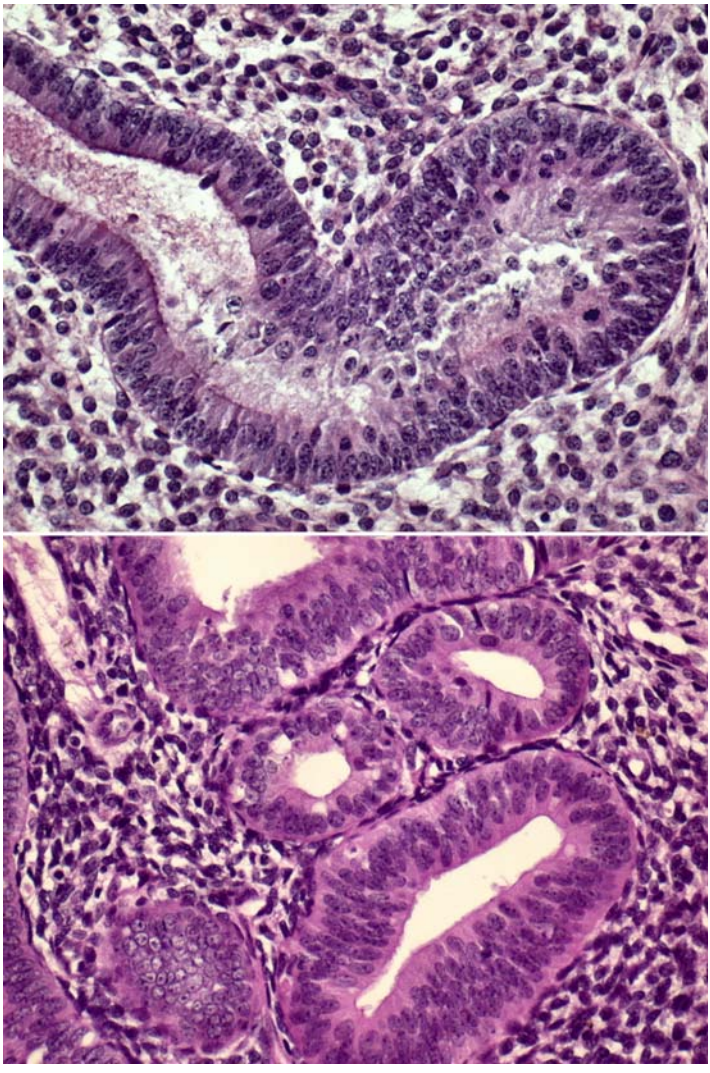

FIG. 6.1. Disordered proliferative endometrium is an exaggerated or hypermature version of normal proliferative endometrium, and, as such, much of the tissue is similar to that seen in normal proliferative endometrium (which is shown in the *top* image). The pathognomonic feature of persistent estrogen stimulation is architectural changes of individual glands distributed randomly throughout the entire hormonally responsive region of the endometrium (*bottom*). These changes express themselves as variability in gland size and in gland distribution.

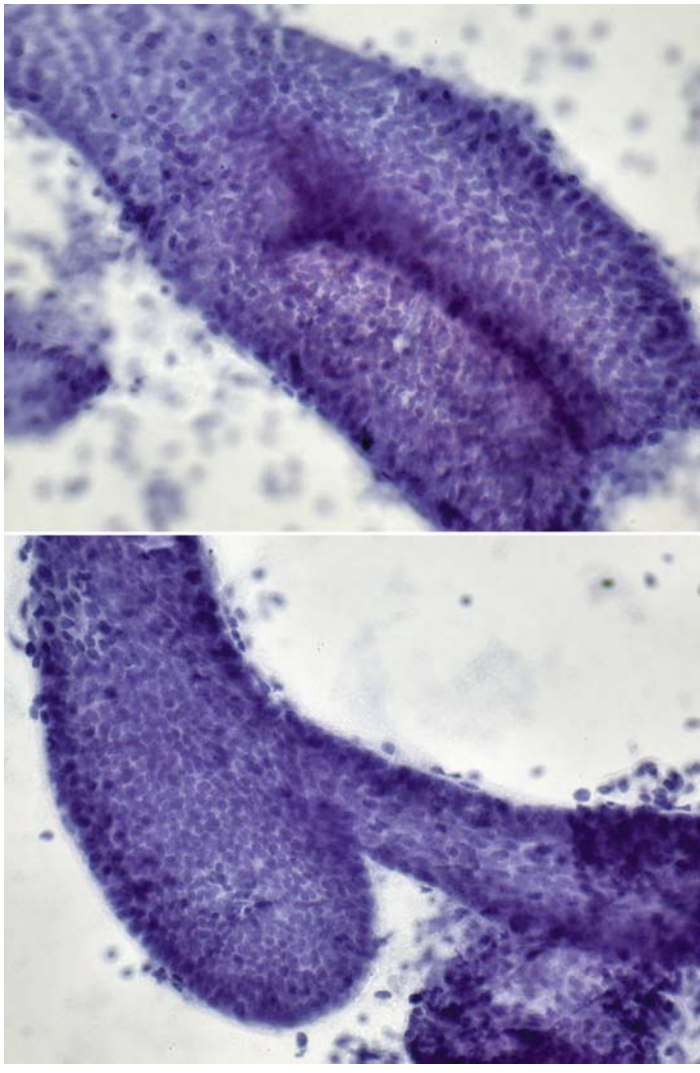

FIG. 6.2. The changes of disordered proliferation involve the entire functional endometrial compartment, and are evident, even in cytology brush preparations, at low magnification, where sac-like dilations and irregular glandular branching and reduplication are seen randomly scattered among tubular glands. These features are admixed with otherwise prototypical proliferative glands. As can be seen in these low-power images, the gland superstructure is altered but gland contours remain smooth and regular. Nuclear size appears relatively constant.

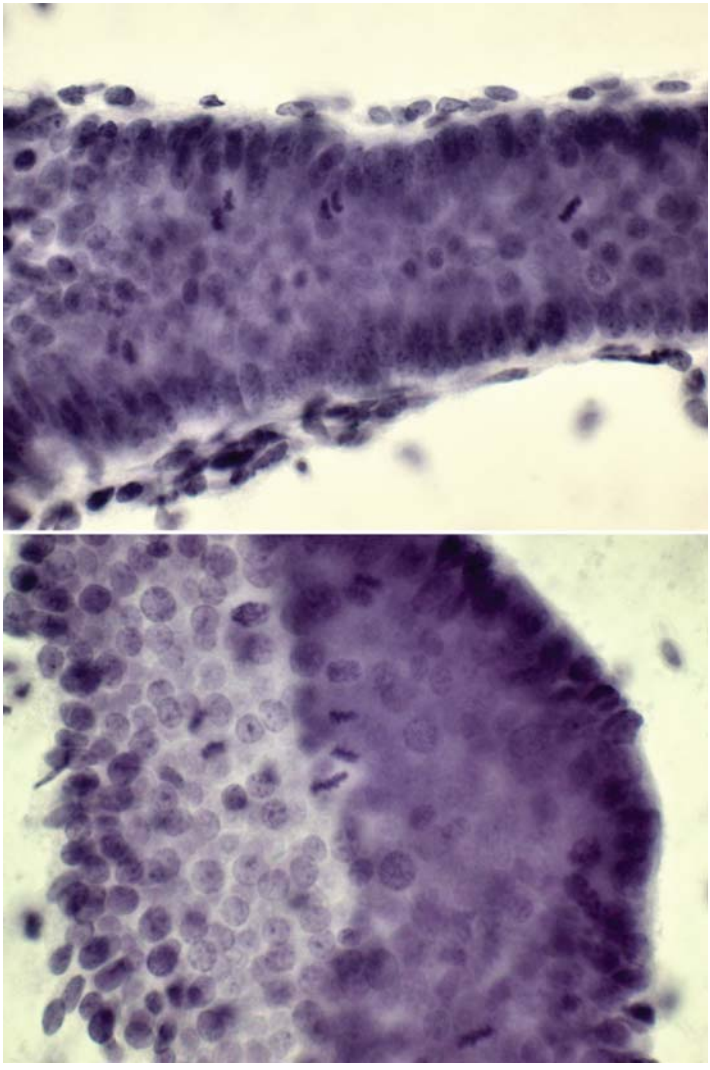

FIG. 6.3. The mitotically active glands of disordered proliferative endometrium are similar to proliferative phase glands, with which they are generally admixed (*top*); both components are composed of a simple pseudostratified epithelium. Nuclear shape and stromal characteristics of disordered proliferative endometrium as well as those of hyperplasia are generally similar to those of proliferative endometrium as well. Indeed, it is the time over which epithelial growth is allowed that distinguishes disordered proliferation and hyperplasia from normal proliferation; thus, hyperplasia can be thought of as "hypermature" proliferation (*bottom*).

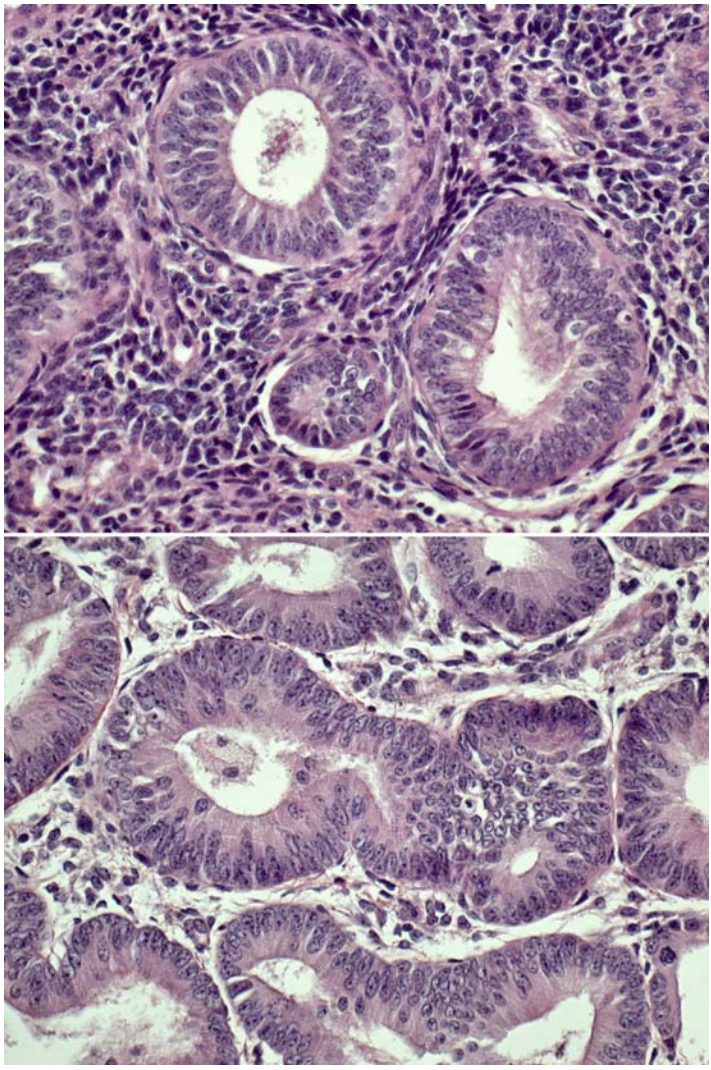

FIG. 6.4. Benign endometrial hyperplasia develops from disordered proliferative endometrium under the continued influence of unopposed estrogens. The entire endometrial compartment contains variable gland densities caused by remodeling of stroma and glands to the extent that in some areas the gland to stroma ratio exceeds 1 (*bottom*); it is the change in gland density that sets off benign hyperplasia from disordered proliferative endometrium.

Individual glands of benign endometrial hyperplasia may be tubular, cystic, or branching and are generally commingled, affording a "regularly irregular" pattern. Again, these features lay beyond the scope of cytological examination that is not augmented by cell block examination. The cytology of benign endometrial hyperplasia does not change between architecturally dispersed and crowded areas, and this speaks for the systemic hormonal etiology of the process that similarly exposes the entire endometrium (Figs. 6.5–6.7).

Absolute cytological presentation may change over time with the evolving hormonal state of the patient and with the superimposition of local factors such as breakdown and repair. During the established phase of active estrogen effect, glands are proliferative and interposed ciliated cell change is common, extending below the level of the endometrial surface, coming to involve both tubular and dilated cystic glands.

Sometime after the initiation of cystic gland dilation, exaggerated vascular dilation occurs and the endothelial lining of dilated superficial endometrial venules may be damaged, leading to luminal fibrin thrombosis (Fig. 6.8). In contrast to the short-lived venular thromboses that may be seen with menstruation, the thromboses of hyperplasia may persist and show evidence of recannalization, which speaks to the chronicity of the process. Unopposed estrogen states such as those related to benign endometrial hyperplasia are the most common setting in which fibrin thrombi are seen in the endometrium. Fibrin thrombi are rarely seen in architecturally normal late secretory endometrium, and there is no evidence that vascular thrombosis is a primary mechanism of cyclical synchronized menstrual shedding. Fibrin thrombi are often intimately associated with discrete areas of surrounding stromal breakdown, affording nonsynchronous endometrial breakdown that is clinically expressed as spotting and intermenstrual bleeding (Fig. 6.9).

When the estrogen level declines slowly, massive breakdown does not occur. As the glands become inactive, the endometrium retains the architectural features of thickness with altered gland architecture, but the glands demonstrate an inactive appearance. With prolonged low estrogen levels, endometrial bulk declines toward an atrophic pattern, sometimes with cysts, so-called "Swiss cheese" endometrium (see Figs. 5.35, 5.36).

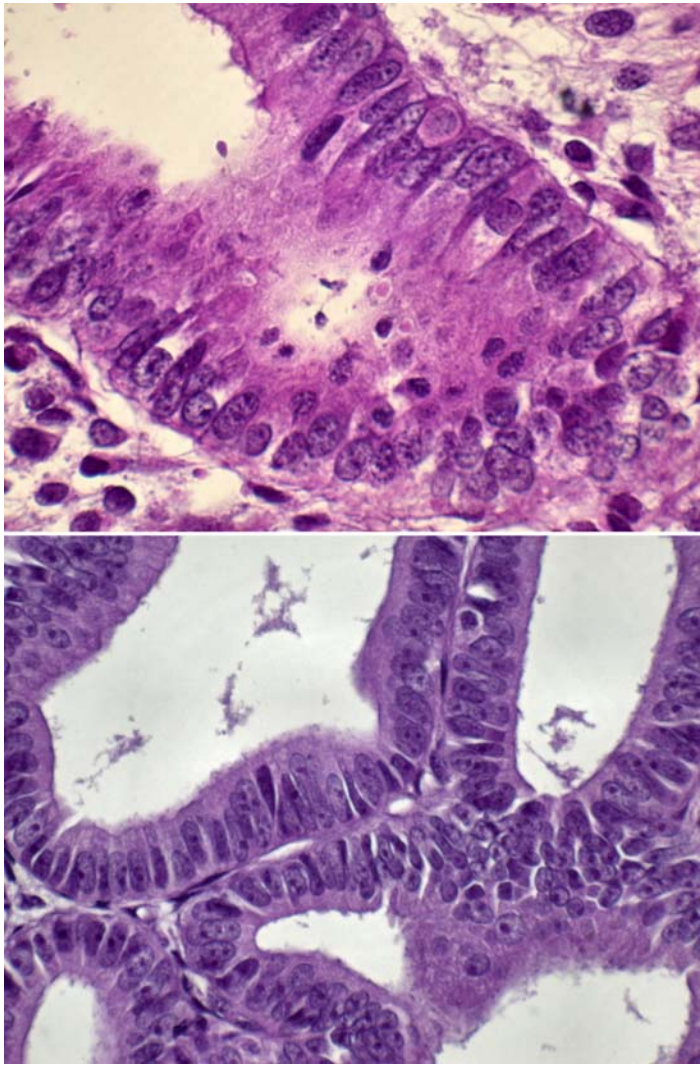

FIG. 6.5. The cytology of benign endometrial hyperplasia does not change between architecturally dispersed and crowded areas, and this speaks for the systemic hormonal etiology of the process that similarly exposes the entire endometrium. For example, in this case, the cellular morphology of tubular proliferative glands (*top*) and tightly packed hyperplastic glands (*bottom*) is essentially equivalent.

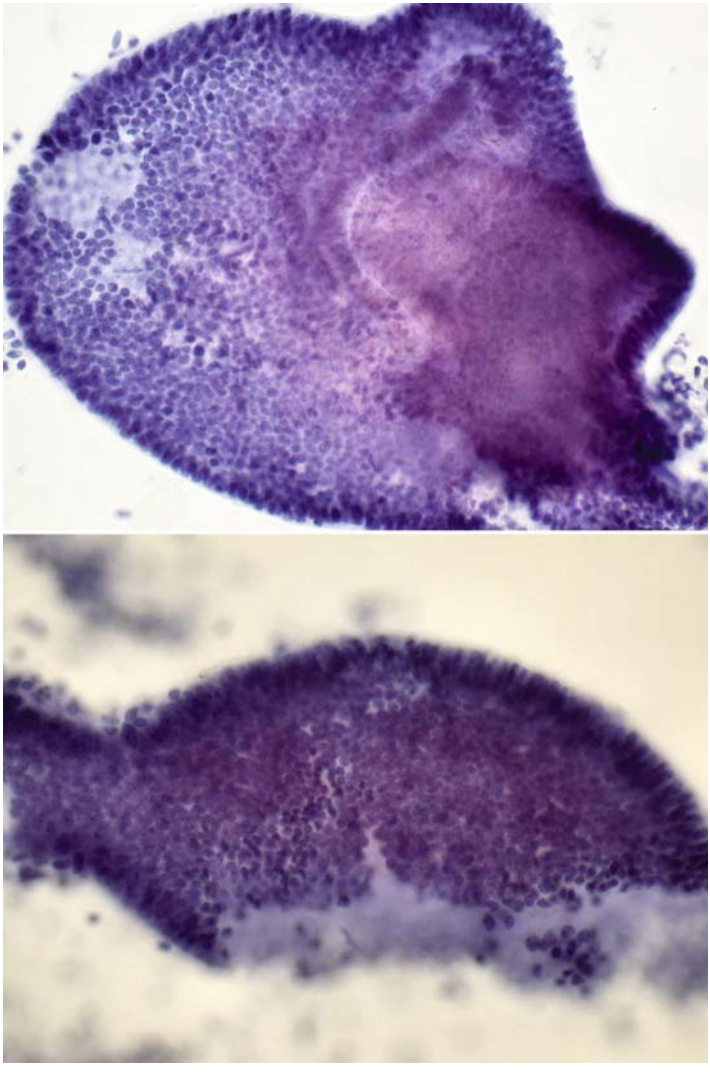

FIG. 6.6. Low-power views of brush cytology preparations from a case of hyperplasia show partly fractured cystic glands with cellular characteristics of otherwise benign proliferative endometrium. If one imagines these glands as variably sized glass balls with smooth contours; and, if one imagines the process of brush collection as equivalent to dropping a box of these balls; then, the products of the collection process can be thought of as the variably shattered remnants of smashed glass balls, with portions of their walls missing because of the traumatic force of the collection process.

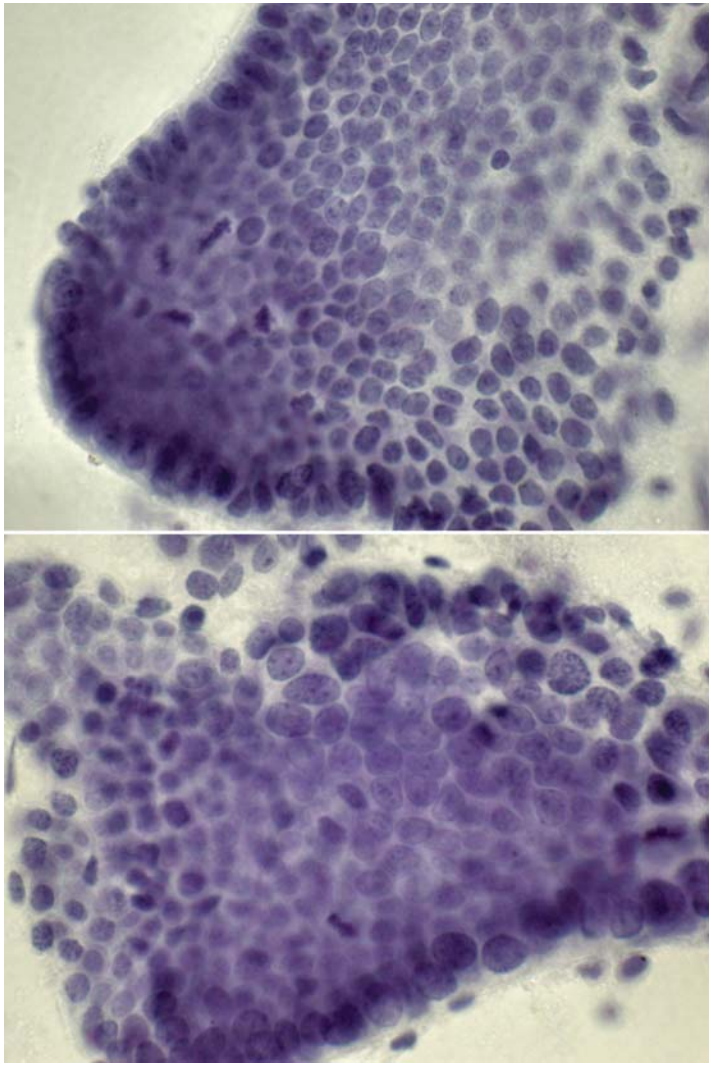

FIG. 6.7. High-power views of brush cytology preparations from a case of hyperplasia show partly fractured cystic glands with cellular characteristics seen in otherwise benign proliferative endometrium and disordered proliferative endometrium. If the diagnosis disordered proliferation/hyperplasia is limited to those cases whose nuclear features do not exceed changes generally associated with proliferative-phase endometrium, then these entities are slightly undercalled; but, this is not a problem, because they are physiological events—not preneoplastic lesions.

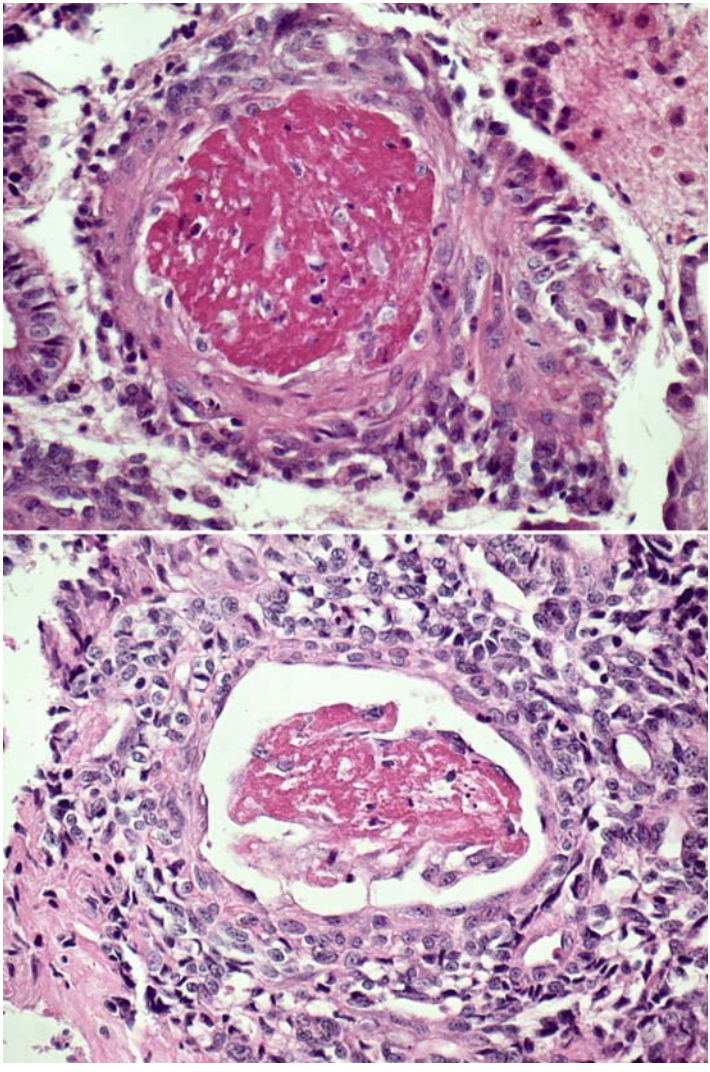

FIG. 6.8. Sometime after initiation of cystic gland dilation, exaggerated vascular dilation occurs and the endothelial lining of dilated superficial endometrial venules may be damaged leading to luminal fibrin thrombosis. What is unique about the microthrombi of dysfunctional uterine bleeding is that they may last long enough to reorganize before the process of vascular thrombosis leads to stromal–epithelial infarction and bleeding. This is not the case with menstrual endometrium, which is a programmed physiological event dependent more on apoptosis than on infarction.

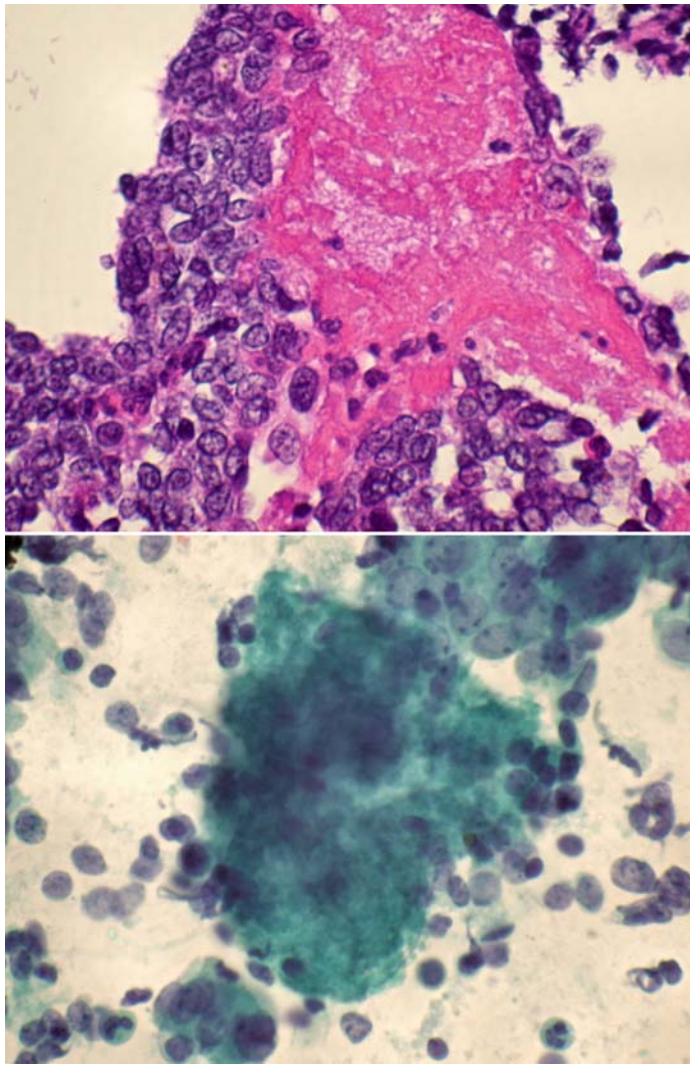

FIG. 6.9. Fibrin thrombi are often intimately associated with discrete areas of surrounding stromal breakdown, affording nonsynchronous endometrial breakdown that is clinically expressed as spotting and intermenstrual bleeding. The *upper image* is that of a stromal fibrin thrombus. The *lower image* shows a fibrin thrombus decorated by adherent stromal cells. Dysfunctional uterine bleeding derives from vascular injury and waning hormonal support for the endometrium. It comprises an admixture of ischemia and, to a lesser extent, apoptosis.

Histological preparations of women with dysfunctional uterine bleeding resulting from anovulation usually disclose endometrial glandular and stromal breakdown products similar to those seen in early menstrual endometrium (Fig. 6.10) and papillary syncytial metaplasia (Figs. 6.11–6.13), generally on the endometrial surface epithelium. Cytopathology slides show abnormal cell clumps composed of metaplastic cells that support irregular small projections. The metaplastic epithelial clumps seen with irregular protrusions and condensed stromal clusters in women with dysfunctional uterine bleeding originate from syncytial metaplasia. Chronic bleeding may eventuate in the accumulation of either hemosiderin-laden macrophages (Fig. 6.14) or stromal hemosiderin (Fig. 6.15).

Disordered endometrium rarely shows nuclear anaplasia, the exception being activated epithelial sheets that may be associated with epithelial irritation caused by polyp or leiomyoma. In our matched hysterectomy series, only 1 of 117 cases histologically diagnosed as disordered proliferative endometrium showed any significant degree of nuclear atypia in mildly dysdifferentiated epithelium. Disordered endometrium and nonatypical hyperplasia form a continuum of anatomic changes associated with endometrial hypermaturity and accounted for 79% of all benign atypia in our hysterectomy cases. Disordered endometrium, nonatypical hyperplasia, as well as other benign changes exhibiting the accumulation of cystic glands comprised 74% of all benign atypia in our office biopsy cases.

What distinguishes disordered proliferation and benign hyperplasia from benign proliferative endometrium is a dichotomy of gland size and structure. Thin, straight, tubular glands of varying diameter coexist with cystic, dilated, bulbous glands. Large, bulbous gland structures may be traumatically ruptured by brush collection and may present as broad cups or sheets of endometrial epithelium whose edges are raised up and appear out of the plane of focus. Estrogen-associated cellular metaplastic changes may occur within these bulbous structures, ciliated cell metaplasia being among the most common, and nuclear atypia may be seen. Coexisting noncyclical gland and stromal breakdown is common and may, at times, be florid, eventuating in syncytial change. Because syncytial change can give rise to clusters or tufts of surface epithelium, it may be mistaken for neoplasm. Sometimes, spurious ovulation or the administration

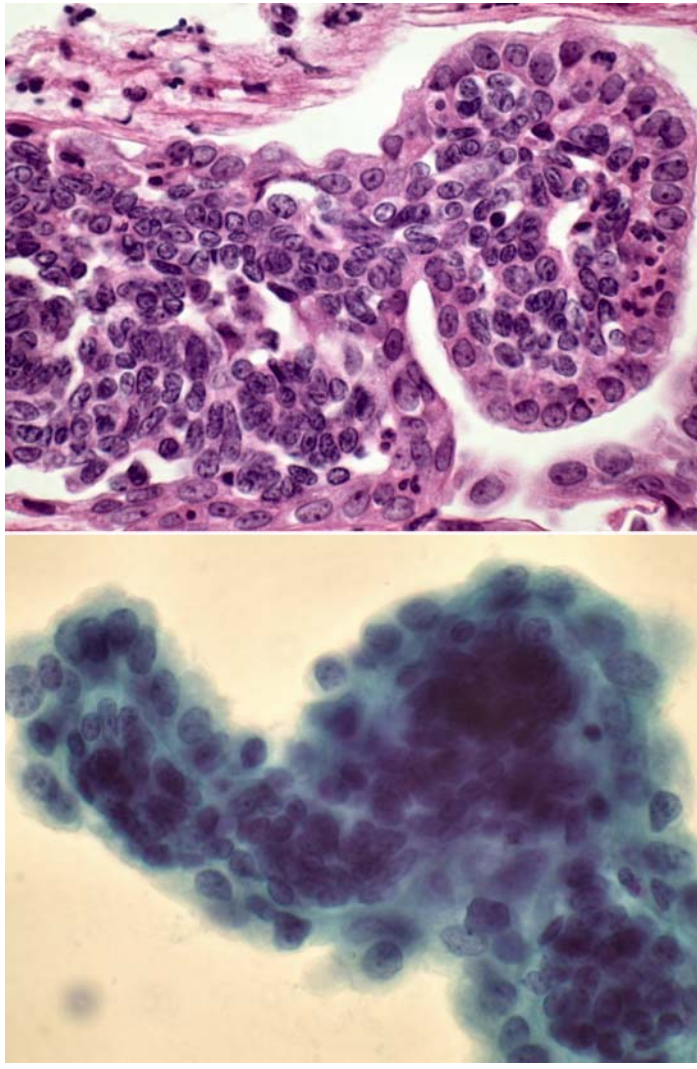

Fig. 6.10. Surface endometrial changes of dysfunctional uterine bleeding are not dissimilar from those seen with menstruation; however, they occur in the absence of cyclical bleeding. Stromal and epithelial cell aggregates with features of exodus balls are seen and may appear in gynecological cytology preparations of women with noncyclical endometrial breakdown and bleeding.

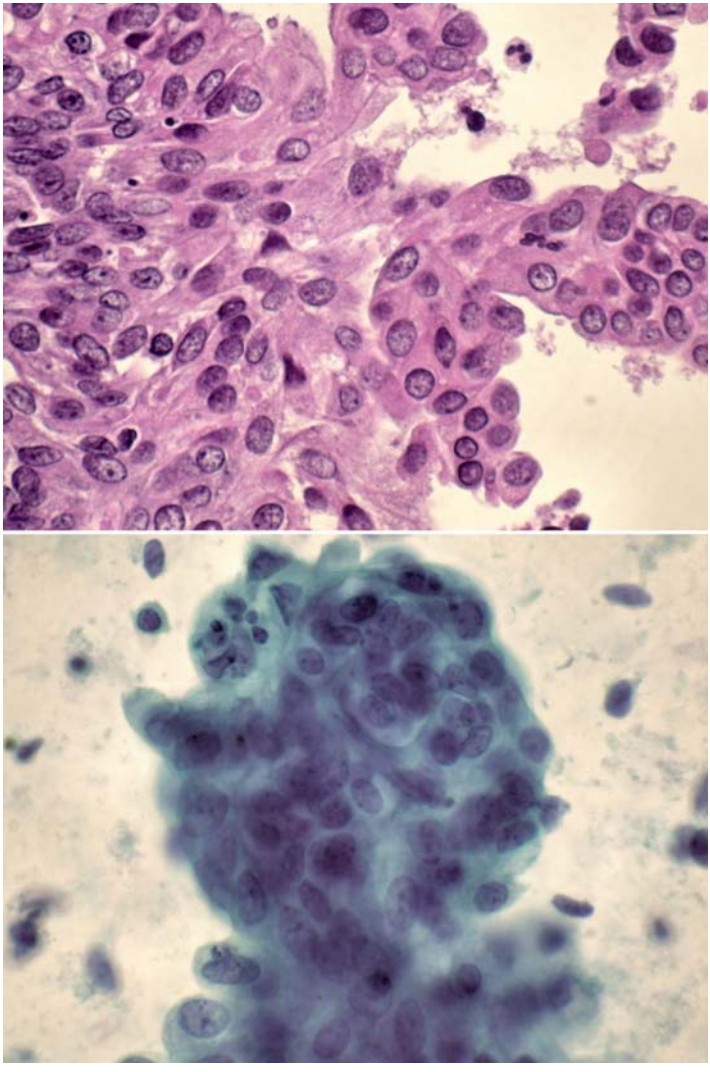

FIG. 6.11. Metaplastic, or perhaps more correctly retrogressive, epithelial clumps with irregular protrusions comprise the usual changes of papillary syncytial metaplasia, also known as syncytial metaplasia or change. The change is represented by sheet-like plaques of regenerating epithelial cells, often eosinophilic, without discrete cell boundaries. Nuclear debris, neutrophils, and rounded clumps of endometrial stromal cells usually are present; however, there are no true papillae with fibrovascular cores, and this is more a reparative process than a form of metaplasia.

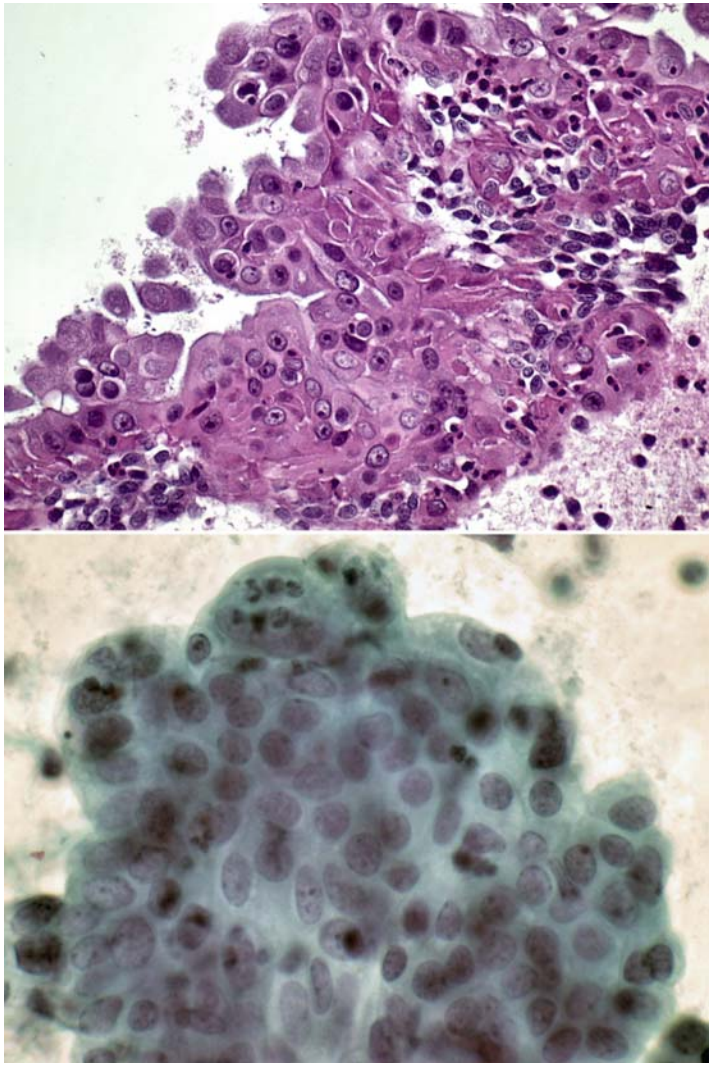

FIG. 6.12. Metaplastic epithelial clumps with irregular protrusions comprise the usual changes of papillary syncytial metaplasia. Sometimes, syncytial change presents as an overwhelming epithelial-proliferative phenomenon and can be mistaken for surface-proliferative changes that may be seen with endometrioid neoplasms. In contrast to neoplastic epithelium, the Ki-67 labeling index of syncytial change is low. The literature refers to its confusion with lesions such as serous carcinoma, but rarely if ever does syncytial change show nuclear features otherwise associated with type 2 cancers.

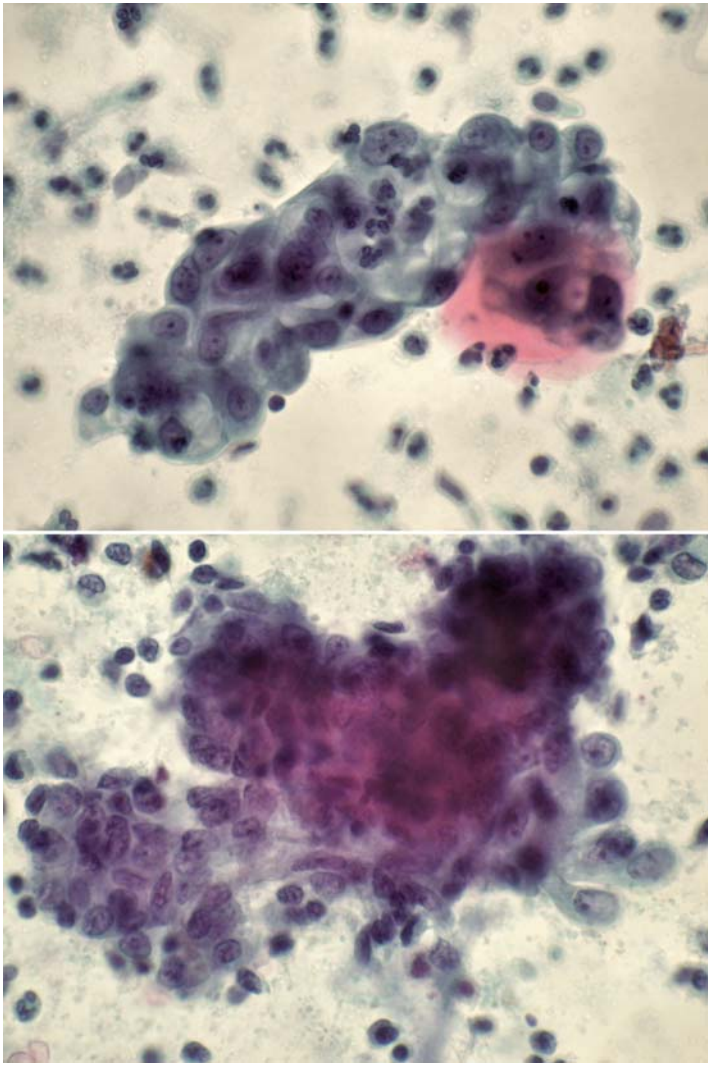

FIG. 6.13. Epithelial clumps with irregular protrusions comprise the usual changes of papillary syncytial metaplasia. These images illustrate how budded-off structures of syncytial change can be misinterpreted as the epithelial tufting of endometrioid neoplasms. In addition to dedifferentiation, these structures also may show clustered polymorphonuclear leukocytes (PMNs) and present in a dirty and bloody background. Both these images show grade 1 nuclei, and the *lower image* shows features of hobnailing at the epithelial tuft's 3 o'clock position.

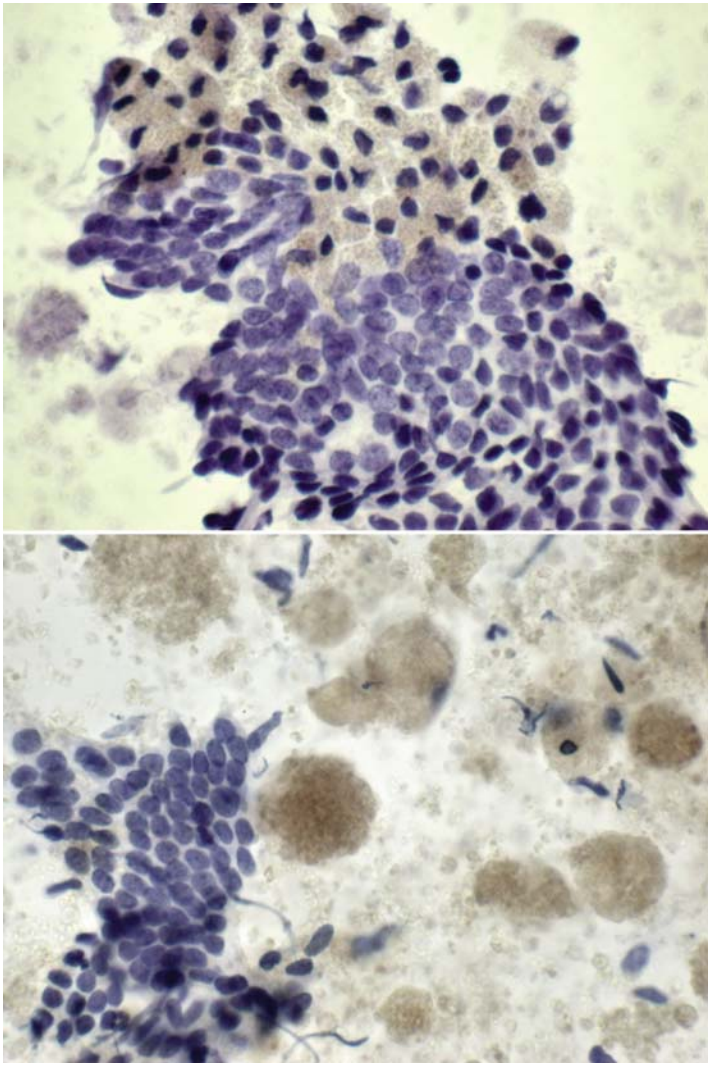

FIG. 6.14. In this case of protracted, chronic endometrial bleeding, foamy macrophages are laden with finely granular hemosiderin pigment. As can be surmised from the atrophic features of the endometrium, this change occurred in a postmenopausal woman who presented with a brown, watery discharge. Brown watery discharge may also be seen in women with endometrial intraepithelial cancer (EIC), again, probably caused by low-level chronic bleeding; a careful search of the slide for nuclear atypia is warranted in such cases. If malignancy is not discovered, another examination may be advisable.

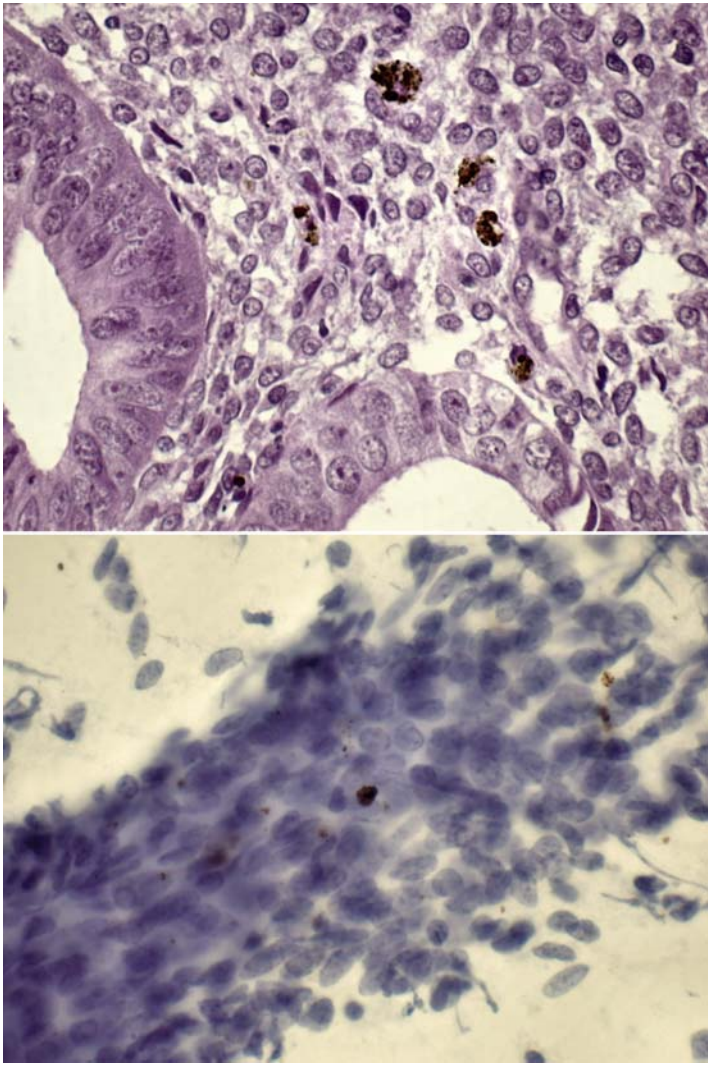

FIG. 6.15. With chronic, prolonged bleeding, hemosiderin granules may be found in the endometrial stroma, probably representing the deposits of long-dead hemosiderin-laden macrophages. Iron stains are not generally applied to endometrial biopsies, but they may be useful when assessing intermittent or prolonged bleeding. Rarely, stromal pigmentation may be caused by nevus cells.

of progestins may initiate the appearance of endometrial cycling, and the resultant "secretory" epithelium may appear disordered and show an admixture of smooth cystic glands and curvilinear tubular glands. Generally, gland/stromal dyssynchrony is seen.

The proliferative characteristics of benign hyperplasia vary, ranging from few to many mitoses and from sporadic to significant nuclear labeling with Ki-67. Variations depend on the woman's age and on her degree of estrogen stimulation. Cytologically, the continuity of structure that comprises the spectrum of disordered proliferation and endometrial hyperplasia conforms to a stable nuclear morphology and an intact epithelial polarity in the context of ever-increasing quantities of mildly dysdifferentiated glands that afford cystic epithelial glands, bulbous irregularities of glands, cup-shaped epithelial sheets, or smoothly contoured and/or multiply outpouched glands.

There is no useful cytological parameter that differentiates disordered proliferative endometrium from benign hyperplasia. This differentiation is generally accomplished by examining the cell blocks, which are available in about 85%–90% of cases. Although benign hyperplasia shows cellular aggregates with dilated glandular borders in cellular sheets along with multipart branching of tubuloglandular structures, no useful cytological parameter differentiates simple hyperplasia from what the World Health Organization (WHO) calls "complex" hyperplasia. Again, this differentiation generally requires examination of cell blocks.

Based on histopathologists' inability to consistently separate the spectrum of lesions ranging from disordered proliferation to complex hyperplasia using tissue biopsies, some investigators conclude that simple and complex hyperplasia should be combined into a single histological category, that is, nonatypical hyperplasia. Hyperplasia that is ascribed to extrinsic causes is generally a diffuse process, but morphological changes similar to those seen with hyperplasia can exist diffusely, focally, or within a sessile or pedunculated polyp, situations that are impossible to separate from one another in cytology preparations. Endometrial "hyperplasia," as currently diagnosed, includes, in the main, the changes caused by an abnormal hormonal state and, in addition, those changes caused by a separate category of monoclonal premalignant disease, endometrial intraepithelial neoplasm (EIN).

Benign endometrial hyperplasia involves the entire endometrial compartment and with protracted estrogen exposure shows the progressive development of cysts, remodeled glands, vascular thrombi, and stromal microinfarcts. These features are best construed as a sequence of changes whereby the appearance at any single time point is uniquely dependent on the preceding combination and the duration of hormonal exposures. In contrast, the premalignant clone of an EIN lesion is characteristically offset from the background endometrium by its altered cytology and crowded architecture. Therefore, the use of an internal standard for cytology assessment, combined with the distinctive topography of a clonal process, enables the histological diagnosis of EIN lesions. EIN lesions of at least 1 mm greatest dimension have a long-term cancer risk that is 45 fold greater than that of their benign endometrial hyperplasia counterparts. Histologically, the resolution of hormonal and premalignant subsets of traditional "endometrial hyperplasias" is possible, enabling patient therapy to be appropriately matched with the underlying disease mechanisms. Cytology, on the other hand, is limited by its inability to demonstrate endometrium that is set off from the background endometrium by crowded architecture.

Given this limitation, when the diagnosis of hyperplasia is rendered based on a cytology preparation that is not augmented by a cell block preparation, we offer a disclaimer indicating the spectrum of entities that present with similar cytological findings and make note of any cytological variability among sheets of cells, even if nuclei are not characteristically "malignant appearing." On the basis of our hysterectomy series, true benign hyperplasia was mildly dysdifferentiated (i.e., actually recognizable as hyperplasia and not as proliferative endometrium) in about 90% of cytology preparations. Therefore, by limiting the diagnosis of benign hyperplasia to those circumstances in which proliferative nuclei appear uniform in the face of smoothly expanded glands and cystic epithelial structures, benign hyperplasia becomes a slightly underdiagnosed but outspokenly benign entity. This is a conscious, cautious maneuver that prevents us from overlooking potentially serious lesions, especially in cytology preparations that are not augmented by cell block examination.

Nonatypical simple hyperplasia of the 1994 WHO schema morphometrically resembles disordered proliferative endometrium or

benign forms of hyperplasia (and not EIN) in more than 95% of cases, and in reality are those latter two entities. Architectural polarity is maintained and bulbous structures are admixed with attenuated glands and cell sheets that contain columnar cells. Some cases, especially hyperplastic polyps, may show nuclear anaplasia caused by epithelial surface irritation, and epithelial and stromal breakdown with ensuing papillary syncytial change; they may also show various forms of metaplasia. Endometrial stroma may resemble that of early proliferative, weakly proliferative or atrophic endometrium, varying from succulent to fibrous. Non-atypical complex hyperplasia shows more advanced gland complexity comprising, in addition to large cystic structures, multipart out-pouched and smooth-walled tubulocystic glands. By morpho-metric analysis, the majority of these cases are forms of benign hyperplasia and not EIN

Simple and complex hyperplasias as used in the WHO classi-fication are generally difficult to distinguish from one another or from disordered proliferative endometrium based on a cytology examination that is not augmented by cell block examination. In cases in which we are unsure of where, in the spectrum of estrogen-stimulated endometrial hypermaturity, the diagnosis lies we offer a differential diagnosis: "Benign-appearing endometrium exhibiting proliferative appearing glands with cystic features. The differential diagnosis of this finding includes disordered proliferation, benign hyperplasia, and early posthyperplastic atrophy. Of import, the cancer risk of this lesion is exceedingly low. Similar changes may be seen with benign endometrial polyp." In the case of epithelial changes in the more inactive range of the spectrum, as may be seen in samplings of endometrium from postmenopausal women, we offer the differential diagnosis: "Benign-appearing endometrium exhibiting inactive-appearing glands with cystic features. Although benign hyperplasia may present in this fashion, the differential diagnosis of this finding more commonly comprises posthyperplas-tic or cystic (Swiss cheese) atrophy. Similar changes may be seen with benign endometrial polyp."

Superimposition of progesterone upon a benign endometrial hyperplasia occurs in women with delayed ovulation, idiosyncratic corpus luteum development in the perimenopausal years, or thera-peutic administration of progestins following an extended follicu-

lar phase. Downregulation of estrogen receptors by progestins leads to a dominant progestational effect, regardless of the presence or absence of continued estrogen production. In this environment menstrual shedding is delayed, as progestins have the capacity to directly support the endometrium. Progesterone-related stromal and secretory glandular changes may then develop within the setting of irregular glands previously built up under the influence of estrogens. Although the causal event in benign hyperplasia is unopposed estrogen, the histological appearance at diagnosis may be greatly modified by intermittent or accompanying progestins leading, from time to time, to a condition best described by the term "secretory hyperplasia," not to be confused with the hypersecretory changes of the Arias–Stella reaction.

Endometritis

Inflammatory cells normally comprise 10%–20% of endometrial cells, and their nature varies with the phases of the menstrual cycle. Endometritis exhibits increased numbers and/or an abnormal distribution of endometrial inflammatory cells, frequently accompanied by morphological abnormalities of endometrial glands and stroma. Pathologists have traditionally classified endometritis as either acute or chronic. Acute endometritis shows the presence of microabscesses or neutrophils within the endometrial glands, whereas chronic endometritis discloses variable numbers of plasma cells within the endometrial stroma. Endometritis may lead to abnormal uterine bleeding, the symptoms of which antibiotic therapy may at times alleviate.

The glands are involved in endometritides. There may be an infiltrate of inflammatory cells, predominantly polymorphonuclear leukocytes (PMNs), affecting the epithelium of the glands, and the lumens and walls of the glands may contain pus (Figs. 6.16, 6.17). Then, as the process becomes more advanced, the glandular response to hormonal stimulation becomes impaired and the glands appear inactive. Once an endometrium shows signs of endometritis, precise dating is not possible. Along with an intraglandular accumulation of PMNs, inflammatory cells may be seen adherent to stromal vascular walls (Fig. 6.18, top) and within the stroma itself (Fig. 6.18, bottom). Also, plasma cells are easily identified.

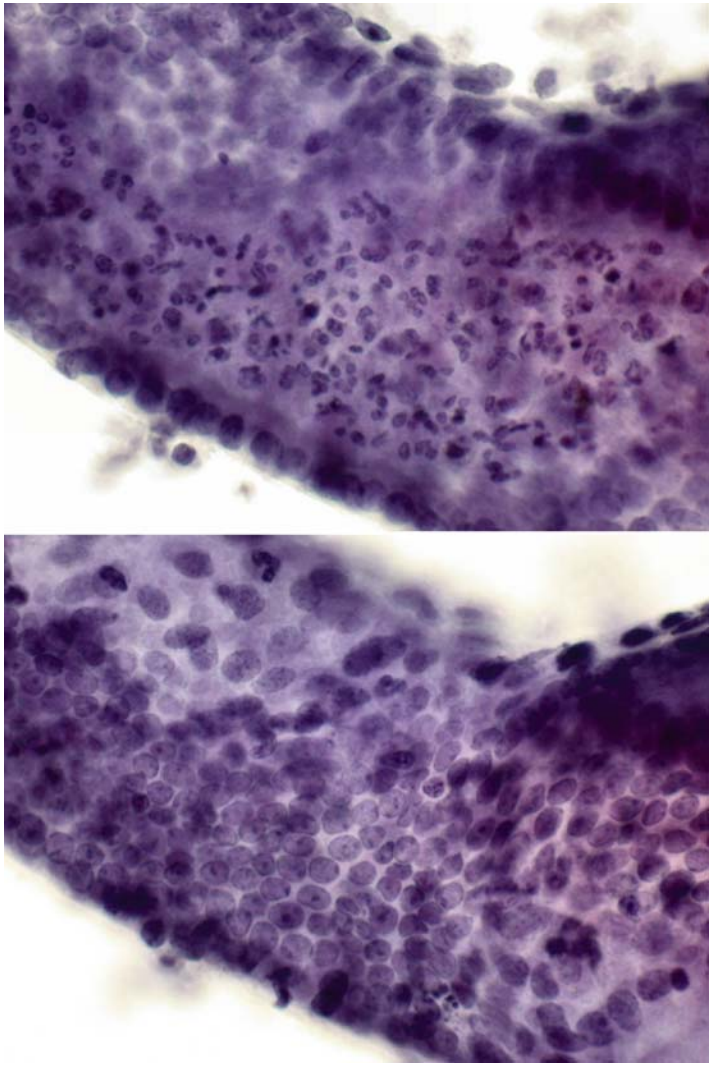

Fig. 6.16. Early on, endometritis may show an infiltrate of inflammatory cells, predominantly neutrophils, affecting the epithelium of the glands, and the glandular lumens may contain pus. In this image, an accumulation of PMNs is seen within the gland lumen; and, as the plane of focus is changed, the gland walls are spared significant inflammatory cell infiltration.

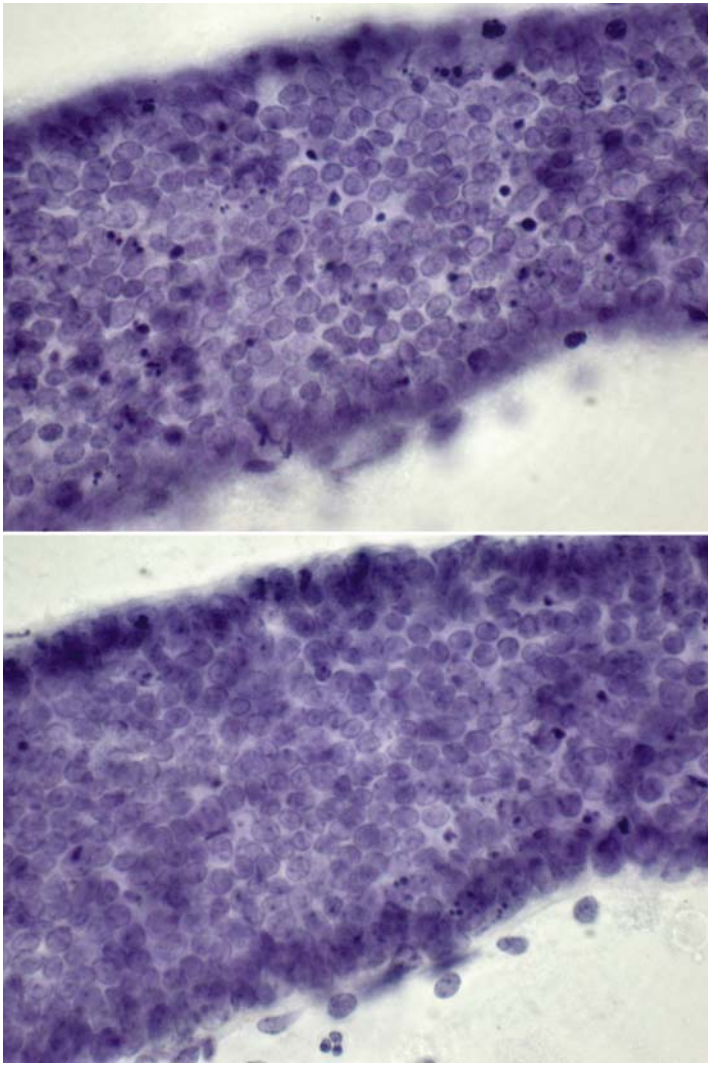

FIG. 6.17. Early on, endometritis may show an infiltrate of inflammatory cells, predominantly neutrophils, affecting the epithelium of the gland, and the lumens may contain pus. In this image, PMNs are seen within the gland wall epithelium. The presence of glandular PMNs is not restricted to endometritis; it may be seen in the course of normal menstruation and with noncyclical bleeding.

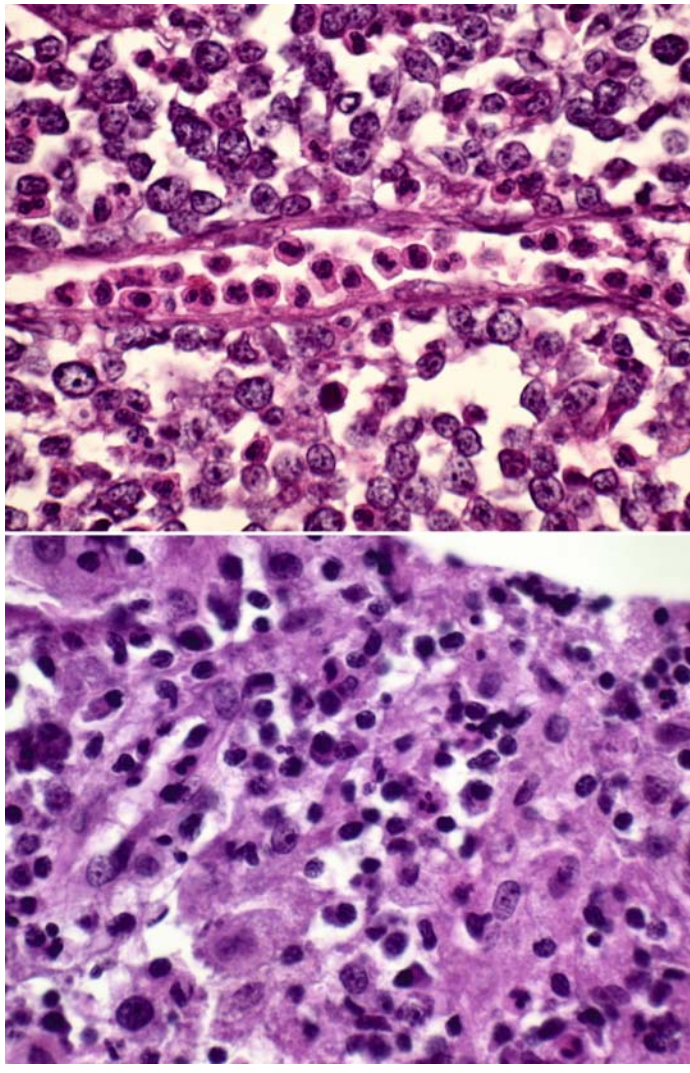

FIG. 6.18. Along with the intraglandular accumulation of PMNs, inflammatory cells may be seen adherent to stromal vascular walls (*top*) and within the stroma itself, in the absence of a deciduoid stromal response as usually accompanies physiological menstruation (*bottom*). Heightened vascular adherence of inflammatory cells is an inflammation-associated phenomenon. Changes of this nature are most common in acute infectious or postabortion endometrial lesions; although, in this case, plasma cells are seen in the *bottom image*.

Although the presence of plasma cells is generally required to diagnose chronic endometritis, contrary to popular opinion, the presence of plasma cells is not specific for the diagnosis. To serve as relevant signposts, plasma cells should accompany other features of endometritis, especially loss of physiological cycling and a fibro-deciduoid stromal response (Figs. 6.19–6.22).

In addition to endometritis, plasma cells may be seen in the endometrium of women with dysfunctional uterine bleeding and focal stromal breakdown. For example, in 61 benign endometrial biopsies described as having disordered/anovulatory patterns with or without stromal breakdown that were compared to control samples of unremarkable proliferative endometrium, the majority of disordered proliferative endometria had plasma cells. Also, two-thirds of proliferative endometrium with breakdown showed plasma cells. Plasma cells were rare in inactive endometrium and seen in only 18% of unremarkable proliferative endometrium. Given the lack of clinical evidence for infection in any of these situations, inflammation with identifiable plasma cells may often represent a physiological, as opposed to infectious, process. Plasma cells may be identified in endometrial material using the methyl green pyronin stain or antibodies to syndecin-1 (CD 138).

More than 70% of chronic endometritis cases result from non-gonococcal, nonchlamydial infections. The common bacteria along with *Mycoplasma* are the most frequent etiological agents of chronic endometritis. Vaginal cultures show poor concordance with endometrial cultures. The Tao brush is very well suited for obtaining direct endometrial cultures in a sterile fashion, and it also may be used to obtain tissue for microbial identification using polymerase chain reaction (PCR) techniques.

In our experience, the most common type of endometritis that we see in cytological preparations is a nodular histiocytic type exhibiting aggregates of histiocytic cells admixed with other inflammatory cells including eosinophils, plasmacytoid lymphocytes, and plasma cells (Figs. 6.23–6.25). Occasionally, histiocytic aggregates require differentiation from neoplasms, including renal cell carcinoma and signet ring cell carcinoma. For example, one of five patients reported whose routine endometrial biopsy specimen contained prominent signet ring cells resembling adenocarcinoma reacted with Mac387 and CD68, indicating they were of histiocytic origin.

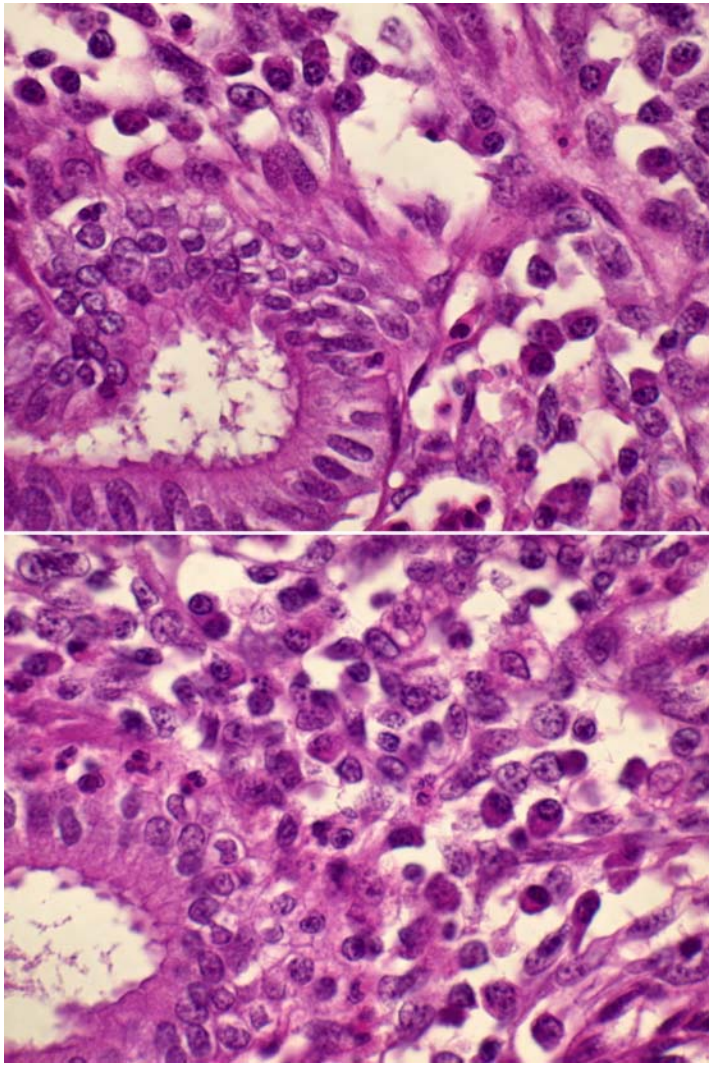

Fig. 6.19. The plasma cells of chronic endometritis generally are seen amidst other features of endometritis, especially loss of physiological cycling and a fibro-deciduoid stromal response. In this case, gland-wall PMNs are seen along with increased stromal plasma cells in the absence of stromal deciduoid response.

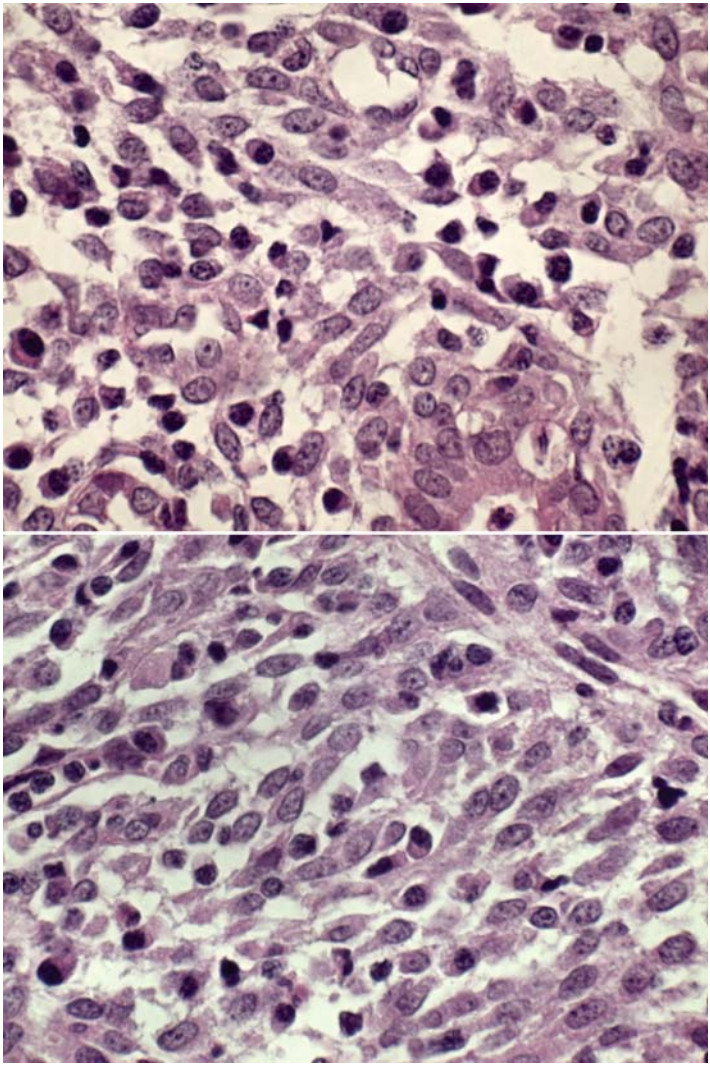

FIG. 6.20. The plasma cells of chronic endometritis generally are seen amidst other features of endometritis, especially loss of physiological cycling and a fibro-deciduoid stromal response. Here, the stroma appears fibrous, and numerous plasma cells lie between the fibroblastic-like stromal cells.

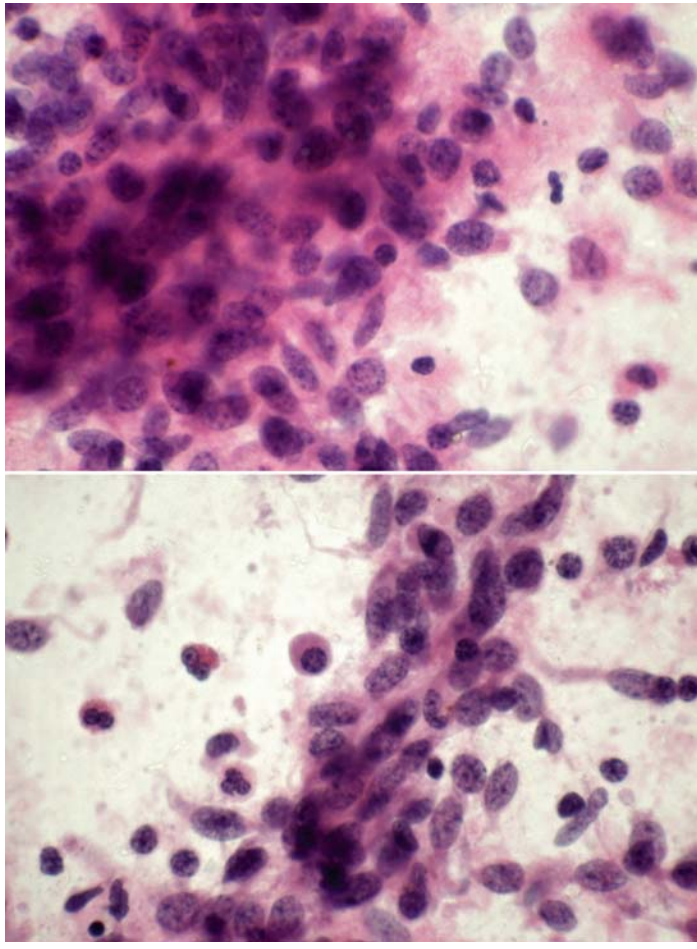

FIG. 6.21. In this brush cytology preparation of chronic endometritis, plasma cells are seen both within (*top*) and without (*bottom*) stromal fragments. Also note the bilobed eosinophils that are seen in the *bottom image*. If eosinophils are seen in anything but a late postovulatory endometrium, look for plasma cells as well.

Other commonly encountered forms of endometritis include intrauterine device (IUD)-associated granulomatous endometritis that may be accompanied by marked epithelial surface activation including

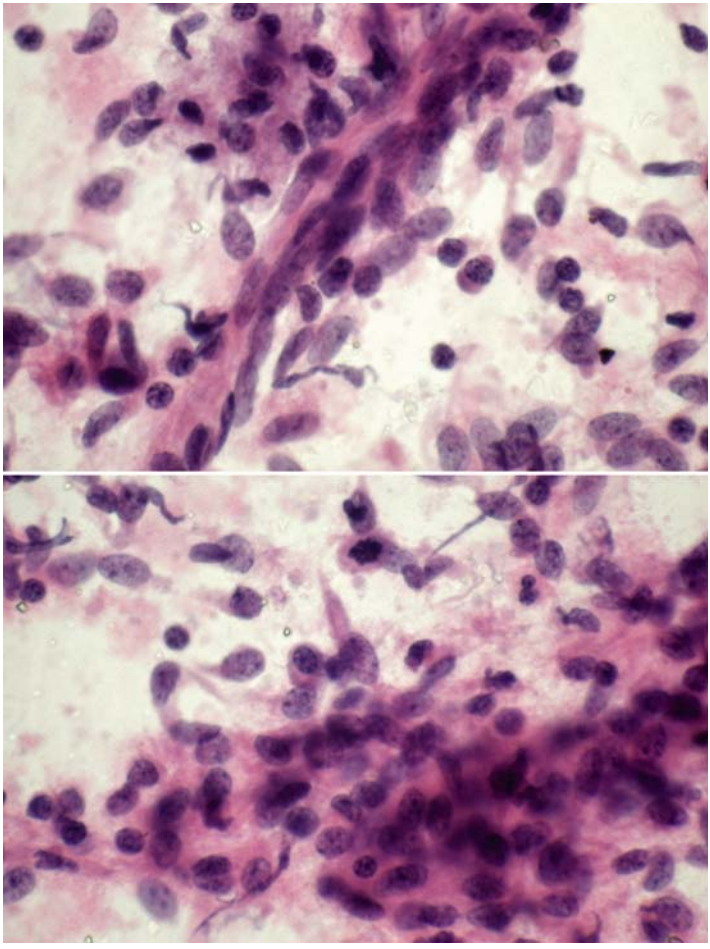

FIG. 6.22. These images from brush cytology preparations of chronic endometritis again show scatterings of plasma cells amidst an otherwise fibrous stroma. Plasma cells may be difficult to find in endometrial brush preparations; and, if they are suspected as being present, anti-CD138 immunostaining is a useful adjunct methodology.

syncytial change and hobnail metaplastic change (Figs. 6.26–6.28) and the presence of frequent lymphoid aggregates interpreted by some as nodular lymphoid endometritis (Fig. 6.29). Extranodal mucosa-associated lymphoid tissue (MALT) lymphoma of the endometrium is

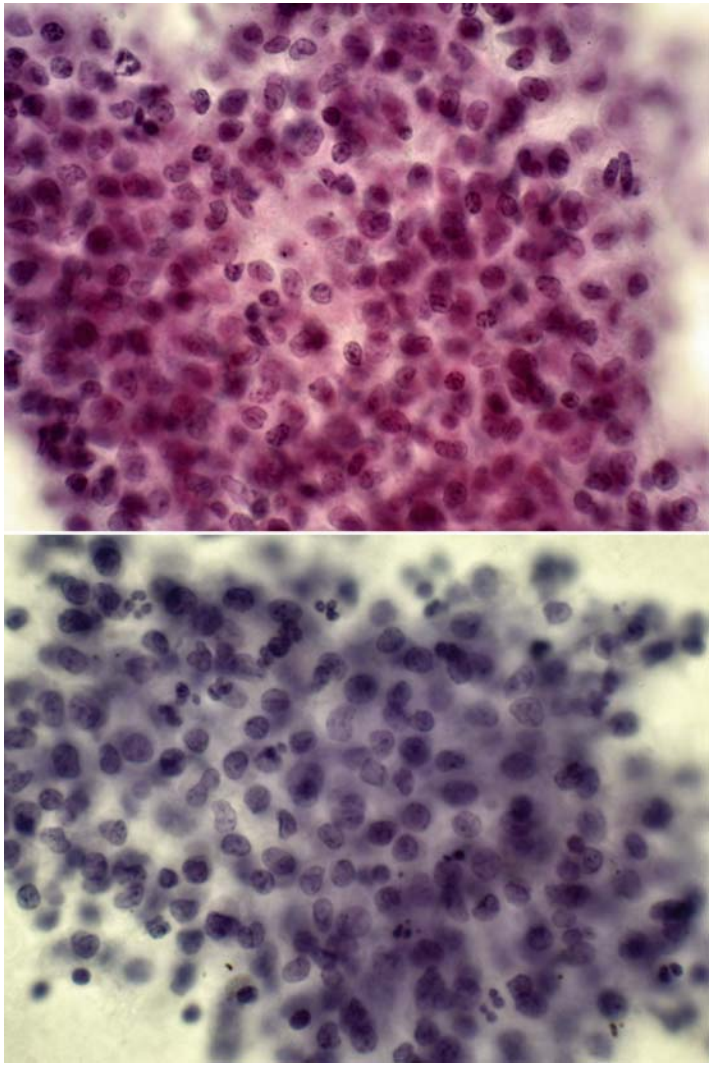

FIG. 6.23. The most common type of endometritis that we can identify in cytological preparations is an often nodular histiocytic type exhibiting aggregates of histiocytic cells admixed with other inflammatory cells including eosinophils, plasmacytoid lymphocytes, and plasma cells.

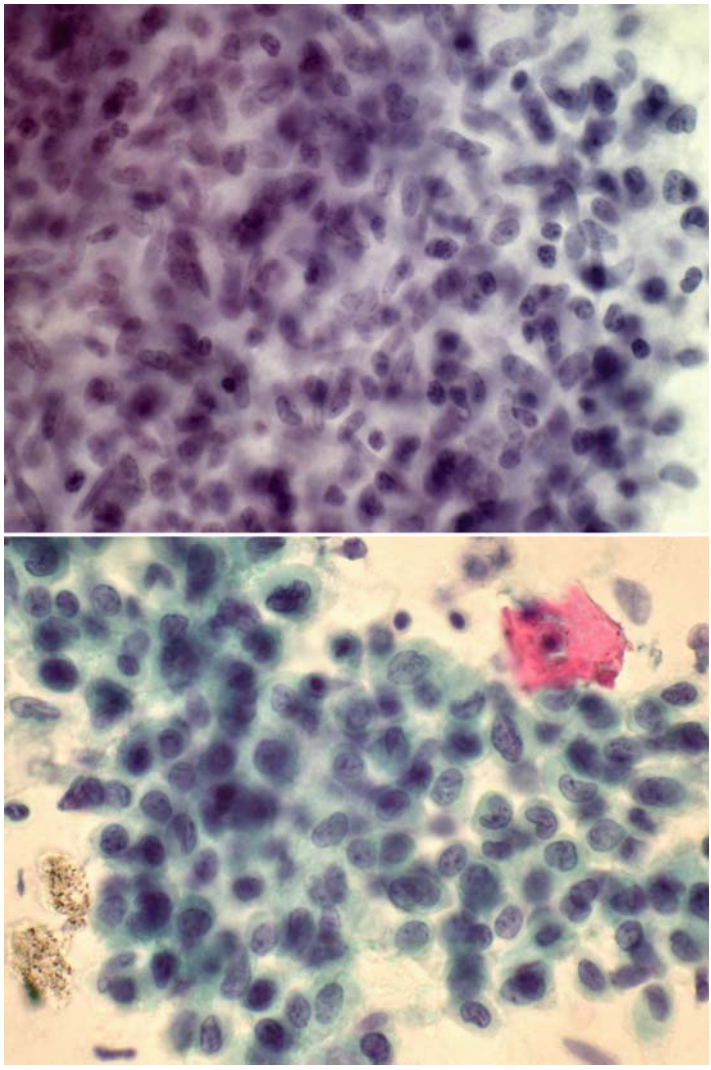

FIG. 6.24. The most common type of endometritis that we can identify in cytological preparations is an often nodular histiocytic type exhibiting aggregates of histiocytic cells admixed with other inflammatory cells including eosinophils, plasmacytoid lymphocytes, and plasma cells. In these images, histiocytes show either fibrocytic (*top*) or epithelioid (*bottom*) characteristics.

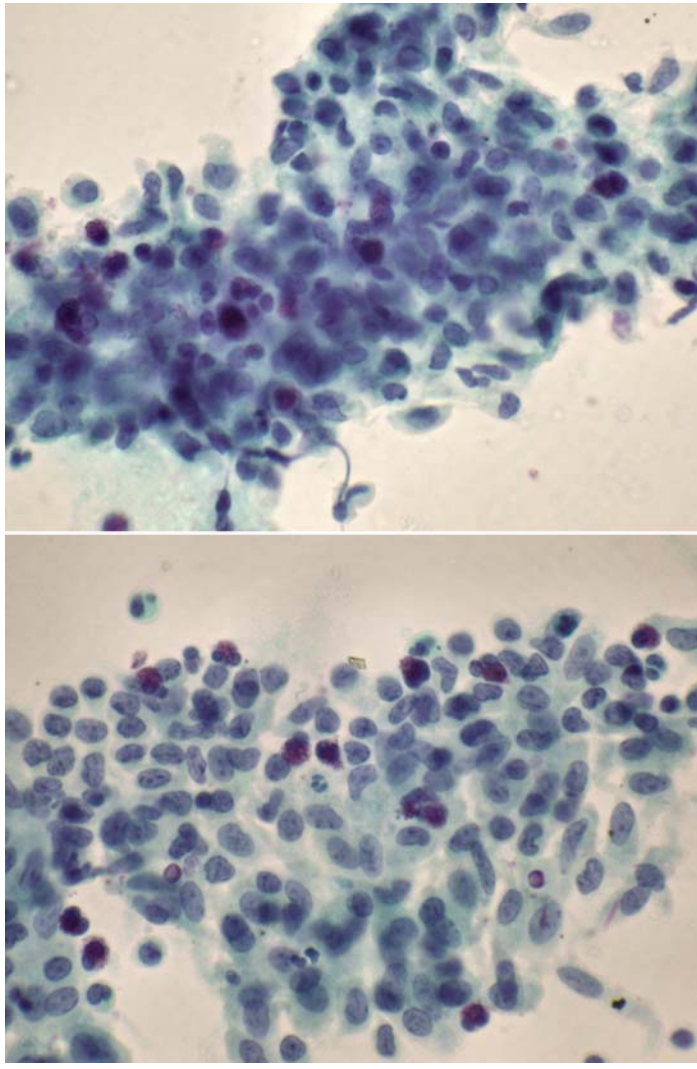

Fig. 6.25. The most common type of endometritis that we can identify in cytological preparations is an often nodular histiocytic type exhibiting aggregates of histiocytic cells admixed with other inflammatory cells including eosinophils, plasmacytoid lymphocytes, and plasma cells. In these images, the histiocytic aggregates show a high content of eosinophils. Immunostaining for Langerhans cell histiocytosis was negative.

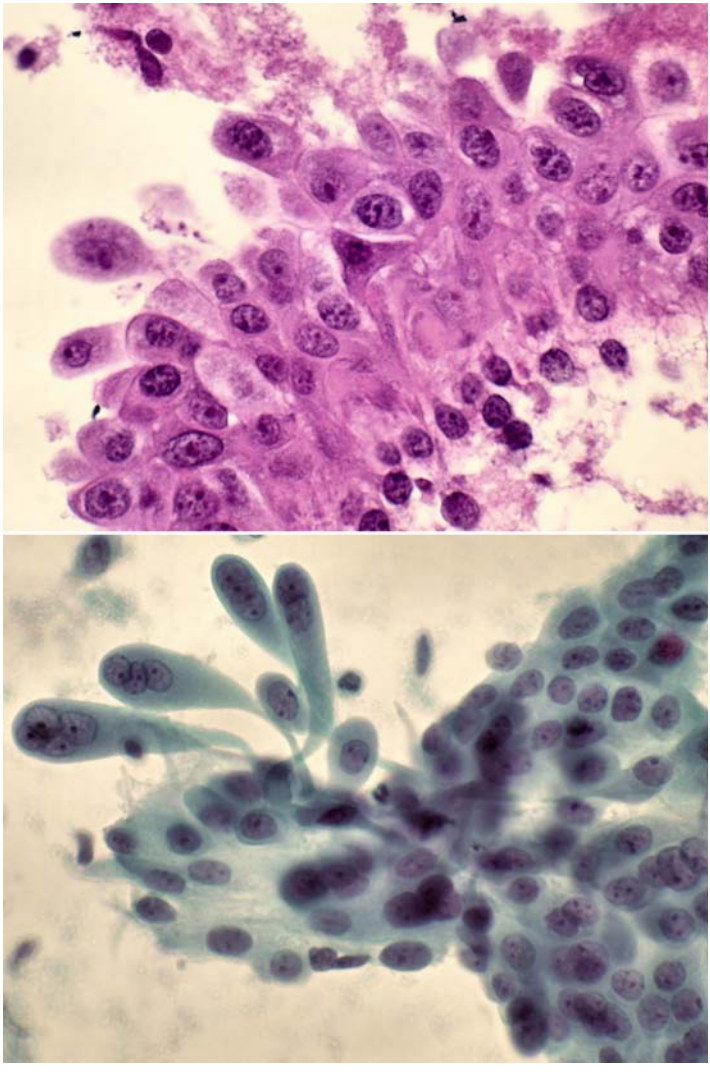

FIG. 6.26. Granulomatous endometritis that was seen at the time of intrauterine device (IUD) removal. The endometrium shows marked surface epithelial activation including a combination of syncytial change and hobnail metaplastic change.

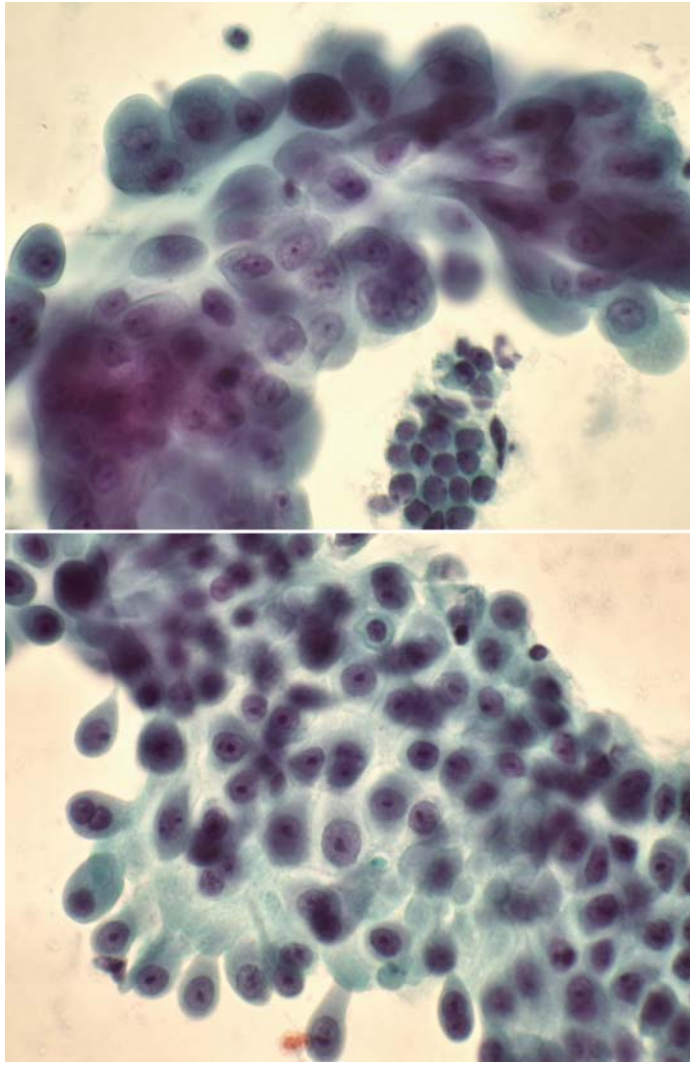

FIG. 6.27. Granulomatous endometritis that was seen at the time of intrauterine device (IUD) removal. The endometrium shows marked surface epithelial activation including a combination of syncytial change and hobnail metaplastic change. We interpret these changes as part of the spectrum of papillary syncytial metaplasia. In the case of extreme hobnailing, clear cell carcinoma may be considered; however, history, an appropriate inflammatory response, and—if necessary—lack of p53/Ki-67 nuclear decoration generally resolve this issue.

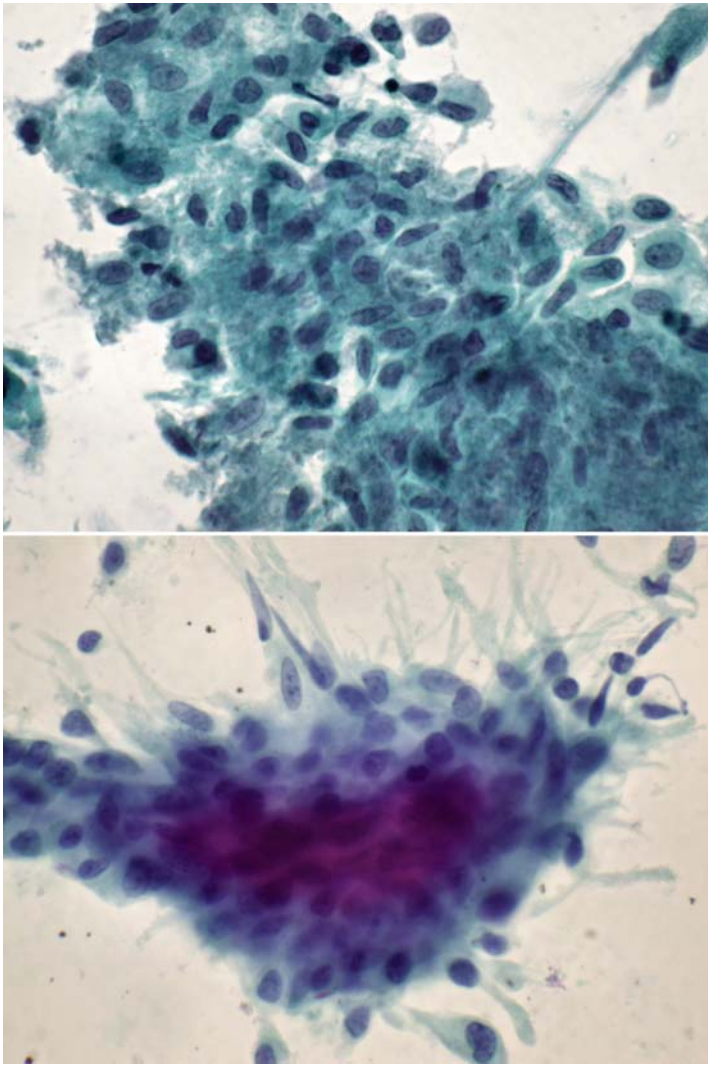

FIG. 6.28. Granulomatous endometritis that was seen at the time of intrauterine device (IUD) removal. Frequent granulomas are present, and the *bottom image* shows a giant cell with stellate processes probably formed by the fusion of histiocytes whose cytoplasm may have partly intercalated with stromal cells.

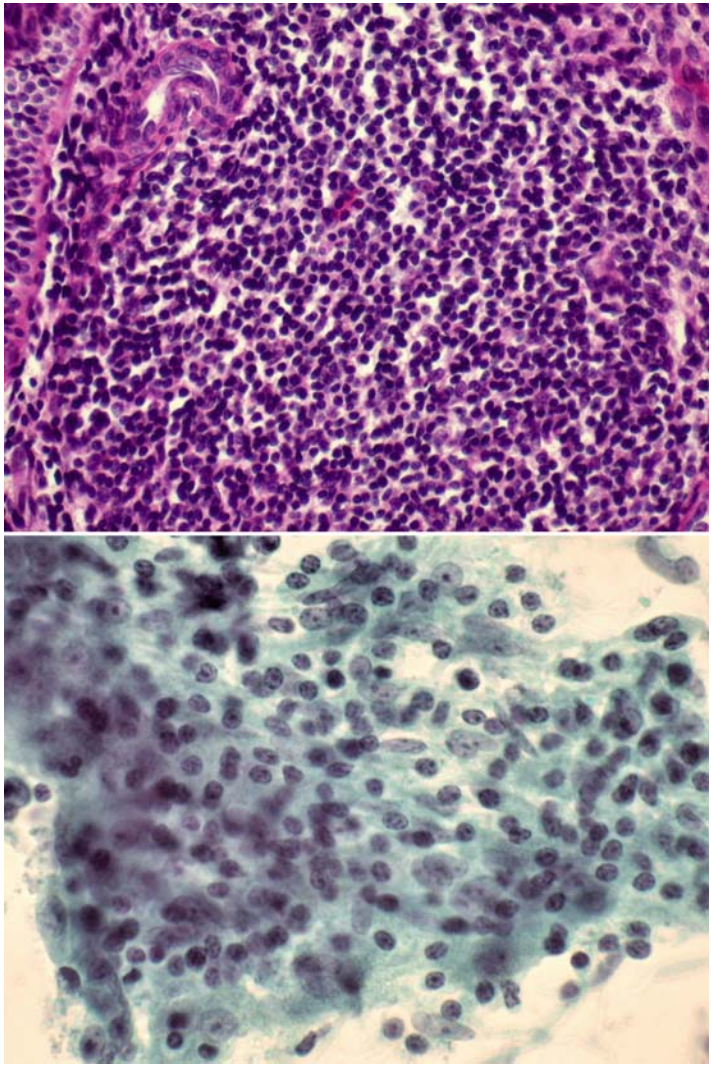

FIG. 6.29. This image shows two of several endometrial mucosal lymphoid aggregates interpreted by some as nodular lymphoid endometritis. Stromal lymphoid nodules may be normal, but it is their excess that implies that they represent more than a resting immune phenomenon.

exceptionally rare. Although most lymphoid aggregates within endometrial curettings result from reactive conditions such as endometritis, the possibility of lymphoma must be considered when dense lymphoid aggregates or atypical lymphoid cells are present.

Inflammation of the endometrium is most dramatic with pyometra as neutrophils fill the uterine cavity (Figs. 6.30, 6.31). Pyometra is more common in dogs than humans. In humans, pyometra is a form of chronic endometritis generally seen in the elderly resulting from a stenotic cervical os and subsequent infection. Common causes of a blocked cervical canal include cervical carcinoma or, more frequently, postmenopausal stenosis. Pyometra may also occur as a sequel of endometrial ablation, colpocleisis, or even introduction of foreign material through fistulous tracts.

Generally, symptomatic chronic endometritis presents with blood-stained discharge, but with pyometra the patient complains of lower abdominal pain. As the term implies, the distended uterine cavity is engorged with pus and the endometrium usually shows the features of severe acute and chronic inflammation. In some cases, where the distension is considerable, pressure markedly thins the endometrium, such that it becomes merely an attenuated layer of stroma covered by surface epithelium. An acute and chronic inflammatory infiltrate may permeate the residual endometrium and extend into the superficial myometrium. Sometimes fatal uterine rupture may occur. Squamous metaplasia may develop in the surface epithelium and glands, and at its most extreme state is called "ichthyosis uteri."

Polyps

An endometrial polyp is a uterine lesion that takes up space within the uterine cavity. Polyps are found in 10% of women. They may be sessile and have a large flat base or be pedunculated and attached to the uterus by an elongated pedicle. They range in size from a few millimeters to several centimeters and if pedunculated, may sometime be found protruding through the cervix into the vagina. Endometrial polyps are biphasic growths of endometrial glands and stroma that arise as monoclonal overgrowths of genetically altered endometrial stromal cells with secondary induction of polyclonal benign glands through as yet undefined stromal–epithelial interactive mechanisms.

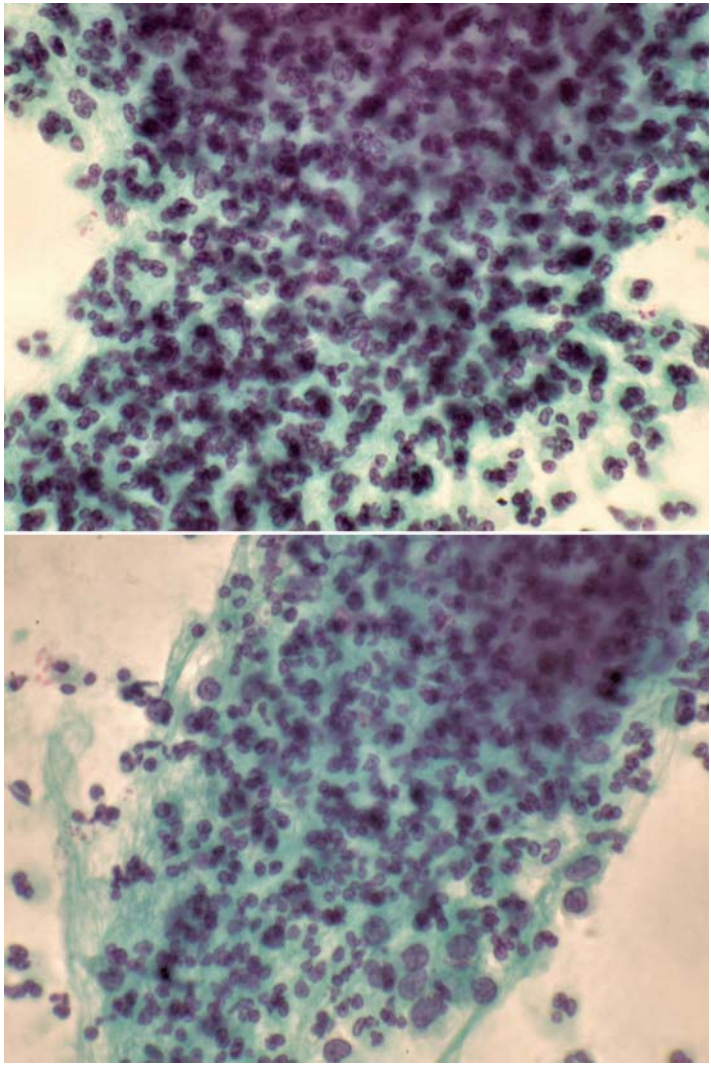

Fig. 6.30. Inflammation of the endometrium is most dramatic with pyometra, wherein PMNs are seen filling the uterine cavity. Brush cytology collections show sheets of pus.

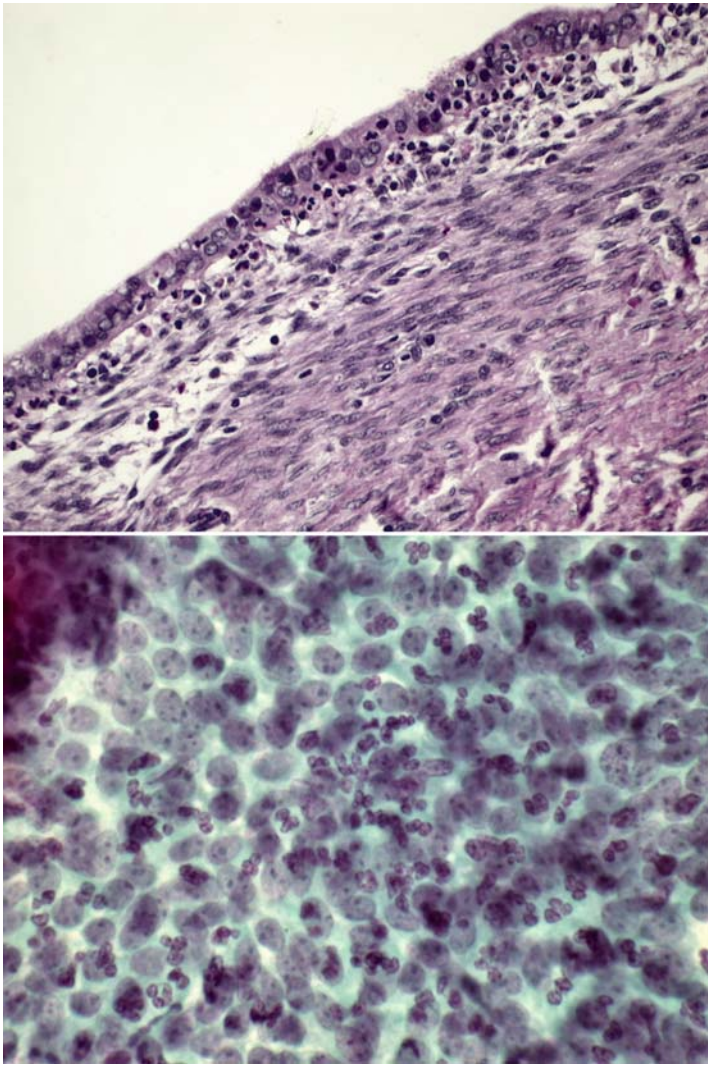

FIG. 6.31. With pyometra, an acute and, less noticeably, chronic inflammatory infiltrate may percolate through residual, flattened endometrial epithelial sheets.

Fibrous polyps, functional polyps, and other entities that cause pressure upon and atrophy of the endometrium, for example, submucous leiomyomas, may cause nuclear atypia of endometrial epithelial sheets, either in a proliferative or atrophic background, depending on the woman's age and the physiological state of her endometrium. In cytology preparations, these benign conditions show fully differentiated to mildly dysdifferentiated epithelium as well as varying degrees of nuclear activation, caused by either repair or irritation (Fig. 6.32). Depending on the extent of epithelial breakdown, various types of inflammatory cells ranging from plasma cells, lymphocytes, and histiocytes to PMN neutrophils may be seen, often percolating through the activated epithelial sheets. Stromal changes also occur and include periepithelial stromal condensation and stromal cell activation. Activated subepithelial or periglandular stroma often presents as sheets of abnormal epithelioid cells that may be confused with neoplasm (Fig. 6.33; also see Figs. 6.35, 6.36).

The common polyp is nonfunctional and lacks the cyclical changes seen in the adjacent normal endometrium. The glands are frequently inactive, showing neither proliferative nor secretory activity. Some or many of the glands may be dilated, slightly branching, or irregularly distributed. This appearance should not give cause for concern or be separately diagnosed as an endometrial hyperplasia, especially when it is uniformly distributed throughout the entire polyp. Unfortunately, the source of these glands cannot often be identified in cytology preparations, and polyps may be misclassified as hyperplasia.

Functioning polyps are less common and are composed of glands that show secretory activity during the secretory phase; however, the glands in the polyp are usually out of step with the rest of the endometrium. Women on tamoxifen have a higher incidence of endometrial polyps than the rest of the population, and their polyps are likely to be larger, more fibrotic, more likely to show mucinous metaplasia, to show bizarre stromal cells, and to contain hyperplasia or carcinoma than polyps of women not taking tamoxifen.

Endometrial polyps, especially when brushed, minced, or fragmented, are commonly misdiagnosed or, almost as commonly, overlooked. Within individual polyp fragments, the irregularly distributed and occasionally branching glands with occasional cysts bear some resemblance to benign endometrial hyperplasia. In histology preparations, the majority of polyps are splintered during sampling so they may have one or no epithelial-covered surface.

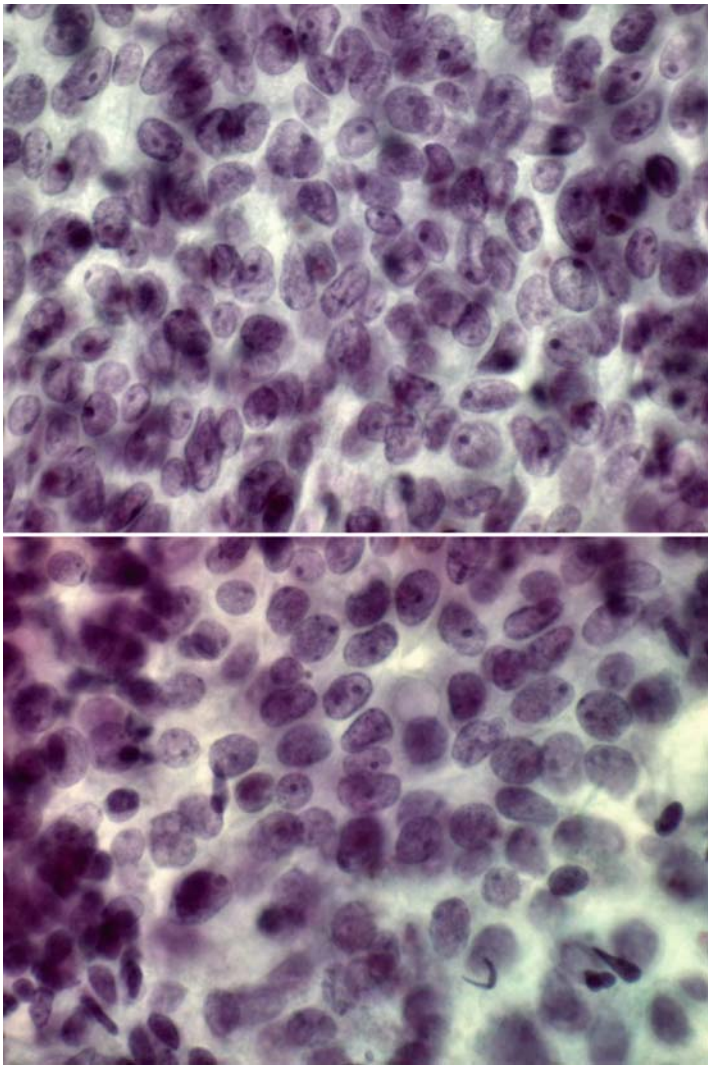

FIG. 6.32. Fibrous polyps, functional polyps, and other entities that cause pressure atrophy of the endometrium, for example, submucous fibroids, also cause nuclear activation of endometrial epithelial sheets. In cytology preparations, these benign conditions show fully differentiated to mildly dysdifferentiated epithelium as well as varying degrees of nuclear activation, caused by either repair or irritation.

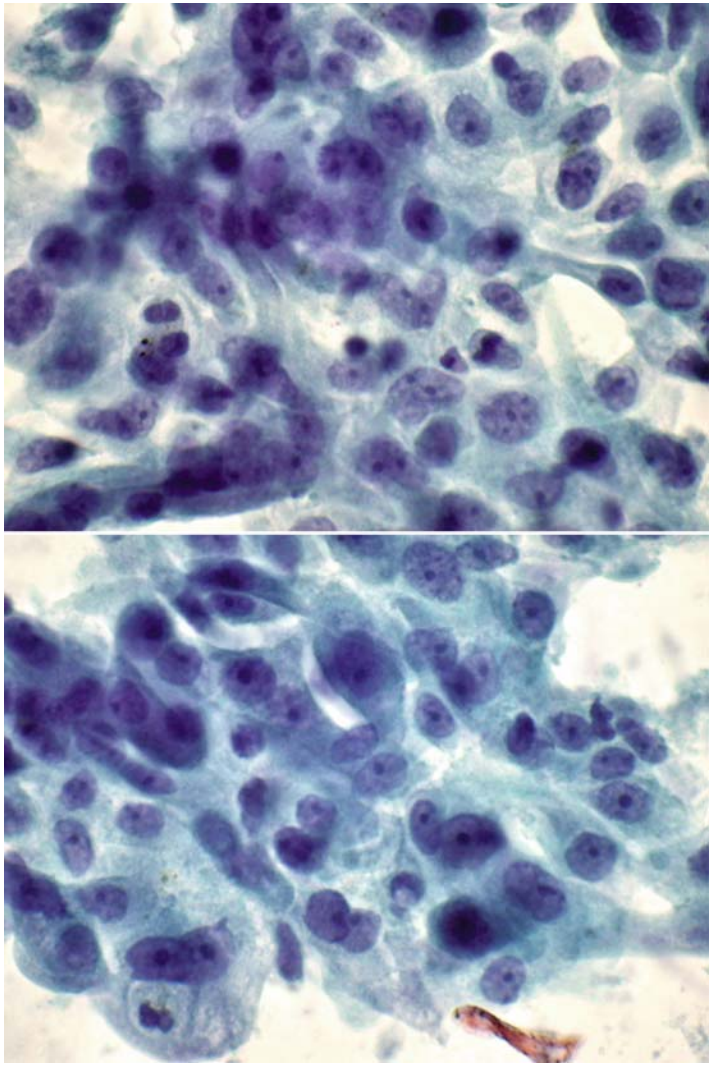

FIG. 6.33. The activated subepithelial or periglandular stroma of polyps often presents as sheets of abnormal epithelioid cells that may be confused with neoplasm.

Thick-walled blood vessels and fibrous stroma commonly seen in polyps are lacking in benign endometrial hyperplasia, and these may be found in cell block preparations of endometrial brushings (Fig. 6.34). Because polyps are localized lesions, specimens obtained by histological or cytological samplings typically contain commingled native endometrium with a variable histological pattern and cytological pattern. Sometimes, the cytological dichotomy may lead falsely to an EIN diagnosis.

Because a polyp is a space-occupying lesion with a tendency for mechanical irritation, the subepithelial stroma of the polyp and of the endometrial wall that the polyp presses upon may be irritated and appear activated to the point of misrepresenting itself as neoplastic tissue (Figs. 6.35, 6.36). This is not the case with benign endometrial hyperplasia where the entire functionalis layer of the endometrium is affected and the stroma is generally proliferative like in appearance.

Atypical stromal cells of the lower female gynecological tract have been specifically described in the vagina, vulva, and cervix, predominantly in the context of polyps (Figs. 6.35, 6.36). Rare cases of markedly atypical stromal cells have also been documented in the endometrium. One series reported 15 examples of atypical stromal cells in the endometrium, 13 in endometrial polyps and 2 within endometrial stroma in curettage/biopsy specimens unassociated with polyps. The patients were 45–82 years old. Immunohistochemical studies helped identify the origin of these atypical cells. Differential diagnoses included adenosarcoma, endometrial stromal sarcoma, and, less likely, carcinosarcoma. Similar to atypical stromal cells reported in other gynecological sites, such cells discovered in the endometrium appear to have a benign clinical course after complete excision or polypectomy (follow-up of 1–44 months). Although accurate recognition of this lesion is essential to avoid excessive surgical treatment, we would advise that the entire polyp be surgically obtained and thoroughly sampled. It is unwise to sign off on the changes as benign on the basis of a cytological specimen alone.

The Tao brush may remove small polyps intact by snaring them and dislodging them at their stalk. Larger polyps may be partly sampled, and sessile polyps (which often show an inversion and exposure of the arteriolar tangles that are generally confined to the basalis portion of the endometrium by a process akin to “herniation”

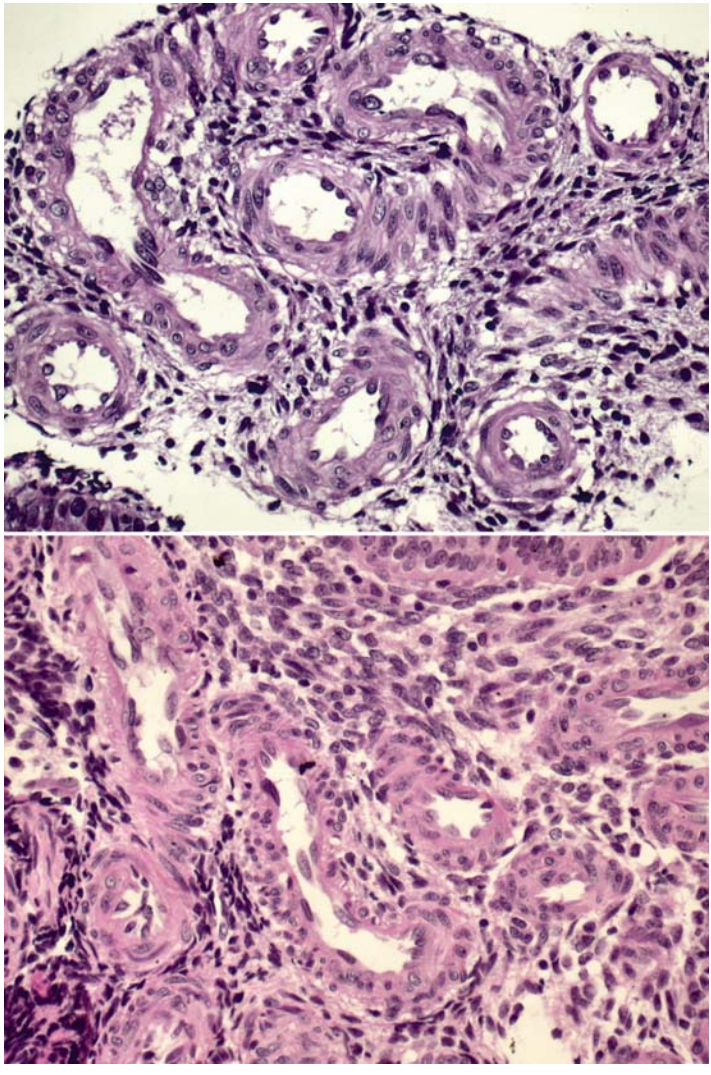

FIG. 6.34. Thick-walled blood vessels and fibrous stroma commonly seen in polyps are lacking in benign endometrial hyperplasia, and these may be found in cell block preparations of endometrial brushings. Brush collections may induce a polyp to twirl about the brush's guidewire, whereby it may be removed intact.

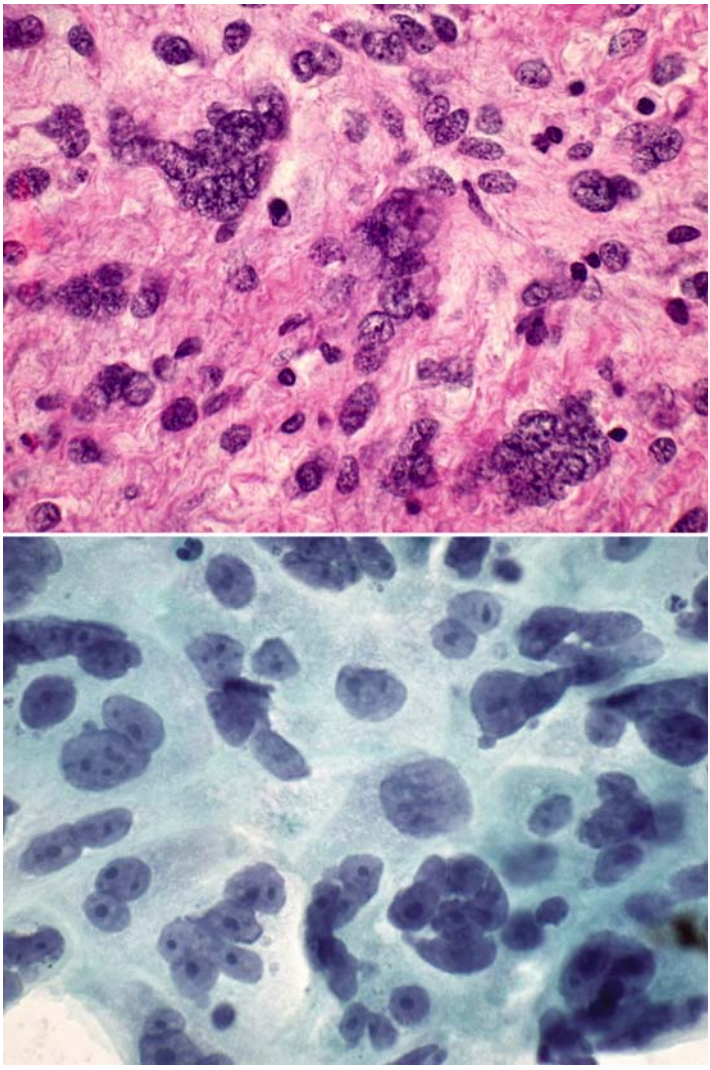

FIG. 6.35. The activated subepithelial or periglandular stroma of polyps often presents as sheets of abnormal epithelioid cells that may be confused with neoplasm (*bottom*). In this case, which was thought to represent an EIC, staining for both p53 and Ki-67 were negative; follow-up hysteroscopy only showed polyp, which, on resection, contained bizarre stromal cells (*top*). The woman had been on tamoxifen for an extended period of time.

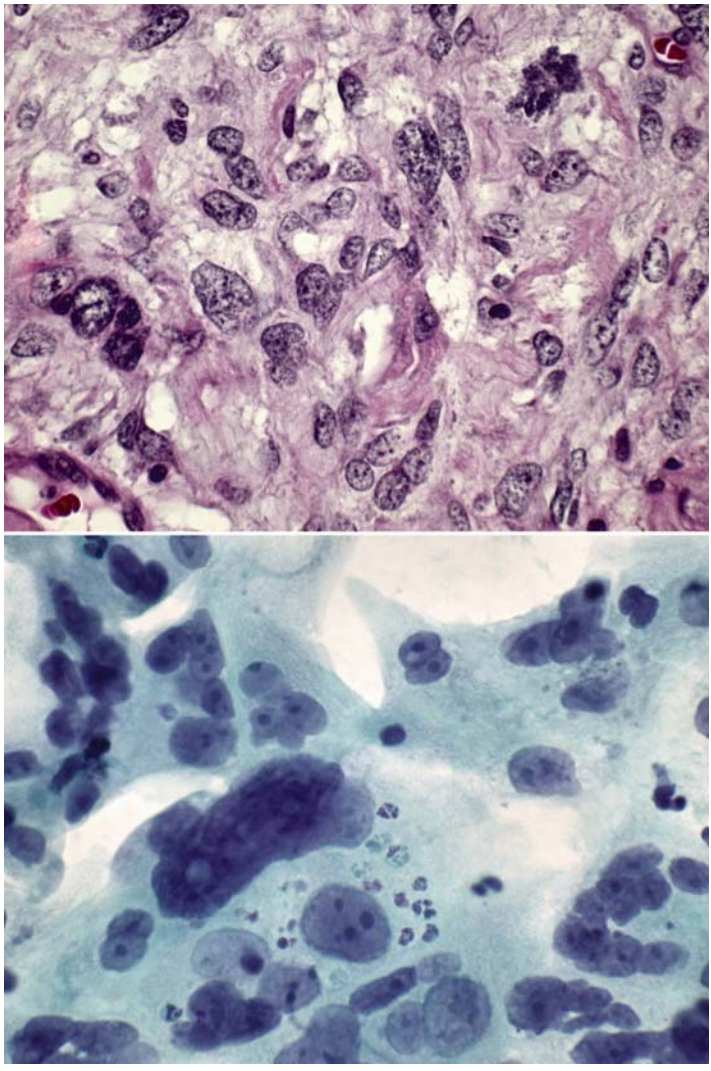

FIG. 6.36. The activated subepithelial or periglandular stroma of polyps often presents as sheets of abnormal epithelioid cells that may be confused with neoplasm (*bottom*). In this case, which was thought to represent an EIC, staining for both p53 and Ki-67 were negative; follow-up hysteroscopy only showed polyp, which, on resection, contained bizarre stromal cells (*top*). The woman had been on tamoxifen for an extended period of time. This case represents one of those "better out than in" situations; and, it is unlikely that we would not advise a clinician to perform a follow-up procedure if we saw another case like this.

of the basalis endometrium) may show thick-walled arteriolar tangles in fibrous stroma.

Endometrial cytology is not a reliable method for obtaining or diagnosing endometrial polyps, leiomyomas, or any pathological condition that resides more than 2 mm beneath the endometrial surface. It is for this reason that gross visualization of the uterus, as, for example, by sonohysterography (transvaginal ultrasonography with cavitary saline infusion) or hysteroscopy is advised as a reasonable and often necessary accompaniment to endometrial sampling in the course of assessing pathological states of the uterine corpus.

Liquid-based endometrial cytology has been examined in the management of endometrial polyps in postmenopausal women, specifically, by capitalizing on the ability of cytology to exclude "hidden" premalignant and malignant changes within the epithelium of these polyps. In one study, 8 of 359 women had malignant or premalignant polyps that were interpreted as benign at hysteroscopy. Endometrial biopsy and liquid-based cytology had a sensitivity of 62% and 88%, respectively, for identifying a neoplastic endometrial state, and both showed 100% specificity for the discovery of such "hidden" neoplasms. Liquid-based cytology has therefore been proposed as a useful tool for establishing the nature of otherwise benign-appearing endometrial polyps in postmenopausal patients.

Postablation Endomyometrial Necrosis

Recently, we have seen five cases of myometrial necrosis in cytology brush preparations from women 39 to 55 years of age (mean age, 43) who had undergone thermal endometrial ablation to control uterine bleeding. Three presented with dysfunctional uterine bleeding, one with a history of endometrial hyperplasia, and one with leiomyomas and menorrhagia.

In each case, necrotic smooth muscle appeared as strands or bands of amorphous material with variably preserved nuclei (Fig. 6.37). Occasionally, the pale outlines of elongated nuclei, typical of smooth muscle, were found in both cytology and histology preparations. The background showed a variable number of PMNs admixed with flocculent material (Fig. 6.38). Granulomatous inflammation was not seen. In one patient (the oldest), foci

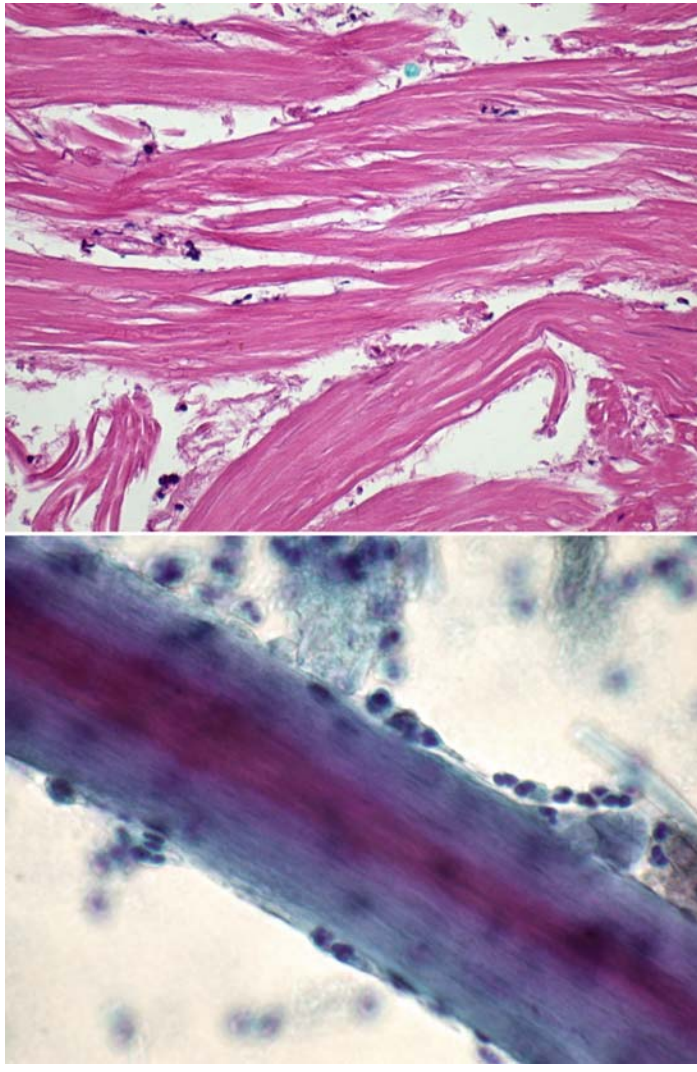

Fig. 6.37. Endomyometrial necrosis can follow endometrial ablation. In this follow-up brushing of a woman who was ablated but continued to bleed, necrotic smooth muscle was found that appeared as strands or bands of amorphous material with variably preserved nuclei and accompanying PMNs.

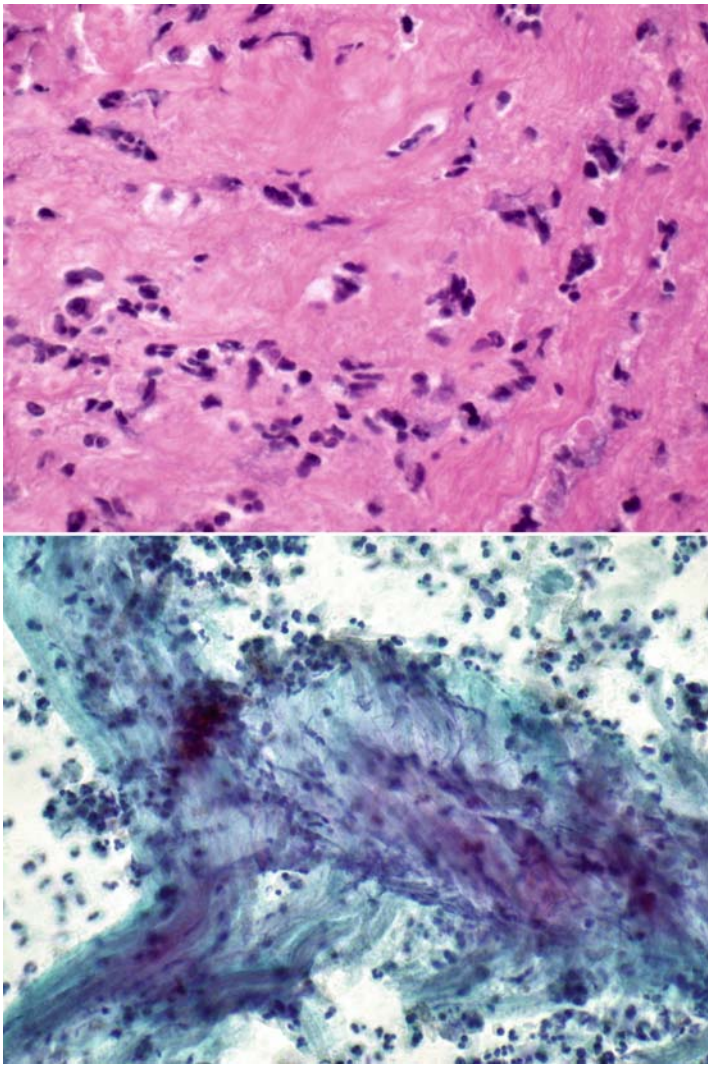

FIG. 6.38. In the typical case of postablation endomyometrial injury, the background shows a variable number of PMNs admixed with flocculent material.

of atypical cells with elongated, enlarged, crowded nuclei were present that we originally took to represent soft tissue neoplasm. Subsequent experience with Pipelle biopsies has shown us that this probably represents stromal scarring (Fig. 6.39).

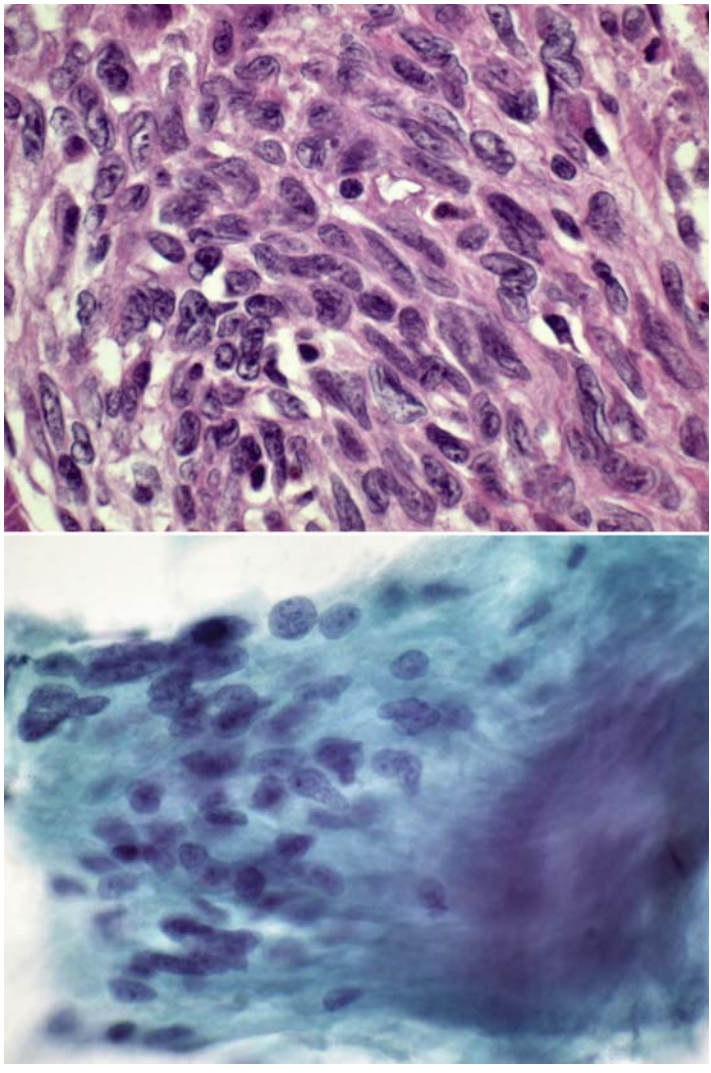

FIG. 6.39. In this case, there are foci of atypical cells with elongated, enlarged, crowded nuclei that we originally thought represented soft tissue neoplasm; however, history, imaging, and subsequent experience with Pipelle biopsies has shown this material to probably represent stromal scar. Differential diagnoses of this finding would include postoperative spindle cell nodule and low-grade uterine sarcoma. As with the case illustrated in Figs. 6.35 and 6.36, it is unlikely that we would not advise a clinician to perform a follow-up procedure if we saw another case like this.

The differential diagnosis of myonecrosis includes myometrial necrosis following endometrial ablation, sampling of a punctured necrotic smooth muscle tumor, sampling of a punctured leiomyoma following arterial embolization, and acute postpuerperal necrotizing endomyometritis. Several case reports and studies have confirmed the phenomenon of smooth muscle necrosis after endometrial ablation. One report noted a granulomatous response to the necrosis that persisted longer than the necrotic myometrium itself while another group reported necrosis, granulomatous inflammation, eosinophils, and pigmented macrophages in the myometrium.

Suggested Reading

Andersen LF, Meinert L, Rygaard C, Junge J, Prento P, Ottesen BS. Thermal balloon endometrial ablation: safety aspects evaluated by serosal temperature, light microscopy and electron microscopy. Eur J Obstet Gynecol Reprod Biol 1998; 79: 63–8.

Appiah-Sakyi K, Byrd L, Slade RJ. Thermal balloon ablation: a rare case of fibroid necrosis. J Obstet Gynaecol 2004; 24: 467.

Bayer-Garner IB, Nickell JA, Korourian S. Routine syndecan-1 immunohistochemistry aids in the diagnosis of chronic endometritis. Arch Pathol Lab Med 2004; 128: 1000–3.

Bergeron C, Nogales FF, Masseroli M, Abeler V, Duvillard P, Müller-Holzner E, Pickartz H, Wells M. A multicentric European study testing the reproducibility of the WHO classification of endometrial hyperplasia with a proposal of a simplified working classification for biopsy and curettage specimens. Am J Surg Pathol 1999; 23: 1102–8.

Berliere M, Radikov G, Galant C, Piette P, Marbaix E, Donnez J. Identification of women at high risk of developing endometrial cancer on tamoxifen. Eur J Cancer 2000; 36: S35–6.

Carberry CL, Hampton BS, Aguilar VC. Pyometra necessitating hysterectomy after colpocleisis in an extremely elderly patient. Int Urogynecol J Pelvic Floor Dysfunct 2007; 18: 1109–11.

Chan KS, Tan CK, Mak CW, Chia CC, Kuo CY, Yu WL. Computed tomography features of spontaneously perforated pyometra: a case report. Acta Radiol 2006; 47: 226–7.

Chiesa AG, Hart WR. Uterine artery embolization of leiomyomas with trisacryl gelatin microspheres (TGM): pathologic features and comparison with polyvinyl alcohol emboli. Int J Gynecol Pathol 2004; 23: 386–92.

Cicinelli E, De Ziegler D, Nicoletti R, Colafiglio G, Saliani N, Resta L, Rizzi D, De Vito D. Chronic endometritis: correlation among hysteroscopic, histologic, and bacteriologic findings in a prospective trial with 2190 consecutive office hysteroscopies. Fertil Steril 2008; 89: 677–84.

Colgan TJ, Shah R, Leyland N. Post-hysteroscopic ablation reaction: a histopathologic study of the effects of electrosurgical ablation. Int J Gynecol Pathol 1999; 18: 325–31.

Colgan TJ, Pron G, Mocarski EJ, Bennett JD, Asch MR, Common A. Pathologic features of uteri and leiomyomas following uterine artery embolization for leiomyomas. Am J Surg Pathol 2003; 27: 167–77.

Fambrini M, Buccoliero AM, Bargelli G, Cioni R, Piciocchi L, Pieralli A, Andersson KL, Scarselli G, Taddei G, Marchionni M. Clinical utility of liquid-based cytology for the characterization and management of endometrial polyps in postmenopausal age. Int J Gynecol Cancer 2008; 18: 306–11.

Ferenczy A. Pathophysiology of endometrial bleeding. Maturitas 2003; 45: 1–14.

Fletcher J, Pinkus J, Lage J, Morton C, Pinkus G. Clonal 6p21 rearrangement is restricted to the mesenchymal component of an endometrial polyp. Genes Chromosomes Cancer 1992; 5: 260–3.

Fogt F, Hinds N, Zimmerman RL. Histologic features of uterine leiomyomata treated with microsphere embolization. Obstet Gynecol 2003; 102: 600–2.

Fukunaga M, Iwaki S. Nodular histiocytic hyperplasia of the endometrium. Arch Pathol Lab Med 2004; 128: 1032–4.

Galan M, Kim YB, Hecht JL. Does physiologic breakdown mask significant pathology in endometrial biopsies? A retrospective case-control study. Arch Pathol Lab Med 2006; 130: 1847–9.

Gilmore H, Fleischhacker D, Hecht JL. Diagnosis of chronic endometritis in biopsies with stromal breakdown. Hum Pathol 2007; 38: 581–4.

Goldstein SR, Zeltser I, Horan CK, Snyder JR, Schwartz LB. Ultrasonography-based triage for postmenopausal patients with uterine bleeding. Am J Obstet Gynecol 1997; 177: 102–8.

Iezzoni JC, Mills SE. Nonneoplastic endometrial signet-ring cells. Vacuolated decidual cells and stromal histiocytes mimicking adenocarcinoma. Am J Clin Pathol 2001; 115: 249–55.

Indman PD, Soderstrom RM. Depth of endometrial coagulation with the urologic resectoscope. J Reprod Med 1990; 35: 633–5.

Iyengar P, Deodhare S. Primary extranodal marginal zone B-cell lymphoma of MALT type of the endometrium. Gynecol Oncol 2004; 93: 238–41.

Katsumori T, Bamba M, Kobayashi TK, Moritani S, Urabe M, Nakajima K, Mihara T, Sugihara H. Uterine leiomyoma after embolization by means

of gelatin sponge particles alone: report of a case with histopathologic features. Ann Diagn Pathol 2002; 6: 307–11.

Kendall BS, Ronnett BM, Isacson C, Cho KR, Hedrick L, Diener-West M, Kurman RJ. Reproducibility of the diagnosis of endometrial hyperplasia, atypical hyperplasia, and well-differentiated carcinoma. Am J Surg Pathol 1998; 22: 1012–9.

McCluggage WG, Ellis PK, McClure N, Walker WJ, Jackson PA, Manek S. Pathologic features of uterine leiomyomas following uterine artery embolization. Int J Gynecol Pathol 2000; 19: 342–7.

Mutter GL, Zaino RJ, Baak JP, Bentley RC, Robboy SJ. Benign endometrial hyperplasia sequence and endometrial intraepithelial neoplasia. Int J Gynecol Pathol 2007; 26: 103–14.

Norimatsu Y, Shimizu K, Kobayashi TK, Moriya T, Kaku T, Tsukayama C, Miyake Y, Ohno E. Endometrial glandular and stromal breakdown, part 2: cytomorphology of papillary metaplastic changes. Diagn Cytopathol 2006; 34: 665–9.

Perrone T, Dehner LP. Prognostically favorable "mitotically active" smooth-muscle tumors of the uterus. A clinicopathologic study of ten cases. Am J Surg Pathol 1988; 12: 1–8.

Schlumbrecht M, Balgobin S, Word L. Pyometra after thermal endometrial ablation. Obstet Gynecol 2007; 110: 538–40.

Sentilhes L, Sergent F, Verspyck E, Gravier A, Roman H, Marpeau L. Laparoscopic myomectomy during pregnancy resulting in septic necrosis of the myometrium. Br J Obstet Gynaecol 2003; 110: 876–8.

Song J, Rutherford T, Naftolin F, Brown S, Mori G. Hormonal regulation of apoptosis and the Fas and Fas ligand system in human endometrial cells. Mol Hum Reprod 2002; 8: 447–455.

Tai LH, Tavassoli FA. Endometrial polyps with atypical (bizarre) stromal cells. Am J Surg Pathol 2002; 26: 505–9.

Tews G, Ebner T, Apfalter P. Acute postpuerperal necrotizing endomyometritis. Int J Gynaecol Obstet 2004; 86: 39–40.

Tresserra F, Grases P, Ubeda A, Pascual MA, Grases PJ, Labastida R. Morphological changes in hysterectomies after endometrial ablation. Hum Reprod 1999; 14: 1473–7.

Weichert W, Denkert C, Gauruder-Burmester A, Kurzeja R, Hamm B, Dietel M, Kroencke TJ. Uterine arterial embolization with Tris-acryl gelatin microspheres: a histopathologic evaluation. Am J Surg Pathol 2005; 29: 955–61.

7
Endometrial Epithelial Metaplasias and Foam Cells

Metaplasia comes from the Greek meaning "change in form." It is the reversible replacement of one differentiated cell type with another differentiated cell type. The change implies the reprogramming of a common stem cell, and not the metamorphosis of one differentiated cell type into another. Metaplasia occurs as a result of reprogrammed stem cells that are nudged along a different pathway of differentiation by cytokines, growth factors, and other substances in the cell's environment. Where these stem cells come from—either stem cells inherent to the tissue in question, derived from a circulating pool, or generated through a process of regressive differentiation—is a matter of speculation.

The change from one type of cell to another is generally caused by some sort of abnormal stimulus. In simplistic terms, the original cells are insufficiently robust to withstand the environmental change, and so they are replaced by another cell type that is within the repertoire of a common stem cell, but more suited to the new, more rugged environment. If the stimulus that caused metaplasia is removed or ceases, tissues generally return to their normal pattern of differentiation.

Metaplasia may occur in benign, atypical, or malignant tissue. Although metaplasia does not represent carcinogenesis, it is accompanied by a loss of normal epithelial function, and this is undesirable. The undesirability is enhanced by the propensity of metaplastic regions to eventually turn dysplastic or cancerous if

J.A. Maksem et al., *Endometrial Cytology with Tissue Correlations*, Essentials in Cytopathology 7, DOI: 10.1007/978-0-387-89910-7_8,

the irritant remains. Although endometrial epithelial metaplasia is most commonly encountered among postmenopausal women with abnormal uterine bleeding, it is unlikely that the bleeding can be ascribed to metaplasia.

Because metaplastic cells may appear "unusual," their nuclei may appear "atypical" in relationship to native endometrial epithelium. Because metaplastic cells often line architecturally complex or irregular glands, endometrial epithelial metaplasia may be confused with endometrioid neoplasia, including adenocarcinoma. Most metaplasias appear as squamous metaplasia, morules, papillary syncytial change (probably a reactive rather than true metaplastic process), ciliated cell metaplasia, eosinophilic metaplasia, mucinous metaplasia, and hobnail or clear cell metaplasia.

Most women whose endometria demonstrate metaplastic transformation are postmenopausal, and many have received some form of estrogen replacement within 3 months of their endometrial curettage or biopsy. All forms of epithelial metaplasia can be recognized in endometrial brushings, and several types of metaplasia may coexist with one another.

Squamous Metaplasia and Morules

Squamous metaplasia may occur as stratified epithelium lining the uterine cavity, or as expansive round morules within individual glands. Keratinizing squamous metaplasia typically results from chronic irritation or infection. Morular metaplasia is more common in a neoplastic setting. Further endometrial study is generally advised when isolated unexplained morular metaplasia is found. Sometimes, the presence of isolated squamous morules is the only clue that something more serious may be present elsewhere in the endometrium.

"Ichthyosis uteri" defines a pronounced stratified keratinizing squamous uterine lining epithelium that extends downward to individual glands and shows intercellular bridges and keratin pearls. The term originally described the condition where the extensive squamous metaplasia lining the endometrial surface developed after caustic substances such as formalin or iodine were introduced into the cavity. Chronic endometritis, over protracted intervals, is generally the antecedent condition (Fig. 7.1).

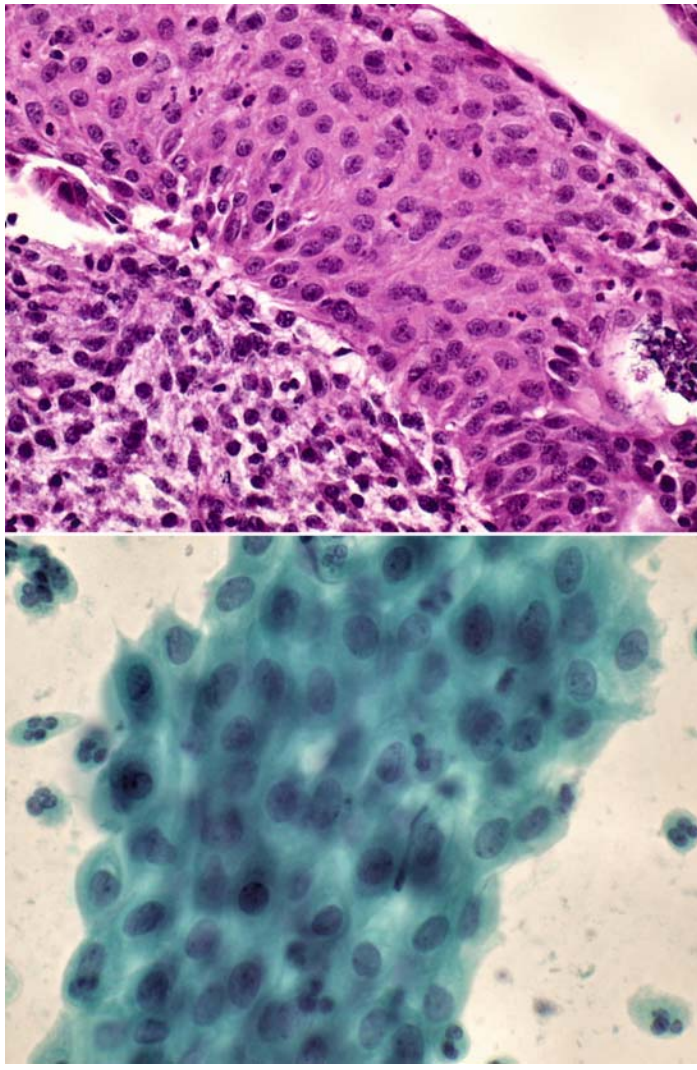

FIG. 7.1. Icthyosis of the uterus defines a stratified keratinizing squamous uterine lining epithelium that may extend downward to individual glands and shows intercellular bridges and keratin pearls. Chronic endometrial irritation that persists over protracted intervals almost always is the antecedent condition.

Icthyosis is a benign condition. Anaplastic and dysplastic changes have been reported rarely, but these cases were often associated with dysplasia or carcinoma of the cervix, suggesting direct extension from cervix to the endometrium. Much more common in the preantibiotic era, icthyosis uteri is now rare and is most commonly encountered in women with anatomic abnormalities favoring recurrent infection, such as fistulous tracts into the uterine cavity.

Morules have distinct histological characteristics. Most investigators consider the morules as a form of nonkeratinizing squamous metaplasia (Fig. 7.2), although one study showed that morules are histologically distinct from keratinizing squamous metaplasia and may actually represent neuroectodermal-like cell clusters. The original description in 1959 defined morules as a characteristic type of metaplasia associated with a group of well-differentiated endometrial glandular lesions showing complex architecture. Keratinizing squamous differentiation was distinguished from morular metaplasia, basing the distinction on both the high degree of differentiation of the glandular component and the low grade of malignancy of lesions associated with morules.

In some cases, as the epithelium within the morules mature, intercellular bridges and central cellular necrosis may appear. The latter is not indicative of malignancy. Intraglandular morules may arise from a clonal population of premalignant endometrial glands, being the mitotically inactive and hormonally depleted products of end-stage differentiation; whereas, the accompanying glandular elements remain mitotically active and responsive to the cancer-promoting effect of estrogens and the involutional effect of progestins (Fig. 7.3). Under this circumstance, with treatment with or exposure to progestins during natural menstrual cycles, the glandular components may involute, leaving residual squamous morules devoid of an accompanying glandular lesion.

Morules lack estrogen and progesterone receptors and have beta-catenin-reactive nuclei. Morules are highly characteristic structures with immunohistochemical and genetic profiles that differ from normal endometrium and endometrioid neoplasms. They most likely are a characteristic, independent type of neoplastic transformation frequently involving a mutation of beta-catenin but not of PTEN, K-*ras*, and microsatellite instability. The beta-catenin

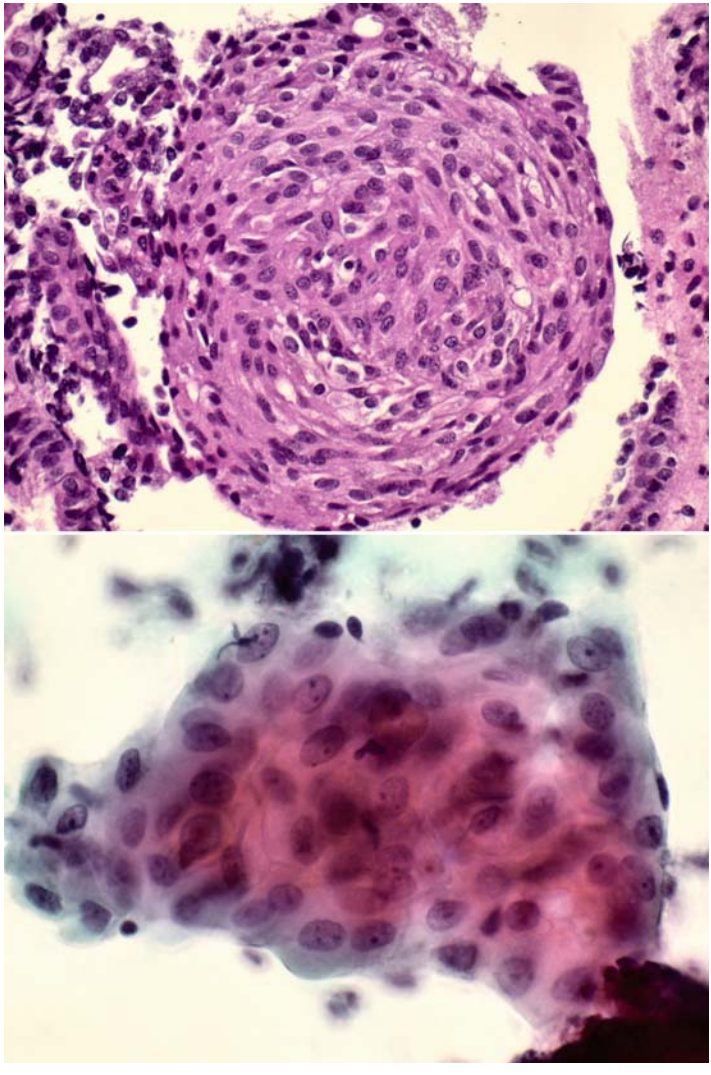

FIG. 7.2. Morules have distinct histological characteristics and are considered by most as a form of nonkeratinizing squamous metaplasia (*top*). Cytologically, they comprise clusters of epithelioid cells with oval to round nuclei and cytoplasm with a waxy texture, akin to the cytoplasm of immature squamous metaplasia of the cervix (*bottom*).

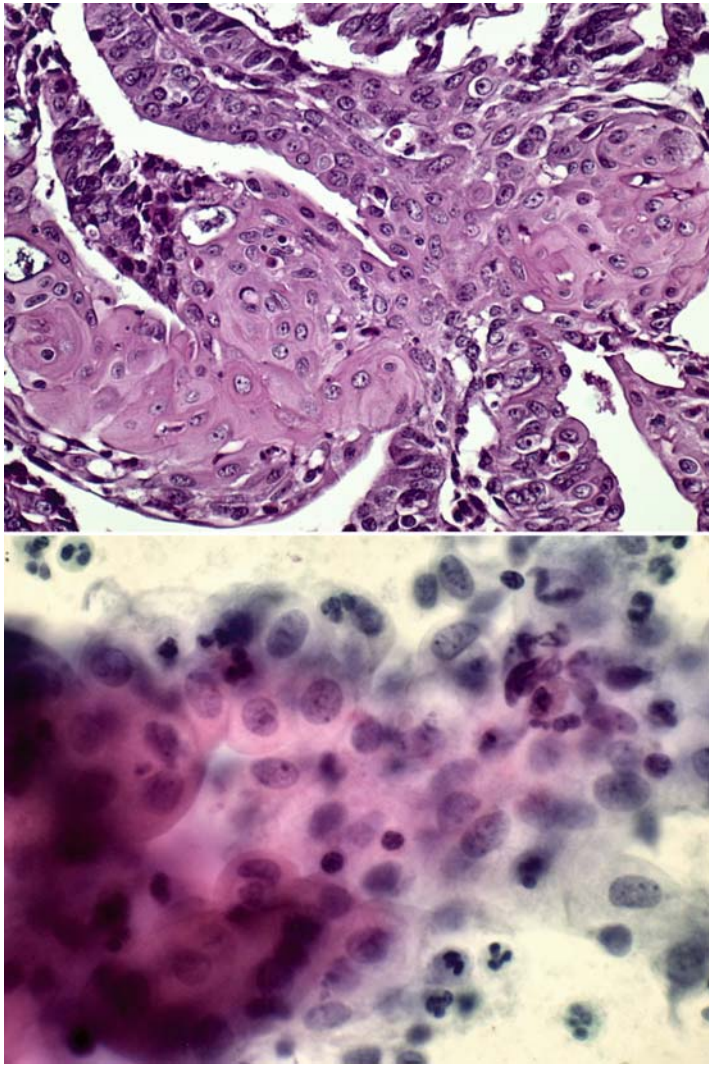

FIG. 7.3. Intraglandular morules may arise from a clonal population of neoplastic endometrial glands, and morules are mitotically inactive and hormonally depleted products of end-stage differentiation; whereas, the accompanying glandular elements remain mitotically active and responsive to the cancer-promoting effect of estrogens and the involutional effect of progestins.

mutation in both the squamous and glandular elements of EIN or adenocarcinoma further suggests both the morules and the glandular elements derive from a common clone.

Keratinizing squamous metaplasia is frequently associated with endometrioid adenocarcinoma and must be differentiated from morular metaplasia in the 20% of cases where both forms of metaplasia coexist. CD10 immunostaining differentiates between morular and keratinizing squamous metaplasia because the latter has a staining pattern similar to glandular epithelium. CD10 staining represents a useful marker of morules in endometrioid neoplasms of the female genital tract. Morules show strongly reactive membranous CD10, which is simultaneously absent in glands, and this allows easy identification of morules at low power in various types of surgical specimens and in curettings. CD10 also highlights early morular metaplasia in glandular epithelium and discriminates between morular and keratinizing squamous types of metaplasia. As is true for some cases of keratinizing squamous differentiation, morules do not show HPV infection.

Approximately 25% of endometrial adenocarcinomas display focal squamous differentiation. In the late 1960s a distinction was made between tumors where the squamous component was well or poorly differentiated. The former tumors were called "adenoacanthoma" (Fig.7.4) and the latter "adenosquamous carcinoma" (Figs. 7.5–7.7). Numerous studies have confirmed the significantly better prognosis associated with adenoacanthoma (about 90% survival at 5 years) than adenosquamous carcinoma (65%). Although the survival relates to the state of differentiation, the grading has been found to be more reliable when judged by the glandular component of the tumor. In most publications, the well-differentiated squamous component is often reported as being benign, but, in reality, it is well-differentiated neoplasm.

Ciliated or Tubal Metaplasia

Ciliated or tubal differentiation is the most common form of endometrial metaplasia (Fig. 7.8). Ciliated cells are distinctive and evident at low magnification or in en face preparations of endometrial epithelial sheets whereby they comprise cells with both increased

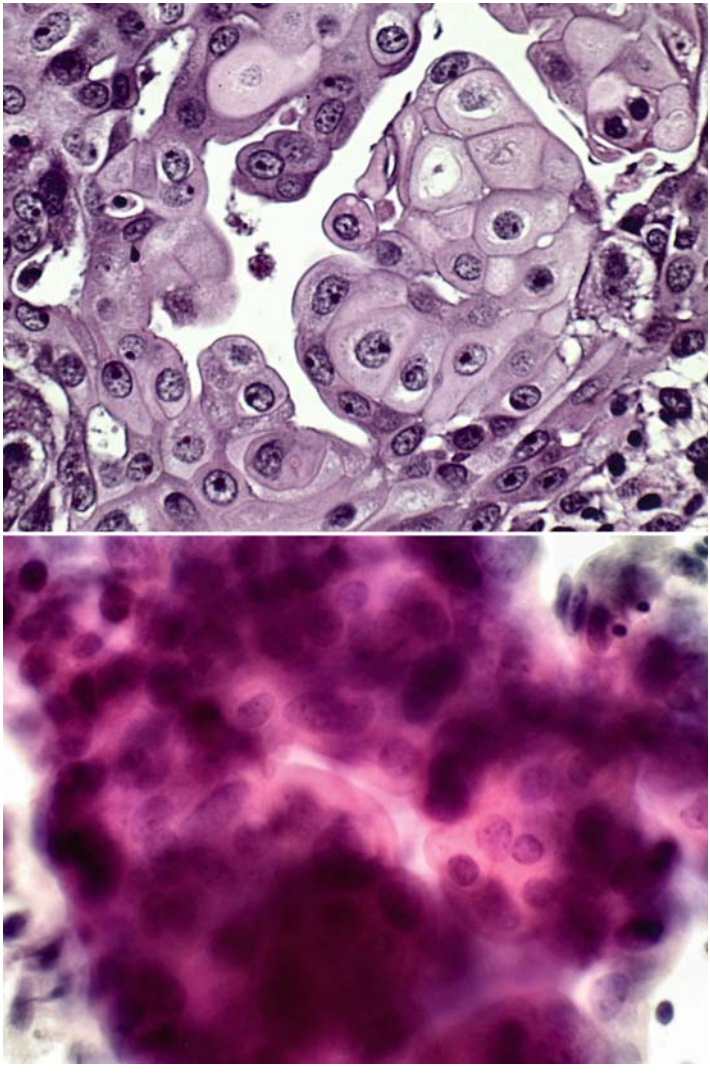

FIG. 7.4. Although a well-differentiated squamous component is often reported as being benign, it is, however, because of its derivation from proliferative gland epithelium, neoplastic and metaplastic at the same time.

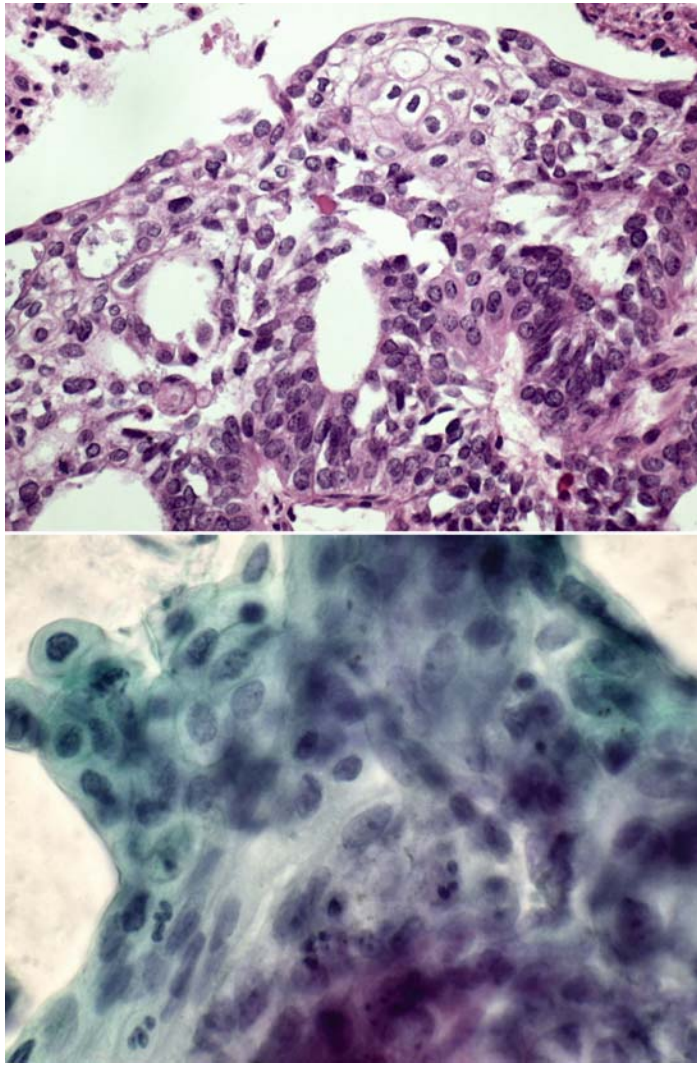

FIG. 7.5. In this example of adenocarcinoma with squamous elements, the squamous component has an immature metaplastic appearance.

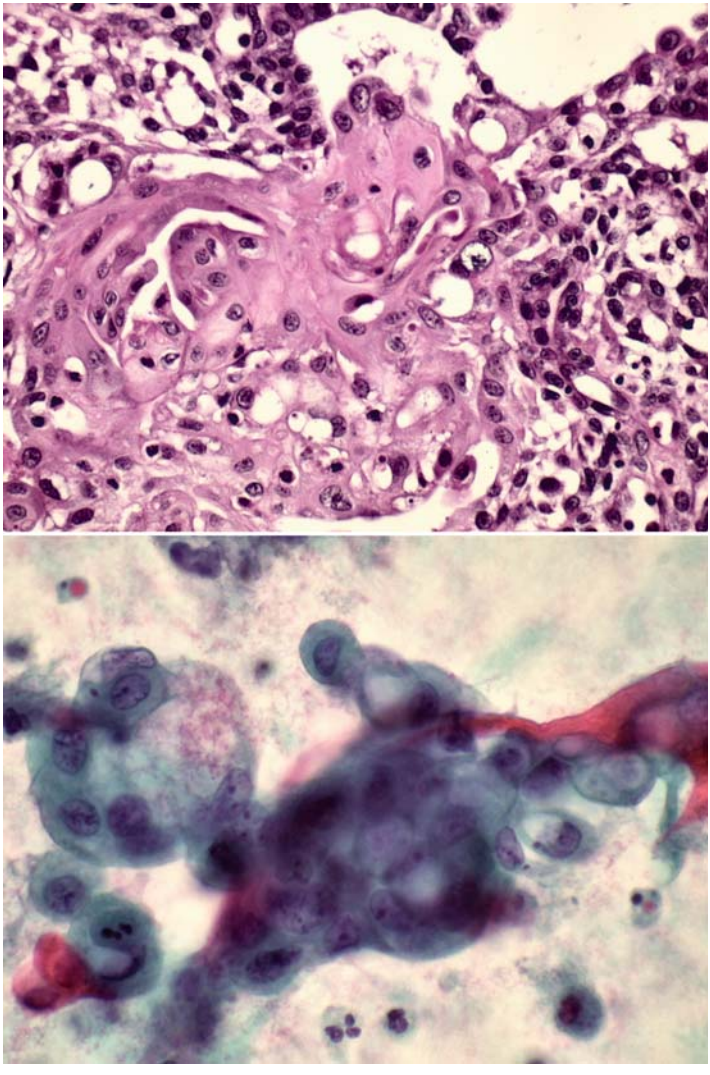

FIG. 7.6. Here, the squamous component of the carcinoma is admixed with signet ring elements of adenocarcinoma.

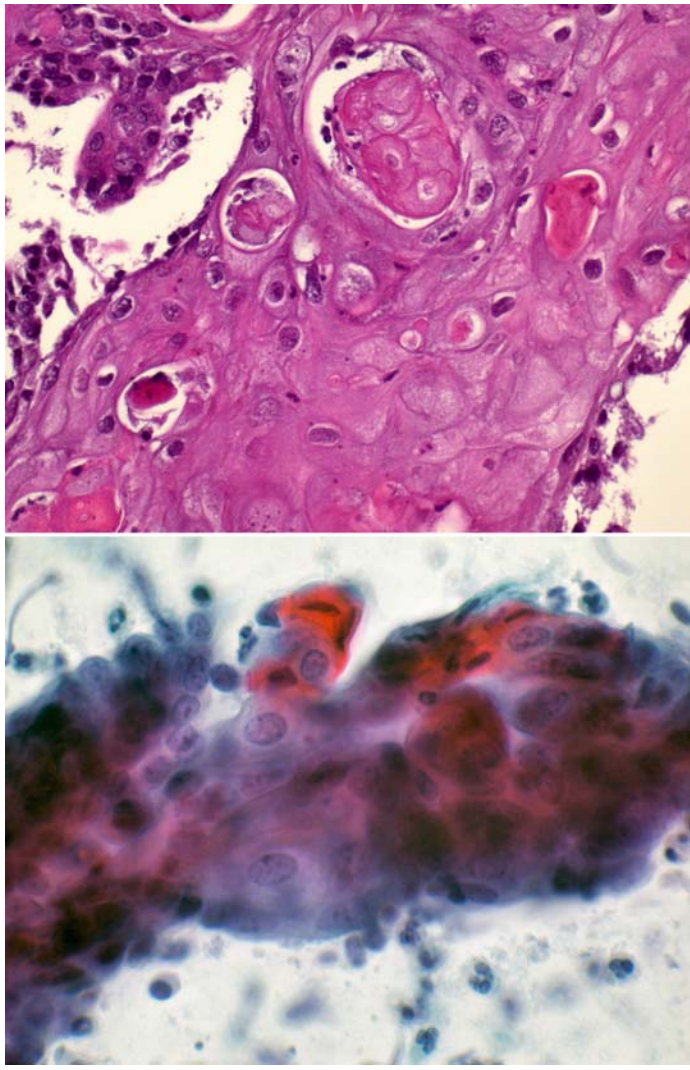

FIG. 7.7. This case shows keratinizing squamous change with small parakeratotic cells and pearl-like formations.

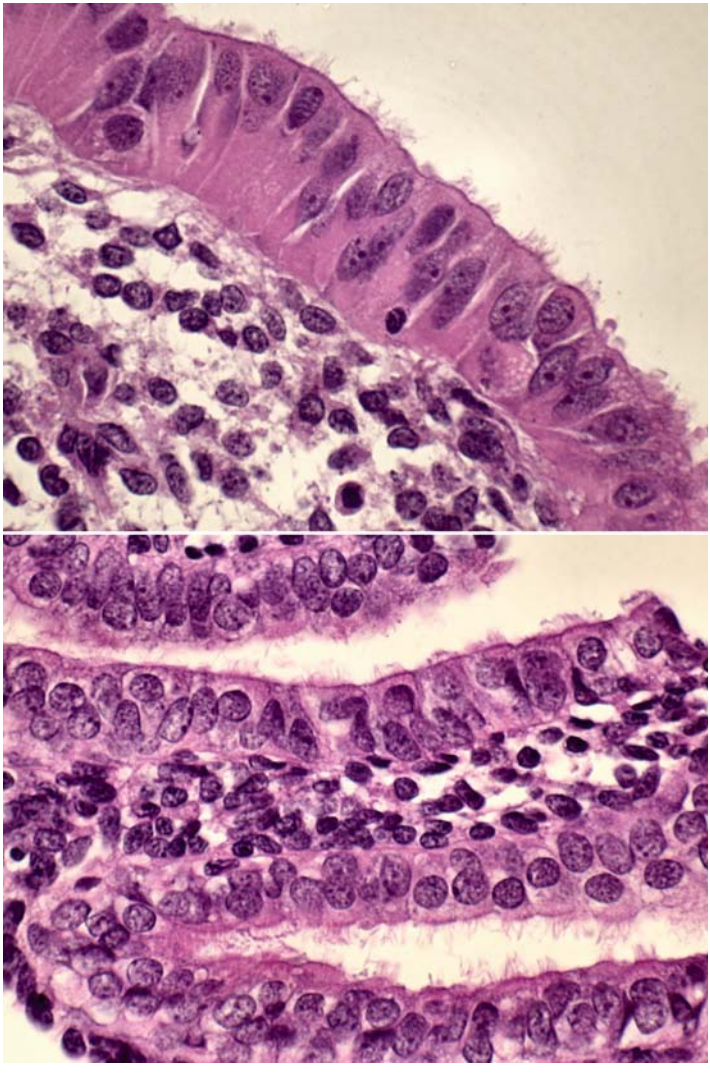

FIG. 7.8. Ciliated or tubal differentiation is the most common form of endometrial metaplasia. In the *lower image*, groups of cystic endometrial glands are lined by ciliated cells.

cytoplasmic volume and altered eosinophilic staining properties (Fig. 7.9). Occasional secretory cells are interspersed between the ciliated cells in a pattern resembling that of the normal fallopian tube.

Tubal differentiation may be induced by estrogen exposure or may be a manifestation of a neoplastic clone. A difficult, but practical, problem is the significance that should be given to finding tubal metaplasia. Ciliated cell change is frequent in the normal proliferative endometrium, but it also occurs in about half of well-differentiated endometrioid endometrial adenocarcinomas. Endometrial intraepithelial neoplasm (EIN) lesions with localized clonal architecture may show tubal differentiation within or even delimiting the neoplastic glands. We believe that little significance should be attributed to ciliation other than it is a sign of a high degree of differentiation and usually is related to an estrogen stimulus.

Eosinophilic Cell Change, Mucinous, Clear Cell, and Hobnail Metaplasia

Eosinophilic cell change is one of the most common endometrial metaplasias occurring in both nonneoplastic and neoplastic endometrium. When focal and associated with a simple lining epithelium, the change is benign (Fig. 7.10). This change is most commonly encountered within endometrial polyps, but it may occur within the endometrial glands of the functionalis layer, usually in an inflammatory setting. It is frequently seen in endometrial hyperplasia and carcinoma. Eosinophilic cell change can be associated with a variety of endometrial alterations such as mucinous, ciliated cell, squamous, and papillary metaplasia.

Endometrial changes exhibiting cytoplasmic eosinophilia have been proposed to represent subtypes of immature mucinous metaplasia. Among endometrial carcinomas, eosinophilic cell change is frequently associated with mucinous metaplasia. The two types of metaplastic cells may occasionally be intermingled in a single neoplastic gland. Eosinophilic cell change may represent an immature stage of mucinous metaplasia that expresses mucin core protein which has not been fully glycosylated. In noncancerous

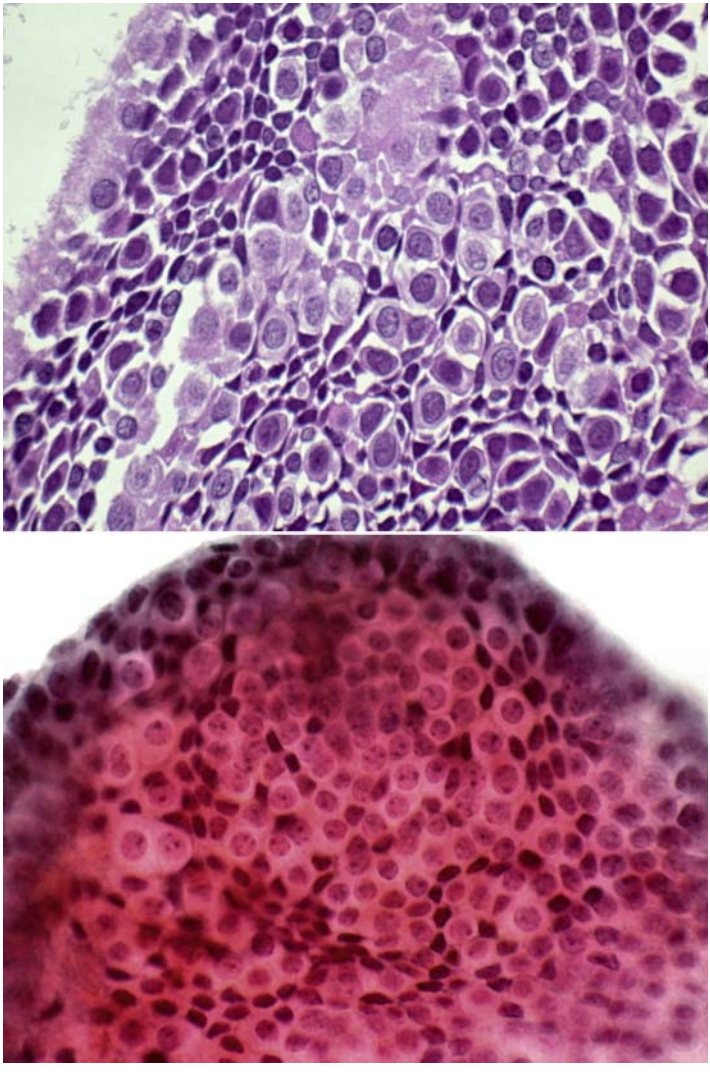

FIG. 7.9. Ciliated cells are distinctive and evident at low magnification or in *en face* preparations of endometrial epithelial sheets whereby they comprise both increased cytoplasmic volume and increased eosinophilic staining properties.

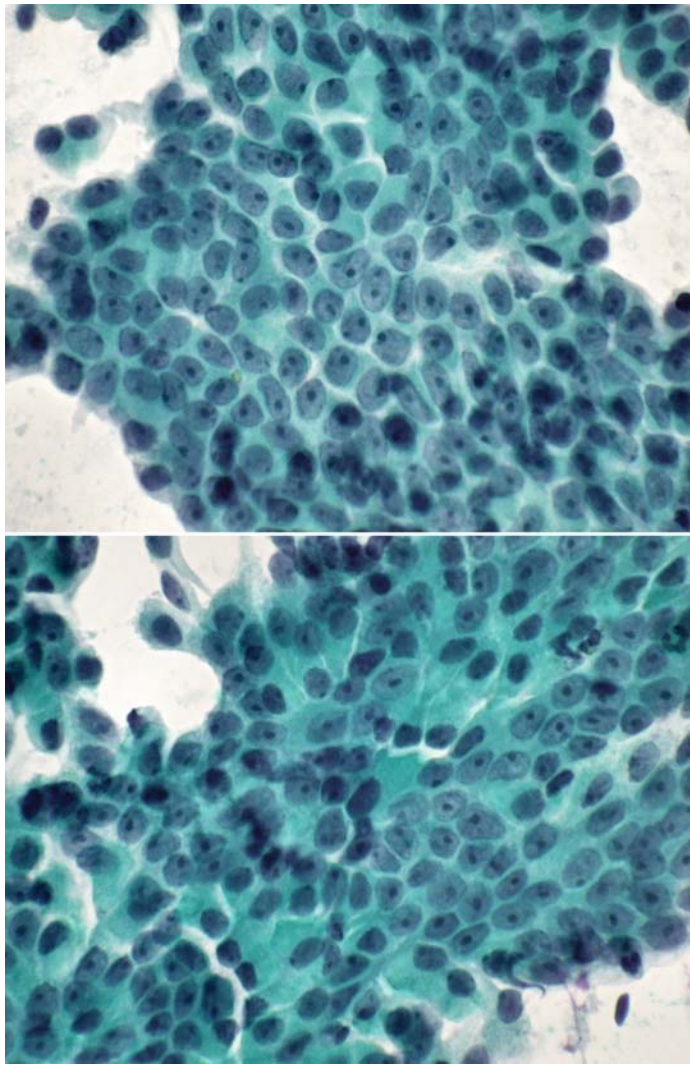

FIG. 7.10. Many of the endometrial changes characterized by cytoplasmic eosinophilia may be subtypes of immature mucinous metaplasia that express a mucin core protein but are not fully glycosylated; and, among endometrial carcinomas, eosinophilic cell change is frequently associated with mucinous metaplasia and the two types of metaplastic cells may occasionally be intermingled in a single neoplastic gland.

endometrium, the cells of eosinophilic metaplasia may be stable. In endometrial carcinoma, because of the relative genetic instability, a transition between eosinophilic cell change and mucinous metaplasia may occur.

Mucinous proliferations with a simple architecture are benign; however, more complex growth patterns have a cancer risk that is roughly proportional to their degree of complexity. Attempts have been made to categorize the mucinous proliferations. In one three-class designation, type A shows mucin-containing epithelial cells, present singly or in small tufts, within architecturally benign glands or involving the endometrial surface (Figs. 7. 11, 7.12). Type B exhibits proliferations that are more complex, consisting of mucin-containing epithelial cells forming small pseudoglands with rigid, punched-out spaces and no supporting stroma (Figs. 7.13, 7.14). Type C demonstrates proliferations showing conspicuous cytological atypia or architectural features such as a filiform growth pattern (Fig. 7.15 and Fig. 7.17). The cancer risk of these proliferations was estimated as 0%, 65%, and 100%, respectively. Thus, the lack of glandular crowding, the absence of a complex exophytic architectural pattern, and the lack of nuclear atypia are key elements in separating benign mucinous change from a neoplastic process.

When especially florid and inflamed, microglandular hyperplasia of the uterine cervix enters into the differential diagnosis of mucinous metaplastic states of the endometrium, especially well-differentiated and microglandular mucinous adenocarcinoma of the endometrium (Fig. 7.16). Subnuclear vacuoles, luminal squamous metaplasia, and luminal neutrophils are seen with microglandular hyperplasia whereas stromal foam cells appear exclusive to mucinous adenocarcinoma. Vimentin is expressed in 90% of mucinous adenocarcinomas of the endometrium but is absent in microglandular hyperplasia (Fig. 7.17). A higher percentage of mucinous adenocarcinoma cells are reactive with KI-67 (MIB-1) (>10% nuclear decoration) compared to microglandular hyperplasia (<1% nuclear decoration).

Mucinous (Fig. 7.18) and clear cell (Fig. 7.19) metaplasia occur in women taking tamoxifen. In two series of 10 and 12 cases in which tamoxifen therapy for breast carcinoma was associated with endometrial carcinoma or endometrial carcinoma appeared after

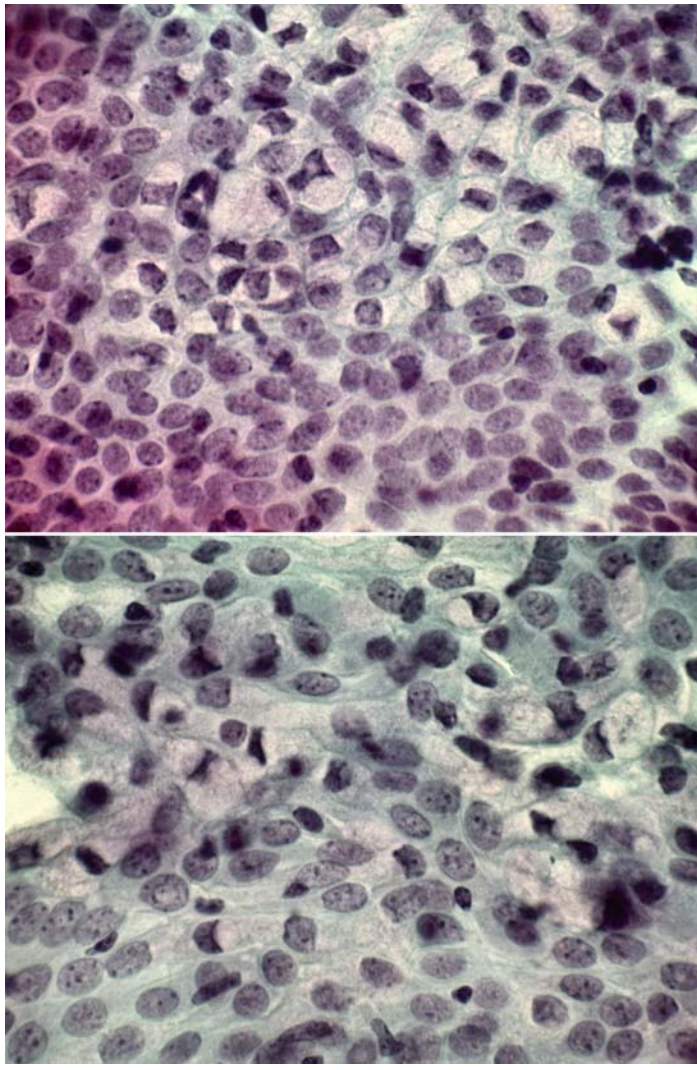

FIG. 7.11. Type A endometrial mucinous metaplasia (of Nucci). Benign tubular glands or flat uterine lining surfaces sometimes show mucinous epithelium with a simple architecture; these may be single layers of columnar epithelium with focal cytoplasmic mucin droplets.

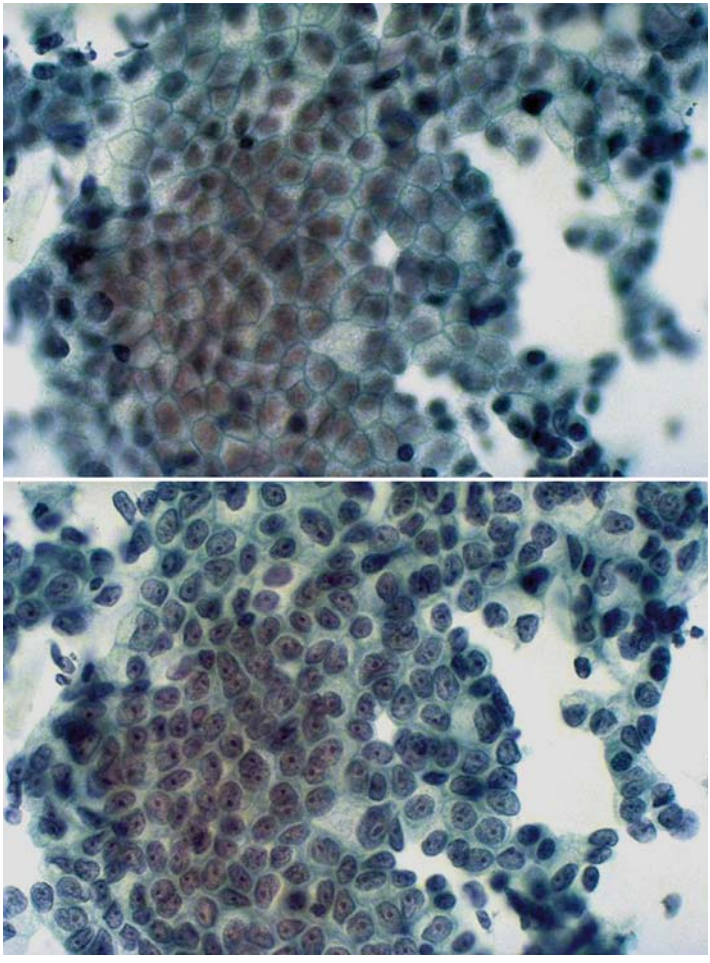

FIG. 7.12. Type A endometrial mucinous metaplasia. Less commonly mucinous metaplasia comprises rows or sheets of tall, columnar cells that are identical to those seen in the endocervix with basally located nuclei and abundant vacuolated, mucin-rich cytoplasm.

therapy was begun with synthetic gestagens, respectively, all but 1 case were of the mucinous or clear cell type, and all arose in atrophic endometria containing foci of mucinous (endocervical-like) and clear cell metaplasia.

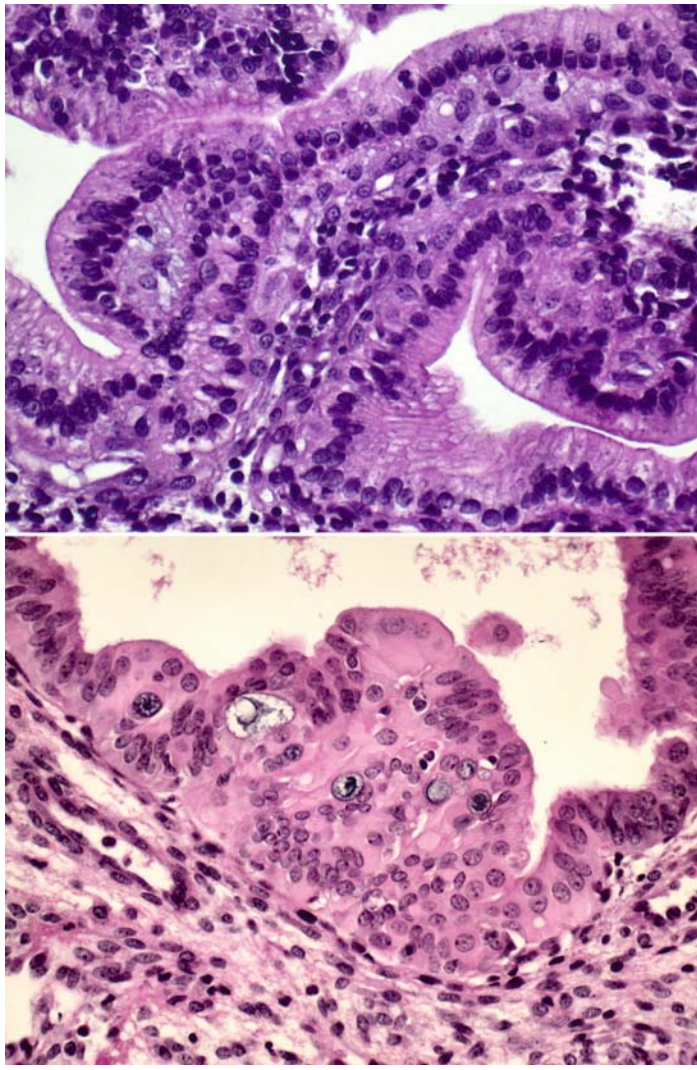

FIG. 7.13. Type B endometrial mucinous metaplasia. This type comprises proliferations that are more complex, consisting of mucin-containing epithelial cells forming small pseudoglands with rigid, punched-out spaces and no supporting stroma.

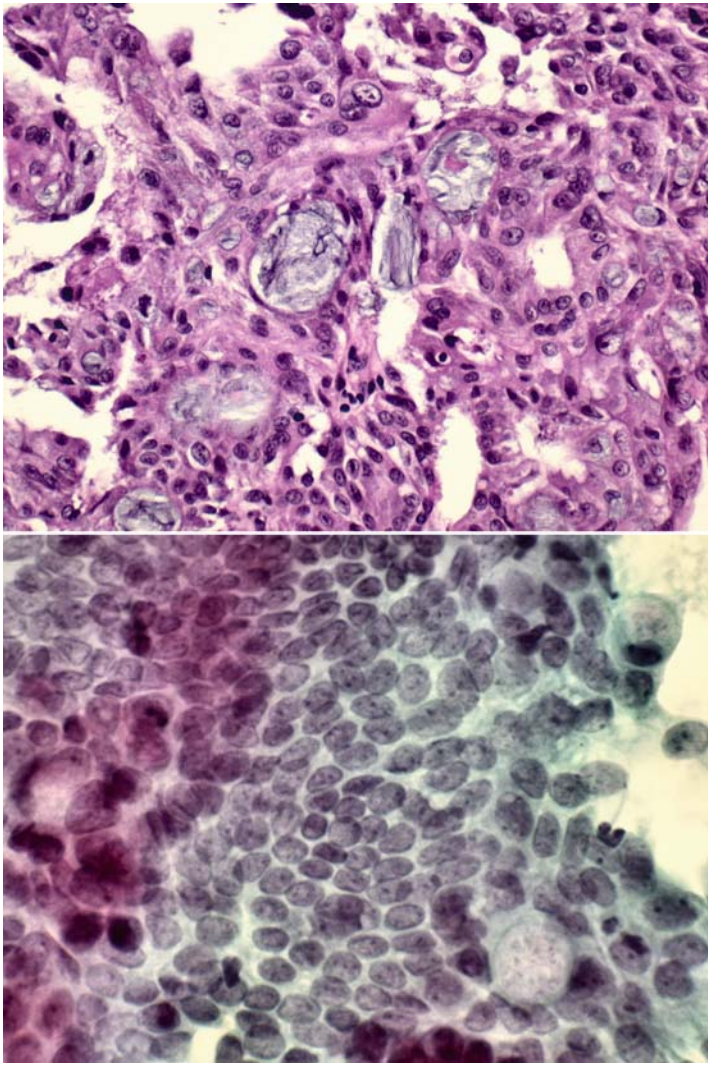

FIG. 7.14. Type B endometrial mucinous metaplasia. This proliferation shows a nearly cribriform growth pattern in the cell block (*top*) but a rather low-grade nuclear atypia in the cytology brush material (*bottom*). The complexity of the architecture is reflected in the piled-up appearance of cells in the *left side* of the *bottom image*.

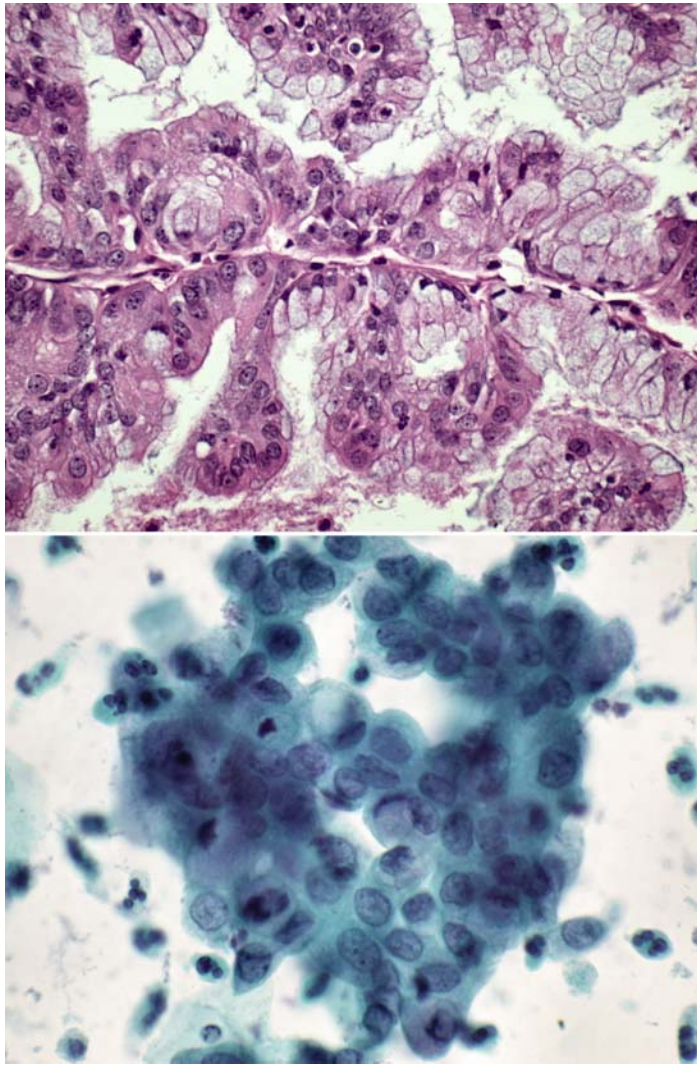

FIG. 7.15. Type C endometrial mucinous metaplasia. In this case, there is a filiform or villous architecture, and the brush cytology preparations reflect the complex architectural arrangement and the intracytoplasmic production of mucin.

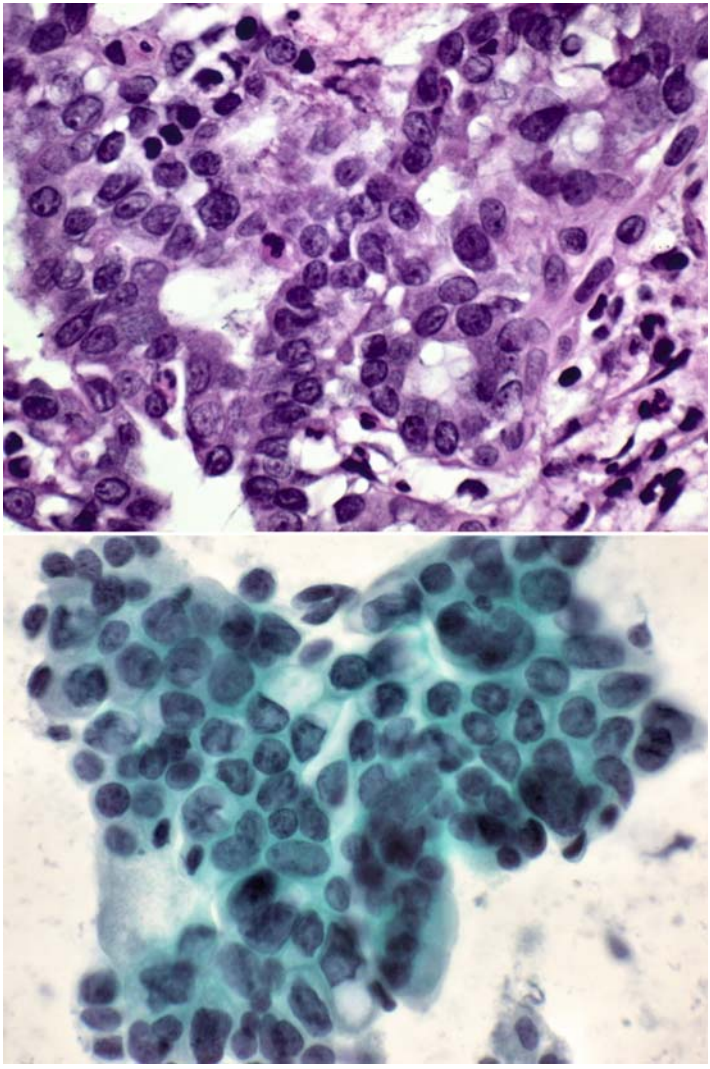

FIG. 7.16. When especially florid or inflamed, microglandular hyperplasia of the uterine cervix figures into the differential diagnosis of mucinous metaplastic states of the endometrium, especially well-differentiated mucinous or microglandular adenocarcinoma of the endometrium. Compare the similarity of these cells with those shown in Fig. 7.15. The immunostaining pattern of the endocervix is generally CEA > > vimentin (which is generally negative) and low Ki-67.

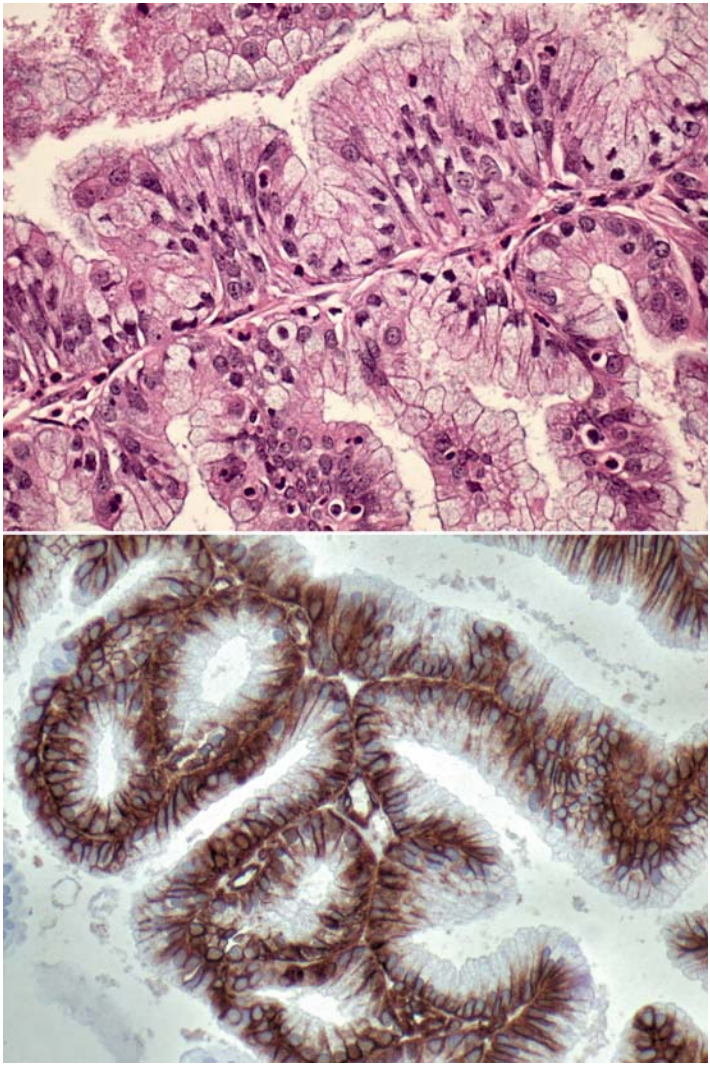

FIG. 7.17. Type C endometrial mucinous metaplasia; hematoxylin and eosin stain (*top*) and vimentin immunostain (*bottom*). Vimentin is expressed in >90% of mucinous adenocarcinomas of the endometrium but is virtually absent in microglandular hyperplasia of the endocervix.

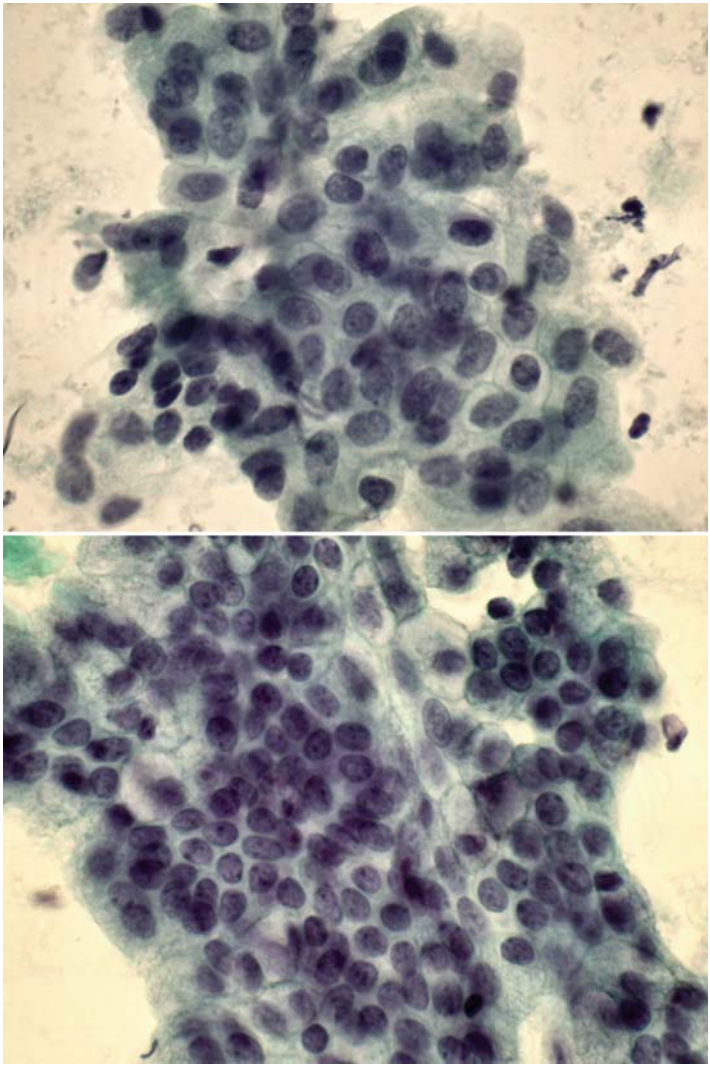

FIG. 7.18. Brush cytology from a woman being monitored while on tamoxifen. These images show nonatypical type A mucinous metaplasia.

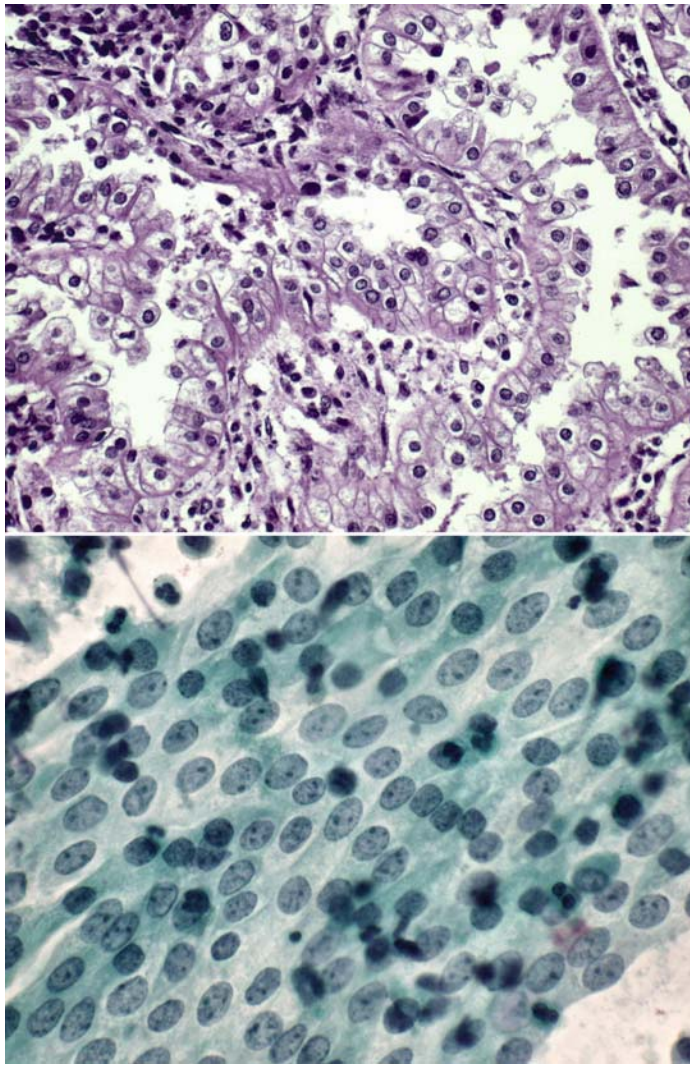

FIG. 7.19. Cell block preparation (*top*) from a woman being monitored while on tamoxifen. These images show clear cell metaplasia. The cytology (*bottom*) shows distinct islands of epithelial cells with clear cytoplasm were thought to correspond to the foci of clear cell metaplasia. Note the orderly architecture and the benign-appearing nuclear features. The cytoplasm almost has secretory-like characteristics. A follow-up hysteroscopy and biopsy showed no evidence of clear cell carcinoma.

In the endometrium, the anti-estrogenic action of tamoxifen closely resembles the antiproliferative action of synthetic gestagens on the endometrium. In the endocervix, tamoxifen and synthetic gestagens stimulate the endocervical mucosa to proliferate. In the endometrium, both tend to produce mucinous (endocervical-like) and clear cell metaplasia. A weak estrogenic action of tamoxifen augments its anti-estrogenic gestagen-like action. Thus, the anti-estrogenic effect of tamoxifen is most likely responsible for the development of endocervical metaplasia and possibly corresponding endocervical and clear cell types of carcinomas within an atrophic endometrium.

Hobnail cell metaplasia is rare, and likely represents a variety of etiologies including exfoliation artifact, degenerative changes associated with underlying necrosis, pseudo-Arias–Stella-like changes and changes associated with endometrial polyps. On morphological grounds, hobnail cell metaplasia exhibits glandular cells with rounded, apical nuclear protrusions that extend beyond the apparent cytoplasmic borders of the individual epithelial cells (Fig. 7.20). Clear cell metaplasia shows cells with abundant, clear cytoplasm (Fig. 7.19). Before considering the diagnosis of clear cell metaplasia, the diagnosis of clear cell adenocarcinoma and the Arias–Stella phenomenon of pregnancy need to be excluded.

Metaplasia in Neoplasia

Various epithelial metaplasias occur with endometrioid neoplasia. Cilia are seen more often with precancerous states and with well-differentiated adenocarcinomas. This is not surprising, because ciliation reflects a highly differentiated estrogen-primed cell. In one study, more than half of women with endometrial cancer had one or more areas of metaplasia in the endometrium adjacent to their neoplasm, and the presence of ciliated cells was by far the most common (73%).

Women with both endometrial carcinoma and metaplasia, as compared to those with carcinoma without metaplasia, were significantly younger and presented with well-differentiated tumor, absence of myometrial invasion, and lack of pelvic lymph node metastases. As an estrogenic event, the presence of metaplasia

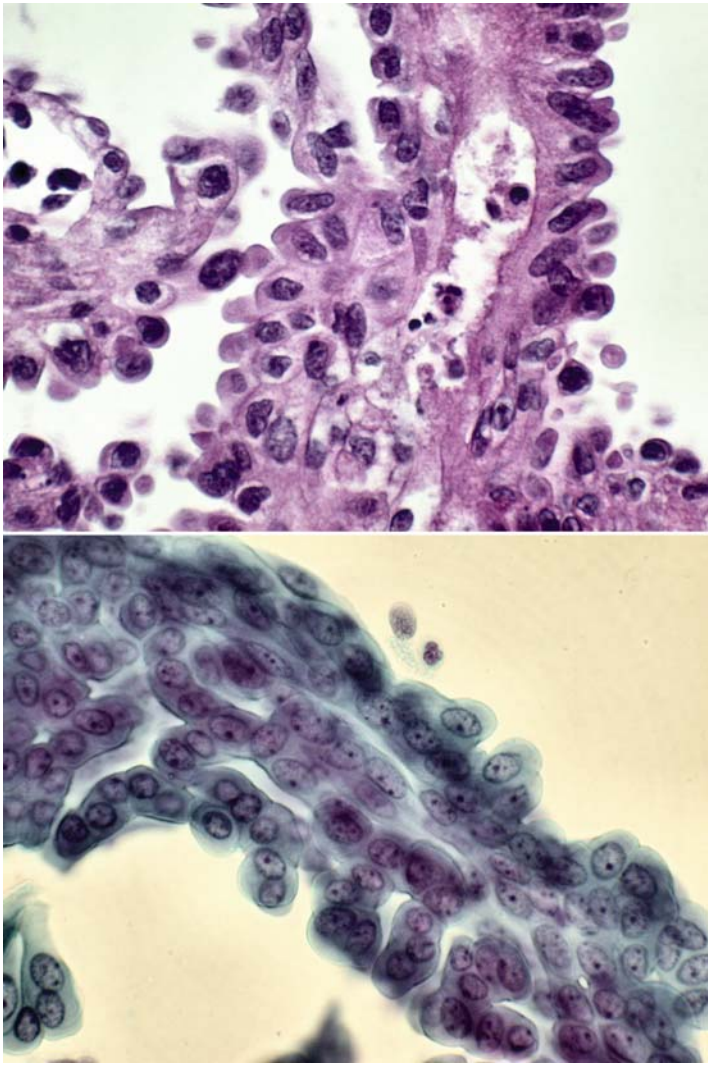

FIG. 7.20. Hobnail cell metaplasia exhibits glandular cells with rounded, apical nuclear protrusions that extend beyond the apparent cytoplasmic borders of the individual epithelial cells.

significantly correlated with the presence of coexisting endometrial hyperplasia.

Foam Cells

Foam cells may be seen with endometrioid neoplasms and with benign endometrial abnormalities. Historically, it was thought that two histologically similar-appearing foam cells existed: a histiocytic-like, "nonestrogenic" reactive type capable of phagocytosis whose cytoplasm contains lipofuscin and/or hemosiderin (Fig. 7.21); and a stromal-like, "estrogenic" nonphagocytic type whose cytoplasm lacks such pigmentation. The "estrogenic" type was thought to be associated with hyperplasia or carcinoma (Fig. 7.22; see Fig. 7.24), and the "nonestrogenic" type was thought to be associated with endometrial breakdown.

In reality, the foam cells are macrophages and not endometrial stromal cells, as confirmed by immunoreactivity with macrophage-specific antibodies. What is most important about foam cells is that, when found without underlying pathology, further study of the endometrium needs to be undertaken to rule out cancer.

Foam cells generally have relatively large, centrally placed, ovoid to bean-shaped nuclei with small nucleoli and a fine chromatin pattern. In some cases of endometrial hyperplasia and endometrial breakdown we have seen, foam cells exhibit filiform cytoplasmic processes appearing to comprise weakly associated granulomas. We interpret the filiform processes as nondestructive intercalation of the foam cell cytoplasm with stromal tissue. Foam cells appear relatively uniform when compared one to another on a case-by-case basis but may show substantial heterogeneity between cases. As is the case with other histiocyte accumulations, foamy giant cells may be seen (Fig. 7.23).

Foamy histiocytes are commonly seen in the endometrial stromal compartment of patients with a carcinoma. Up to 20% of carcinoma cases contain stromal cells that are lipid laden, but no correlation exists between the presence of these cells and the grade of the tumor or the survival of the patient. This change is simply a reactive response to tumor cells that have died. The presence of such histiocytic cells in endometrial biopsies showing any atypia

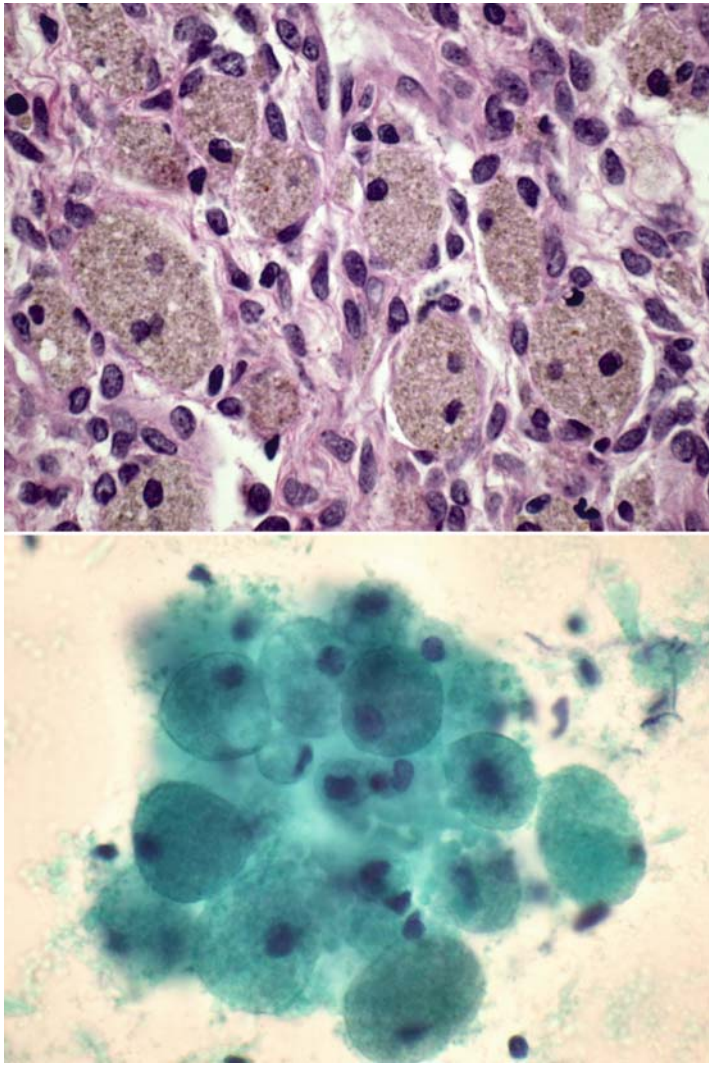

FIG. 7.21. A histiocytic-like, "nonestrogenic" reactive-type foam cell capable of phagocytosis, whose cytoplasm contains lipofuscin and/or hemosiderin.

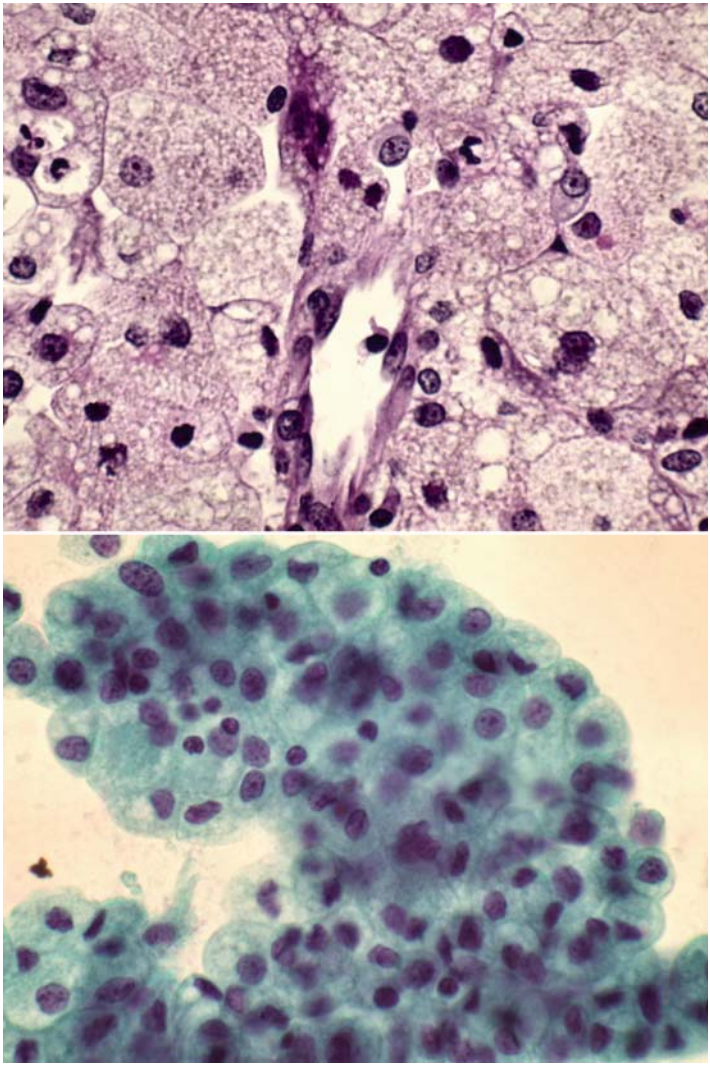

FIG. 7.22. A stromal-like, "estrogenic" type of foam cell. This "estrogenic" type of foam cell was thought to arise from endometrial stroma and to be associated with hyperplasia or carcinoma; however, present evidence indicates that all foam cells are of histiocytic origin.

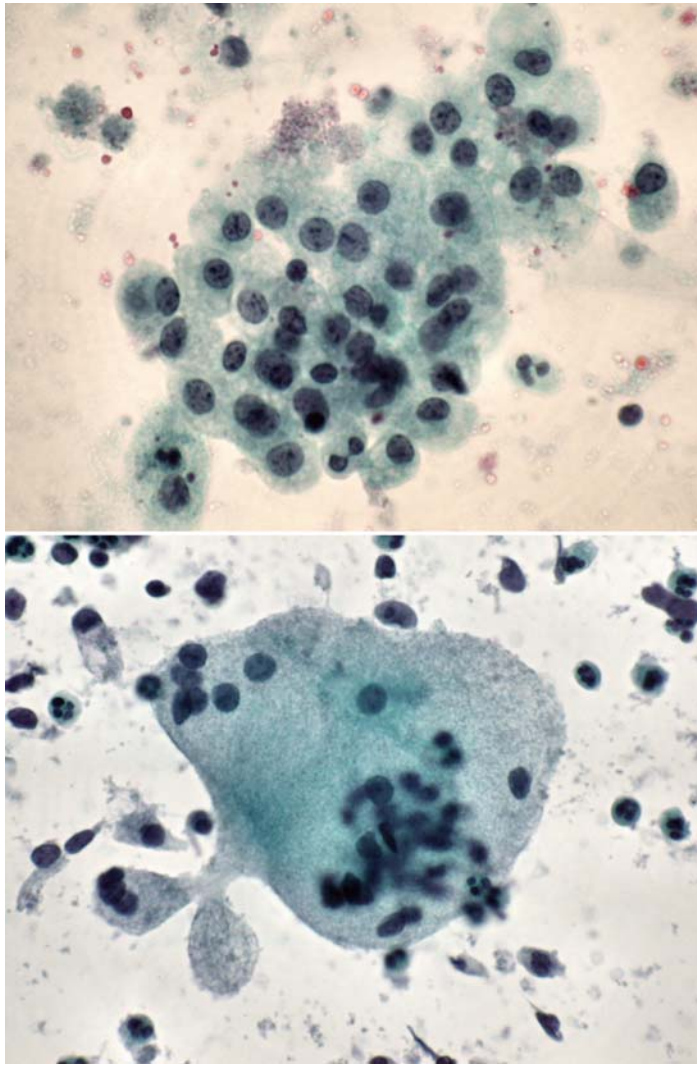

FIG. 7.23. Foam cells appear relatively uniform when compared one to another on a case-by-case basis, but may show a good deal of heterogeneity between cases (*top*). Foamy giant cells may be seen (*bottom*).

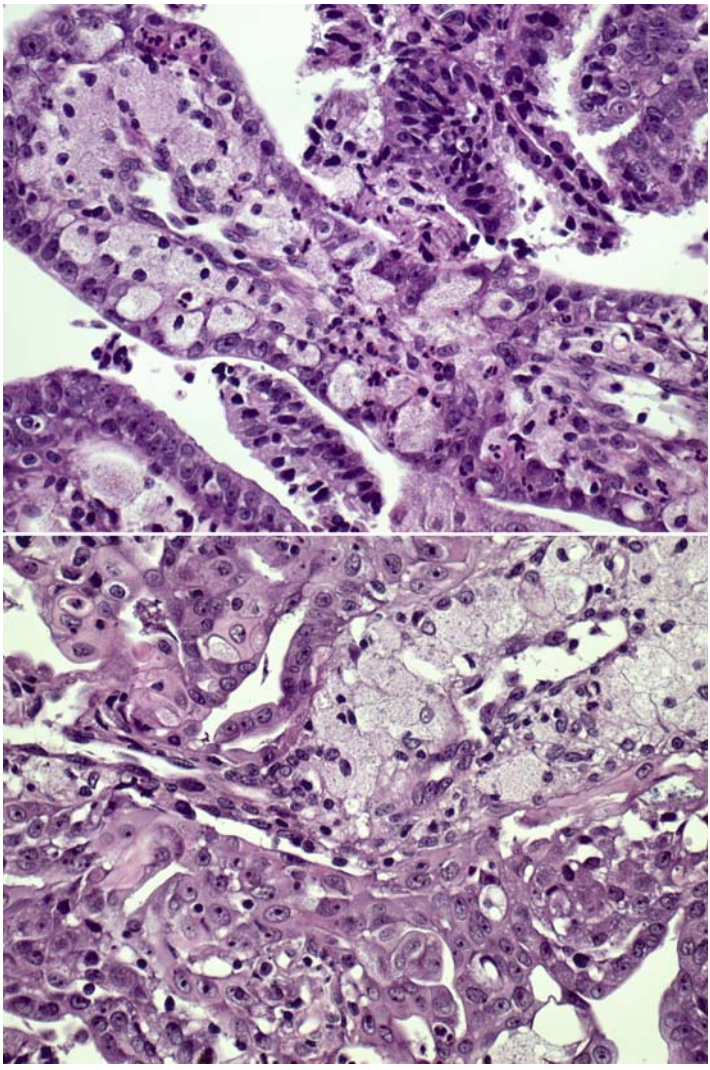

FIG. 7.24. As many as 20% of carcinoma cases contain stromal cells that are laden with lipid, but there is no correlation between the presence of these cells and the grade of the tumor or the survival of the patient. This change is simply a reactive response to tumor cells that have died. The presence of such histiocytic cells in endometrial biopsies showing any atypia should always lead to further diagnostic workup to exclude the presence of a coexistent carcinoma.

should always lead to further diagnostic workup for coexistent carcinoma (Fig. 7.24) especially as one-third of patients with endometrioid carcinoma also have areas of simple or atypical hyperplasia in the endometrium.

Suggested Reading

Ashkenazy M, Lancet M, Borenstein R, Czernobilsky B. Endometrial foam cells. Non-estrogenic and estrogenic. Acta Obstet Gynecol Scand 1983; 62: 193–7.

Brachtel EF, Sanchez-Estevez C, Moreno-Bueno G, Prat J, Palacios J, Oliva E. Distinct molecular alterations in complex endometrial hyperplasia (CEH) with and without immature squamous metaplasia (squamous morules). Am J Surg Pathol 2005; 29: 1322–9.

Chiarelli S, Buriticá C, Litta P, Ciani S, Guarch R, Nogales FF. An immunohistochemical study of morules in endometrioid lesions of the female genital tract: CD10 is a characteristic marker of morular metaplasia. Clin Cancer Res 2006; 12: 4251–6.

Chinen K, Kamiyama K, Kinjo T, Arasaki A, Ihama Y, Hamada T, Iwamasa T. Morules in endometrial carcinoma and benign endometrial lesions differ from squamous differentiation tissue and are not infected with human papillomavirus. J Clin Pathol 2004; 57: 918–26.

Connelly PJ, Alberhasky RC, Christopherson WM. Carcinoma of the endometrium. III. Analysis of 865 cases of adenocarcinoma and adenoacanthoma. Obstet Gynecol 1982; 59: 569–75.

Crum CP, Lomo L, Lin MC, Nucci MR, Mutter GL. Morular metaplasia of the endometrium revisited: a follow-up study. Lab Invest 2004; 84:195A.

Dallenbach-Hellweg G, Hahn U. Mucinous and clear cell adenocarcinomas of the endometrium in patients receiving antiestrogens (tamoxifen) and gestagens. Int J Gynecol Pathol 1995; 14: 7–15.

Dallenbach-Hellweg G, Schmidt D, Hellberg P, Bourne T, Kreuzwieser E, Dören M, Rydh W, Rudenstam G, Granberg S. The endometrium in breast cancer patients on tamoxifen. Arch Gynecol Obstet 2000; 263: 170–7.

Dutra FR. Intraglandular morules of the endometrium. Am J Clin Pathol 1959; 31: 60–5.

Fadare O. Dysplastic ichthyosis uteri-like changes of the entire endometrium associated with a squamous cell carcinoma of the uterine cervix. Diagn Pathol 2006; 1: 8.

Gilmore H, Fleischhacker D, Hecht JL. Diagnosis of chronic endometritis in biopsies with stromal breakdown. Hum Pathol 2007; 38: 581–4.

Hendrickson MR, Kempson RL. Endometrial epithelial metaplasias: proliferations frequently misdiagnosed as adenocarcinoma. Report of 89 cases and proposed classification. Am J Surg Pathol 1980; 4: 525–42.

Kaku T, Tsukamoto N, Tsuruchi N, Sugihara K, Kamura T, Nakano H. Endometrial metaplasia associated with endometrial carcinoma. Obstet Gynecol 1992; 80: 812–6.

Kaku T, Silverberg SG, Tsukamoto N, Tsuruchi N, Kamura T, Saito T, Nakano H. Association of endometrial epithelial metaplasias with endometrial carcinoma and hyperplasia in Japanese and American women. Int J Gynecol Pathol 1993; 12: 297–300.

Kendall BS, Ronnett BM, Isacson C, Cho KR, Hedrick L, Diener-West M, Kurman RJ. Reproducibility of the diagnosis of endometrial hyperplasia, atypical hyperplasia, and well-differentiated carcinoma. Am J Surg Pathol 1998; 22: 1012–9.

Jovanovic AS, Boynton KA, Mutter GL. Uteri of women with endometrial carcinoma contain a histopathologic spectrum of monoclonal putative precancers, some with microsatellite instability. Cancer Res 1996; 56: 1917–1921.

Lehman MB, Hart WR. Simple and complex hyperplastic papillary proliferations of the endometrium: a clinicopathologic study of nine cases of apparently localized papillary lesions with fibrovascular stromal cores and epithelial metaplasia. Am J Surg Pathol 2001; 25: 1347–54.

Maksem JA. Ciliated cell adenocarcinoma of the endometrium diagnosed by endometrial brush cytology and confirmed by hysterectomy: a case report detailing a highly efficient cytology collection and processing technique. Diagn Cytopathol 1997; 16: 78–82.

Moritani S, Kushima R, Ichihara S, Okabe H, Hattori T, Kobayashi TK, Silverberg SG. Eosinophilic cell change of the endometrium: a possible relationship to mucinous differentiation. Mod Pathol 2005; 18: 1243–8.

Nucci MR, Prasad CJ, Crum CP, Mutter GL. Mucinous endometrial epithelial proliferations: a morphologic spectrum of changes with diverse clinical significance. Mod Pathol 1999; 12: 1137–42.

Patton WT, Squares GV. Ichthyosis uteri. A case report. Am J Obstet Gynecol 1962; 84: 858–60.

Pins MR, Young RH, Crum CP, Leach IH, Scully RE. Cervical squamous cell carcinoma in situ with intraepithelial extension to the upper genital tract and invasion of tubes and ovaries: report of a case with human papilloma virus analysis. Int J Gynecol Pathol 1997; 16: 272–278.

Qiu W, Mittal K. Comparison of morphologic and immunohistochemical features of cervical microglandular hyperplasia with low-grade mucinous adenocarcinoma of the endometrium. Int J Gynecol Pathol 2003; 22: 261–5.

Saegusa M, Okayasu I. Frequent nuclear beta-catenin accumulation and associated mutations in endometrioid-type endometrial and ovarian carcinomas with squamous differentiation. J Pathol 2001; 194: 59–67.

Saegusa M, Hashimura M, Yoshida T, Okayasu I. Beta-catenin mutations and aberrant nuclear expression during endometrial tumorigenesis. Br J Cancer 2001; 84: 209–17.

Scholten AN, Creutzberg CL, van den Broek LJ, Noordijk EM, Smit VT. Nuclear beta-catenin is a molecular feature of type I endometrial carcinoma. J Pathol 2003; 201: 460–5.

Silver SA, Sherman ME. Morphologic and immunophenotypic characterization of foam cells in endometrial lesions. Int J Gynecol Pathol 1998; 17: 140–5.

8
Endometrial Precancer

A Background to Endometrial Precancer

A situation generally dismissed as benign by pathologists and gynecologists alike is the clinically/sonographically thin endometrium that affords a paucicellular, essentially nondiagnostic (informatively noninformative) tissue specimen. Normal postmenopausal endometrium is grossly thin and microscopically inactive and oftentimes (in up to 50% of samplings) yields no tissue, but the corollary that grossly thin (sonographically <5-mm thickness) postmenopausal endometrium is microscopically inactive and uniformly benign may be false. Endometrial suction biopsy has a reasonable sensitivity and specificity for detecting symptomatic endometrial cancer in thickened endometrium. However, few data are available regarding its sensitivity and specificity among asymptomatic postmenopausal women.

While it is well understood that every endometrial cancer has a precursor state, the assumption that the precursor of endometrial carcinoma, similar to hyperplasia, must be diffuse and symptomatic is false. Theoretically, the cells of an endometrial precancer should be genetically different from normal endometrium yet should share some but not all the features of malignant endometrium. Precancerous endometrial cells should be neoplastic (Table 8.1) and demonstrate a monoclonal growth pattern and clonally distributed (geographically clustered) mutations. Progression of endometrial

J.A. Maksem et al., *Endometrial Cytology with Tissue Correlations*, Essentials in Cytopathology 7, DOI: 10.1007/978-0-387-89910-7_9,

TABLE 8.1. Neoplastic endometrium.

Diagnosis	Hysterectomy	Average Age (years)	Office Biopsy (% with cell block)	Average Age (years)
Carcinoma	93	68	34 (91%)	66
EIN (atypical hyperplasia)	54	56	14 (71%)	56
EIN (other)	15	62	22 (52%)	65
EIC/EmGD	6	66	1 (0%)	66
Necrotic and inflammatory diathesis			1 (0%)	78
Total	168		72	

EIN, endometrial intraepithelial neoplasm; EIC, endometrial intraepithelial cancer; EmGD, endometrial gland dysplasia.

precancer to carcinoma, effectively a conversion from a benign neoplasm to a malignant neoplasm, would be expectedly accomplished through the acquisition of additional mutations and accompanied by a change in biological behavior most notably characterized by the ability of outspoken cancer cells to invade local tissues and to metastasize.

Many endometrial cancers appear to defy the hyperplasia-carcinoma model and arise in otherwise inactive-appearing or atrophic endometria. For example, in the pre-estrogen era (before 1960), hyperplasia was reported in only 8% of hysterectomies for endometrial cancer. In 1981, we found unsuspected endometrial cancer in 0.27% (24 of 8,998) of autopsied women dying of unrelated causes. Only one woman had been an estrogen user. In a another prospective series spanning two decades, the prevalence of endometrial cancer in asymptomatic women over the age of 45 years was about 7 per 1,000, a rate almost three times that which we reported. Asymptomatic cancers are almost exclusively associated with endometrial atrophy.

Tissue biopsy contributes little to case-finding or case management of thin endometria because, in the absence of gross disease, tissue biopsy may miss, destroy, or obviate the diagnosis of a thin epithelial lesion. Regrettably, the concept that endometrial cancer exists in atrophic endometrium or begins as a focal clonal event has been generally overlooked.

Most aspects of endometrial cytology are straightforward, entailing the diagnosis of typical benign or malignant endometrium. A notable exception involves finding small amounts of epithelium with atypical (cancer-like) nuclei in flat to tubulocystic epithelium, cancer-like patterns in epithelium with proliferative-like nuclei, or epithelium with atypical nuclei and cancer-like patterns scattered amidst much larger amounts of normal-appearing endometrium in the absence of gross epithelial change (as evidenced, for example, by sonohysterography, hysteroscopy, or by correlation to the gross hysterectomy specimen). When these situations occur, it is difficult to "name" the lesion and to advise the clinician on how to manage the patient.

The differential diagnosis of these findings, especially against the background of sonographically normal-appearing postmenopausal endometrial epithelium includes a variety of possibilities. These variations take in "activated" benign endometrium (as caused, for example, by the irritation effects of a polyp or leiomyoma) including sheets of activated subepithelial epithelioid stroma, lower uterine segment collection in which epithelial nuclei may have symplastic nuclear features or features representing a changeover between endocervix and endometrium, including the proliferation of otherwise normal but irritated mucinous elements in the case of microglandular hyperplasia, cancer metastatic to the endometrium (as seen, e.g., with gastric, breast, endocervical or ovarian cancer), isolated foci of endometrial intraepithelial neoplasm (EIN, including what has been called "adenomatous change" and endometrioid microcancer), endometrial intraepithelial cancer/endometrial gland dysplasia (EIC/EmGD; i.e., lesions related to the serous form of endometrial cancer), and putative precursors of endometrial clear cell carcinoma.

Conceptual Basis of EIN

Prolonged estrogen exposure that is unopposed by progestins results in an abnormal endometrial histology that has long been thought to be associated with an increased risk for endometrioid (type 1) endometrial adenocarcinoma. The risk, which is increased 2- to 10 fold with unopposed estrogen exposure in a

dose- and duration-dependent manner, has been calculated from large epidemiological studies of cancer outcomes in women with known hormonal exposure. Endometrial hyperplasia is a condition of excessive proliferation of endometrial epithelial cells. Most cases result from high levels of estrogens, combined with insufficient levels of the progesterone-like hormones that ordinarily counteract estrogen's proliferative effects, as may occur in a number of settings, including the polycystic ovary syndrome and with certain formulations of estrogen replacement therapy.

Endometrial hyperplasia is a significant risk factor for the development of endometrial cancer. Pathological confirmation of endometrial changes secondary to unopposed estrogen exposure helps to establish that there is an abnormal hormonal state, but it is of less value in determining which specific patients are most likely to get cancer and which specific histological changes are the immediate precursors to endometrial cancer.

In the past, both generalized hormonal responses and localized premalignant lesions have been lumped under the term "endometrial hyperplasia" and further subdivided by architectural complexity and cytological atypia. Endometrial hyperplasia without nuclear atypia with either a simple or complex pattern shows irregularity and cystic expansion of glands (simple) or crowding and budding of smoothly outlined glands (complex) without worrisome changes in the appearance of individual gland cells. In one study, 1.6% of patients diagnosed with these abnormalities eventually developed endometrial cancer.

Atypical endometrial hyperplasia, again, with either simple or complex patterns, shows worrisome (atypical) changes in gland cells, including cell stratification, tufting, loss of nuclear polarity, enlarged nuclei, and an increase in mitotic activity. These changes are similar to those seen in true cancer cells, but atypical hyperplasia does not show invasion into the connective tissues, which is one of the defining characteristics of endometrial cancer. The previously mentioned study found that 22% of patients with atypical hyperplasia eventually developed cancer.

Recent molecular studies have provided evidence that the use of the term hyperplasia is conceptually correct for some lesions. However, contrary to long-standing assumptions, the change from benign endometrium to endometrioid adenocarcinoma is not the

consequence of a gradual morphological progression in which a continuous histological spectrum of changes is related to a progressively increased endometrial cancer risk. Rather, it is the precipitous emergence of endometrial intraepithelial neoplasia (EIN) that portends increased cancer risk.

This concept is in keeping with observations that endometrial glands themselves may be composed of monoclonal populations of epithelial cells, and many normal adjacent glands within an area of 1–2 mm diameter may also share clonality. The clonality of the luminal epithelium is also regionally defined. The clonality of the endometrium has been demonstrated in studies of female mice harboring the green fluorescent protein gene on either the maternal or paternal X chromosome. Fluorescent microscopy of uterine sections reveals that individual endometrial glands consist completely of either fluorescent or nonfluorescent cells and that the corresponding surface epithelium exhibits a clear boundary between these cell types, which exist as localized domains of cells on the uterine epithelial surface.

These findings suggest that single or multiple stem cells with uniform clonality exist on the bottom of each endometrial gland. Genetic alterations occurring in such cells play a critical role in endometrial carcinogenesis.

Endometrioid endometrial precancers are monoclonal, noninvasive neoplasms that are prone to malignant transformation, originate focally, and expand in size over time, which is in keeping with a proliferative monoclonal origin. They exhibit closely packed glands with cytology that, although not necessarily malignant appearing, is clearly demarcated from that of the adjacent field. Those cases with a largest diameter of at least 1–2 mm, which is also the putative size of the monoclonal endometrial domain of the mouse model, predict concurrent or future endometrial adenocarcinoma.

Nonphysiological loss of PTEN protein, a product of a tumor suppressor gene mutated in many endometrioid adenocarcinomas, is seen in individual glands of endometrium exposed to unopposed estrogens and in packed clusters of EIN glands. Furthermore, isolated PTEN-free glands in anovulatory endometria may be the earliest stage of endometrial tumorigenesis, but these glands are not readily distinguishable by routine histology or cytology, nor do they have a defined natural history.

In summary, the subset of largely polyclonal proliferations that result from a physiological response of the endometrium to an abnormal estrogenic stimulus defines hyperplasia. In contrast, EIN is a clonal proliferation that has the characteristics of a noninvasive endometrioid neoplasm.

Histological EIN

Histological EIN displays a monoclonal growth of mutated cells, a distinctive histopathological appearance, and a significant risk for concurrent presence or future development of endometrioid adenocarcinoma. Criteria for diagnosing histological EIN include gland area greater than stromal area, cytological change in foci of altered architecture appearing not necessarily atypical but dichotomous with surrounding normal endometrium, lesion size >1 mm, and exclusion of outspoken cancer and cancer mimics. Histological EIN is not graded. It is either present or absent.

Histological EIN can be diagnosed on routine surgical pathology material without the aid of computerized morphometric devices. For example, in a study addressing the subjective reproducibility of histological EIN, the diagnoses of "presence" or "absence" were unanimous among pathologists in 75% of cases, and intraobserver reproducibility was very good.

Cases diagnosed as histological EIN encompass hyperplasias previously diagnosed as atypical or nonatypical, the latter because features other than frankly atypical nuclei figure in its diagnosis. The subjective application of criteria for diagnosing histological EIN correlates well with objective morphometry, predicts disease progression more accurately than the 1994 World Health Organization (WHO) hyperplasia classification, and identifies women with benign changes that would have been regarded as "high risk" according to the WHO classification system. Histological EIN includes lesions within and beyond the atypical hyperplasia to carcinoma spectrum.

Both the relative excess of glands (in comparison to the volume of stroma within the histological EIN focus) and the irregularity of the endometrial gland margins figure strongly in the diagnosis of histological EIN. For example, gland margin irregularity has been

mathematically assessed in hyperplasia, atypical hyperplasia, and well-differentiated endometrial carcinoma to discriminate between these lesions based on the fractal dimension of gland architecture.

A fractal is generally a rough or fragmented geometric shape that can be split into parts, each of which is (at least approximately) a reduced-size copy of the whole, a property called self-similarity. The term, introduced by Benoît Mandelbrot in 1975, is derived from the Latin "fractus," meaning broken or fractured. Thus, a "fractal dimension" comprises an objective measurement of the degree of gland complexity per unit area.

The fractal dimension of hyperplasia has been shown to significantly differ from that of atypical hyperplasia and endometrial carcinoma. However, no difference exists in fractal dimensions between the glands of atypical hyperplasia and of well-differentiated endometrial carcinoma.

Cytological EIN

The dilemma faced by cytopathologists is that frank nuclear atypia is not required for the diagnosis of histological EIN. What is necessary is a dichotomous nuclear morphology as compared with surrounding normal endometrial epithelium. This dichotomy is difficult, if not impossible, to resolve in cytological material; this means that in the absence of outspoken nuclear atypia, what is histological EIN may be underdiagnosed by cytological methods that are not augmented by cell block preparations. As many of these cases are gleaned from postmenopausal individuals, cell blocks may not be available for review.

Flat epithelial sheets with "cancer-like" nuclei can be seen in an atrophic background. We studied 237 late postmenopausal uteri from women 65 years or more of age that were removed for symptomatic prolapse in which the entire endometrium was examined after endometrial brushing. None of the uteri had ovarian or significant uterine muscular abnormalities or polyps. Twenty-seven atypical cytology cases led us to discover 22 abnormal histologies that comprised 6 p53-positive EIC/EmGDs, 12 instances of "adenomatous change" comprising small, geographically localized epithelial foci architecturally resembling atypical hyperplasia,

and 4 endometrioid microcancers. In the 12 cases of "adenomatous change," all lesions were focal, isolated, crowded, and showed significant dichotomy in nuclear features between the lesions and the adjacent atrophic endometrium.

In our office-based brushings series, findings of this type were about twice as common as outspoken atypical hyperplasia (23 vs. 14 discoveries, respectively). Today, with the exclusion of cases that show simultaneous strong p53/Ki-67 nuclear decoration, most of these lesions are classified as EIN. Others have found foci of endometrial epithelium with proliferative-like features (as evidenced by upregulated Ki-67 staining) in half of clinically disease-free postmenopausal uteri removed for prolapse and in more than 80% of postmenopausal endometria harboring adenocarcinoma elsewhere. This indicates a dynamic, yet lurking, potential for atrophic endometrium to provide the bed from which endometrial adenocarcinoma can arise on the basis of focal clonal expansion as opposed to diffuse transformation.

Thus, cytological EIN comprises, in addition to "atypical hyperplasia" or "endometrioid neoplasia," atypical epithelium that may be found in atrophic endometrium, including atrophic regions of endometrial cancers. It may be a microscopic finding without a gross counterpart that features grades 2 or 3 nuclei and presents as isolated glands, gland clusters, or altered surface epithelium amidst otherwise unaltered fibrous endometrial stroma (Figs. 8.1–8.5).

Similar to atypical hyperplasia and endometrial carcinoma, cytological EIN generally is associated with clustered domains of nuclei showing significant upregulated proliferative activity (10%–50% nuclear decoration using Ki-67 immunostaining), oftentimes in a background of endometrial atrophy. The identification of clustered surface epithelial cell domains is in keeping with monoclonal domains of surface epithelium that have been described in normal mouse endometrium.

In addition to flat epithelial sheets with nuclear atypia, cytological EIN may show rare, small epithelial foci architecturally resembling atypical hyperplasia with structures such as intertwining epithelial bridges, epithelial cysts with activated nuclei showing upregulated proliferative activity, or distorted microacini, all indicators less of epithelial structural instability and more of high fractal dimension. Larger amounts of nearly identical structures are seen with brushings

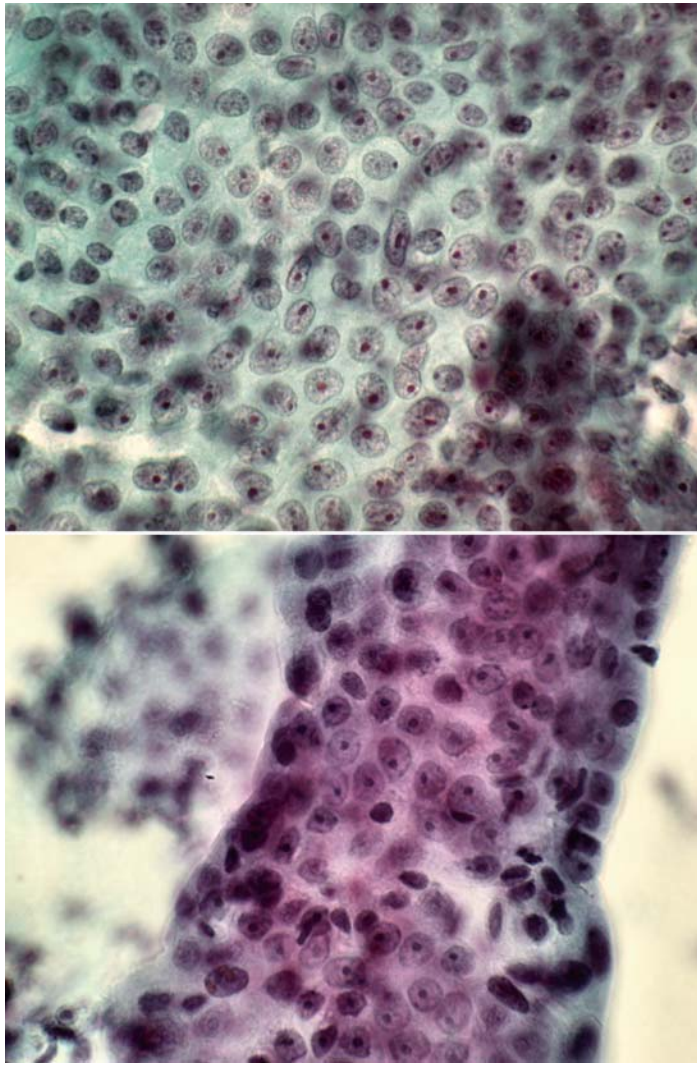

FIG. 8.1. Cytological endometrial intraepithelial neoplasm (EIN). This is an endometrial brush cytology in which occasional atypical-appearing flat epithelial sheets and atypical tubuloglandular structures were scattered amidst larger amounts of otherwise atrophic endometrium with fibrocollagenous stroma. p53 and Ki-67 immunostaining was performed on additional cytology material, as shown in Fig. 8.2.

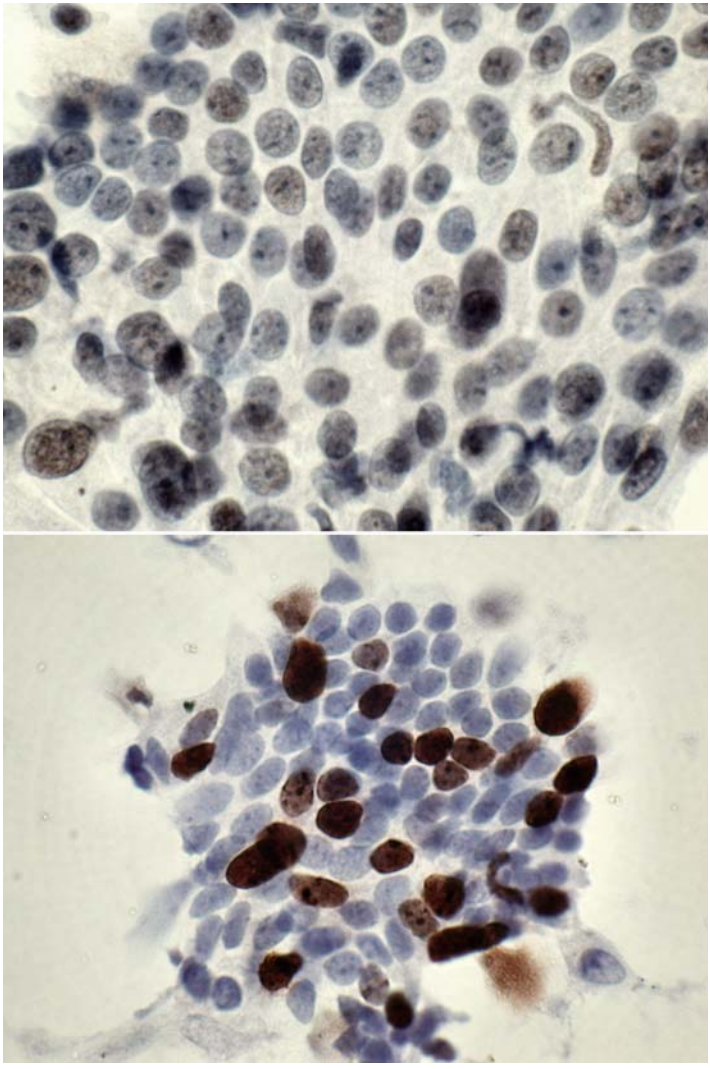

FIG. 8.2. Cytological EIN. Immunohistochemical staining for p53 (*top*) and Ki-67 (*bottom*) show only faint p53 nuclear decoration but significant Ki-67 staining in the nuclei of isolated epithelial sheets, far exceeding the Ki-67 decoration of accompanying atrophic epithelium. The staining pattern of this case is similar to that seen with EIN that would previously been diagnosed as atypical hyperplasia, which is shown for comparison in Figs. 8.3 and 8.4.

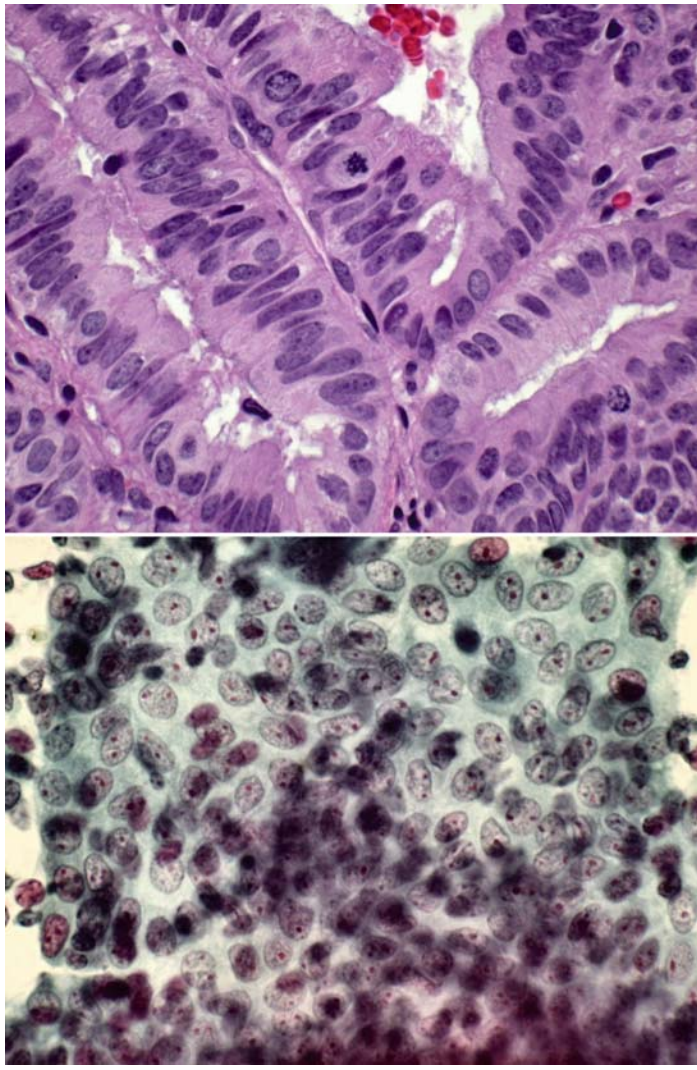

FIG. 8.3. EIN that would previously been diagnosed as atypical hyperplasia. In this case, there is marked gland crowding in cell block material (*top*) and moderate nuclear atypia in the brush cytology preparation (*bottom*). Immunohistochemical staining of the cell block preparation for p53 and Ki-67 is shown in Fig. 8.4.

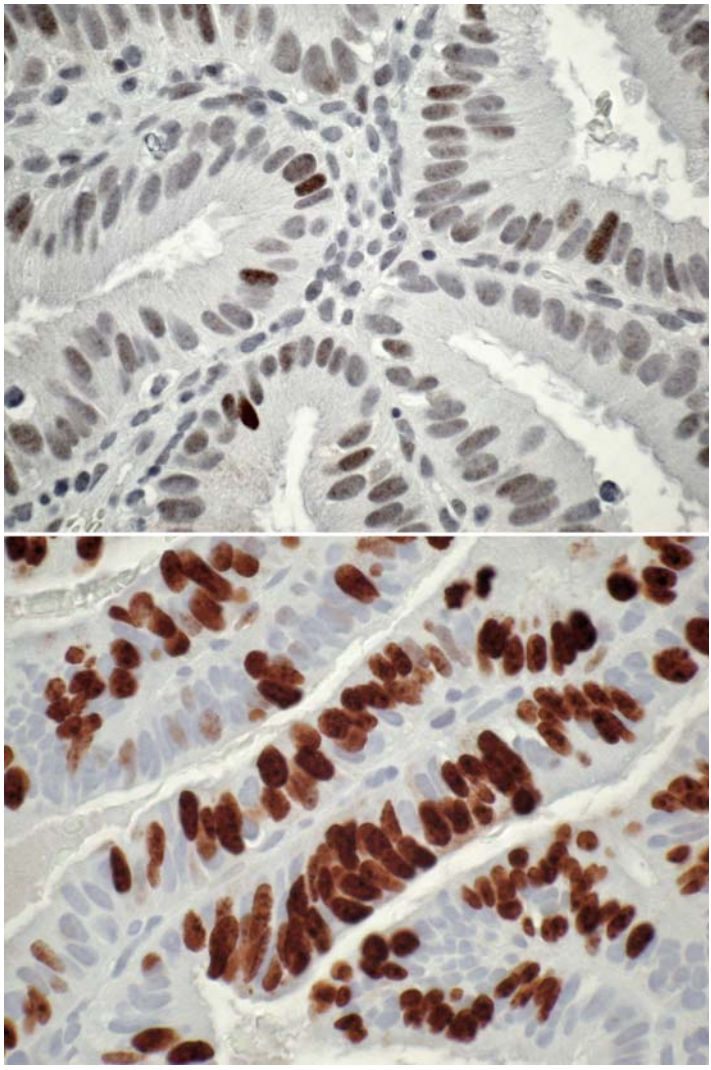

FIG. 8.4. EIN that would have previously been called atypical hyperplasia. Immunohistochemical staining for p53 (*top*) and Ki-67 (*bottom*) show only faint p53 nuclear decoration but significant Ki-67 nuclear staining. The flat sheets of cytological EIN probably arise from surface endometrium overlying deeper foci of histological EIN, expressing the growth pattern of adenomatous epithelium with glandular or cryptal epithelium migrating upward and populating the epithelial surface covering altered underlying glands.

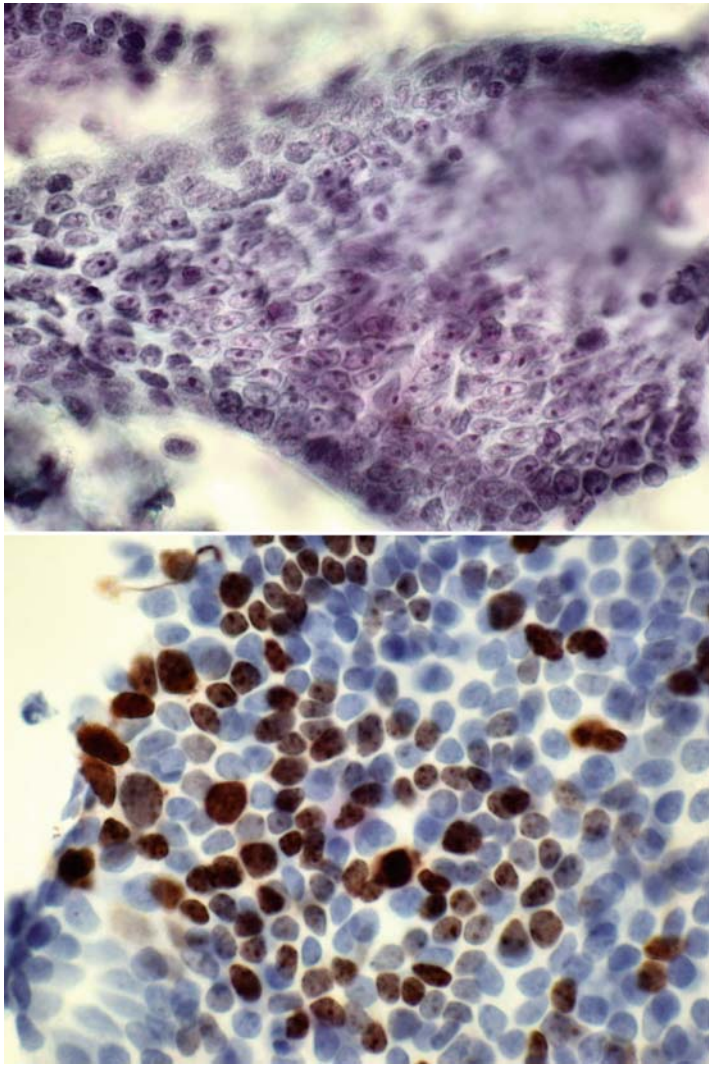

FIG. 8.5. Cytological EIN. In this case from a postmenopausal woman with a thin endometrial stripe by ultrasound, smooth-bordered glands show moderately atypical-appearing nuclei (*top*), and a corresponding Ki-67 immunostain shows profound nuclear decoration. In this case, smooth-bordered glands are different from those of "Swiss cheese" atrophy, which generally show no significant upregulation of Ki-67; in this way, they are functionally dichotomous from the surrounding epithelium.

of histological EIN (previously called atypical hyperplasia) and even with some well-differentiated adenocarcinomas.

The flat sheets of cytological EIN probably arise from surface endometrium overlying deeper foci of histological EIN, expressing the growth pattern of adenomatous epithelium, glandular, or cryptal epithelium migrating upward and populating the epithelial surface covering altered underlying glands. This phenomenon is illustrated in histological sections of PTEN-null endometrium (see fig. 17.4 in Crum et al's. *Diagnostic Gynecology and Obstetric Pathology*).

Although weak and inconstant p53 nuclear decoration is seen among many lesions, including many benign metaplastic lesions, cytological EIN does not feature marked simultaneous and strong nuclear decoration with either Ki-67 or p53 immunostaining. This situation is more consistent with a diagnosis of EIC/EmGD.

In short, although histological EIN progresses from a monoclonal expansion of clustered glands with (at least) nuclear dichotomy from benchmark atrophic or proliferative epithelium, cytological EIN comprises those states in which nuclear dichotomy, generally comprising architecturally differentiated epithelial structures with grade 2 or grade 3 nuclei, is found in brushings of generally thin, atrophic endometrium and where serial sectioning of the endometrium often reveals clustered regions of epithelium with features of atypical hyperplasia or microcarcinoma.

Therapeutic Consequences of EIN

On biopsy, what is interpreted as cytological EIN may eventuate as endometrioid microcancer, but this probably does not significantly affect therapy or outcome. Endometrioid microcancer has been defined as a malignant neoplasm less than 5 mm in largest size showing, at a minimum: periglandular stromal desmoplasia, villoglandular architecture, cribriform architecture, or solid cell masses. As with histological EIN, cytological EIN is a neoplasm whose immediate clinical significance is uncertain, because in the setting of unaltered endometrial thickness, the tissue counterparts of cytological EIN remain incidental findings comprising the spectrum of "adenomatous change" to "microcancer." In the latter situation, because a small endometrioid cancer poses no immediate threat to

an individual, treatment with progestins may be as efficacious as hysterectomy.

In most cases, histological EIN and endometrioid endometrial carcinoma show a loss of immunohistochemically detectable PTEN protein, which acts as part of a chemical pathway that signals cells to stop dividing and causes cells to undergo programmed cell death (apoptosis). The problems with using PTEN immunostaining with cytology specimens is that epithelial decoration is "normal" whereas its absence is "abnormal" and the staining of adjacent internal control tissue that is generally afforded with histology specimens is not present in cytology preparations. Only about 60% of histological EINs stain abnormally, and the PTEN staining procedure is technically difficult.

PTEN tumor suppressor inactivation is among the earliest steps in endometrioid endometrial carcinogenesis, occurring in morphologically unremarkable endometrial glands in half of normal women. Progestin therapy promotes involution or disappearance of PTEN-null endometrial glands relative to their persistence rate seen among normal cycling women; this occurs among PTEN-null glands having a variety of histopathological presentations.

One group of investigators tested the hypothesis that sex hormones positively or negatively select for EIN and examined its emergence, persistence, and regression rates under differing hormonal conditions. Women who underwent hormonal therapy with a progestin-impregnated intrauterine device had the highest rate of EIN regression, with a 62% pretherapy and 5% posttherapy rate of latent precancers, contrasting to nonsignificant changes for the oral progestin-treated and untreated control groups.

Delivery of high doses of progestins locally to the endometrium by an IUD leads to ablation/involution of preexisting PTEN-inactivated endometrium and is a possible mechanism for reduction of long-term endometrial cancer risk known to occur in response to this hormone. Progestin modulation of EIN echoes progestin protection against estrogen-induced hyperplasias reported in the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial.

It may be that cytological EIN could, in the future, be recognized as a pharmacologically treatable preneoplastic event, much in the same way atypical ductal hyperplasia of the breast is prophylactically treated to prevent breast cancer. Although we still cannot

make recommendations about the treatment of cytological EIN, we believe that its discovery should be correlated with sonohysterography and/or hysteroscopy so that gross, treatable lesions such as polyps or grossly detectable neoplastic foci can be surgically addressed. Otherwise, intrauterine progesterone treatment with follow-up cytological sampling seems to be a relatively harmless and theoretically reasonable approach to "treating" cytological EIN.

EIN with Features of Endometrioid Neoplasia Comprising Atypical Hyperplasia and Well-Differentiated Endometrial Adenocarcinoma

In a study designed to assess intraobserver and interobserver agreement on the diagnosis of 56 endometrial specimens by five European expert gynecological pathologists using the WHO classification system and to establish which histological features significantly associated with each classification category, two diagnostic concepts emerged: hyperplasia and endometrioid neoplasia. In endometrial biopsy or curettage specimens, the lack of agreement in the diagnoses of complex hyperplasia and atypical hyperplasia and the lack of reproducibility in the recognition of the histological feature of stromal alterations to differentiate atypical hyperplasia from well-differentiated adenocarcinoma suggested that histological classification should be simplified to include a combined category for simple and complex hyperplasia, called hyperplasia, and a combined category for atypical hyperplasia and well-differentiated adenocarcinoma, called endometrioid neoplasia. This system increases the likelihood that histology from endometrial sampling and from hysterectomy will be concordant for the presence or absence of endometrial carcinoma. These terms are particularly fitting for endometrial cytology diagnoses, which are limited in their ability to discriminate further within the "disordered proliferation to complex hyperplasia" spectrum and within the "atypical hyperplasia to well differentiated (endometrioid) type 1 endometrial cancer" spectrum.

In endometrial brush preparations of EIN with features concordant with what has been called atypical hyperplasia, significant numbers of cells are arranged showing flat sheets with either atypical or cytologically discordant nuclear morphology, pseudopapillae, short papillae, or epithelial cell tufts and distorted acini along with either complete or fragmented bulbous, branched, or outpouched structures. Many of these findings overlap with cytological features of well-differentiated endometrioid adenocarcinoma. In this circumstance, we find it useful to apply the concept of endometrioid neoplasia to brush cytology specimens, especially those not augmented by cell block preparations. We explain to our clinicians that EIN/atypical hyperplasia or endometrioid adenocarcinoma may afford indistinguishable cytological presentations, even when augmented by cell blocks.

One of the earliest changes that are seen among the back-to-back glands of endometrioid neoplasia, especially at the EIN end of the spectrum that was formerly referred to as atypical hyperplasia is clustered nuclear pseudostratification (Fig. 8.6). In brush cytology preparations clustered nuclear pseudostratification appears as small clumps of epithelium with picket-fence-like grade 2 nuclei that are distributed throughout different levels of the epithelial sheet. The nuclear crowding and pseudostratification resembles that seen in colonic adenomas. When fragments of these structures are dislodged by endometrial brushing, they oftentimes retain their associated stromal vascular scaffolding, which shows that they have been harvested from back-to-back glands.

Tufted epithelial structures may appear within glands and along the epithelial surface of the endometrium. As the tufted epithelial aggregates are dislodged by endometrial brushing, they show clustered epithelial cells that resemble the epithelial tufts of syncytial change (Fig. 8.7). Indeed, some of these structures may actually represent retrogressive features similar to syncytial change that originated in neoplastic epithelium (Fig. 8.8). The neoplastic structures may show changes such as the intranucleoplasmic accumulation of glycogen and frankly malignant-appearing nuclei (Fig. 8.9). Because these changes are not specific for cancer, they must be interpreted in the context of the entire cytology slide and/or cell block preparation.

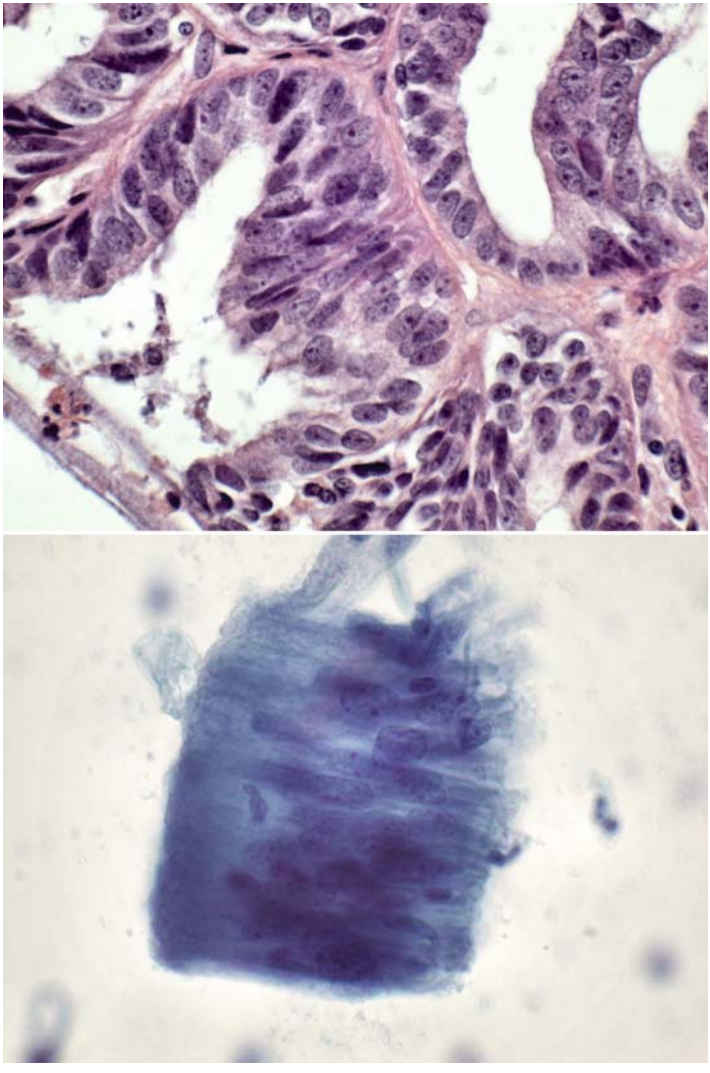

FIG. 8.6. One of the earliest changes seen among the back-to-back glands of endometrioid neoplasia, especially at the EIN end of the spectrum that was formerly referred to as atypical hyperplasia, is clustered nuclear pseudostratification that leads to the formation of mounds of epithelial cell nuclei. For those who look at colonic brushings, similar nuclear stratification and "picket-fencing" may be seen with adenomatous colonic polyps.

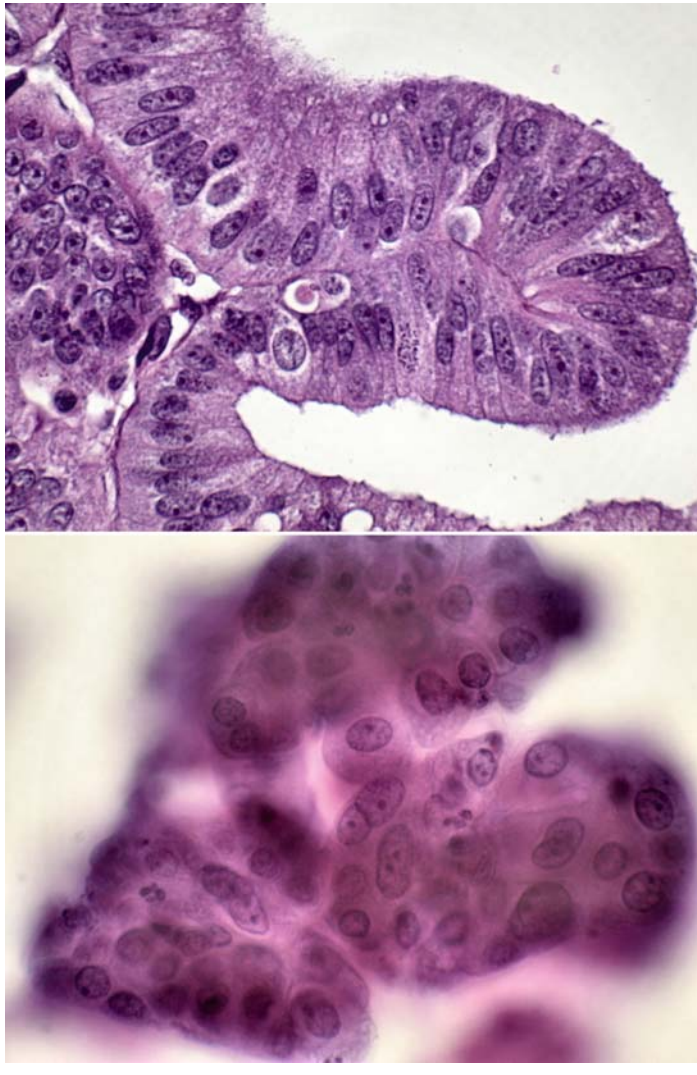

FIG. 8.7. With time, tufted epithelial structures appear within glands and along the epithelial surface of the endometrium. As these are dislodged by endometrial brushing, they show clustered epithelial cells that may resemble the epithelial tufts of syncytial change.

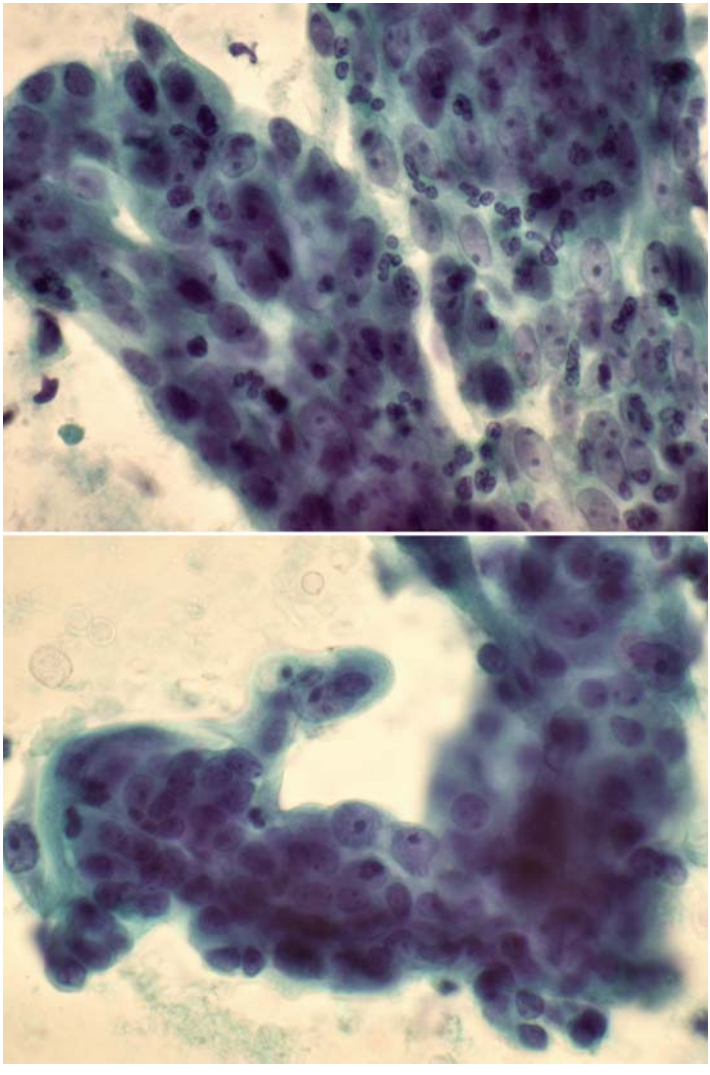

FIG. 8.8. Some of the tufted epithelial structures associated with endometrioid neoplasia and outspoken endometrial carcinoma may actually represent retrogressive syncytial-like change, probably originating from neoplastic epithelium.

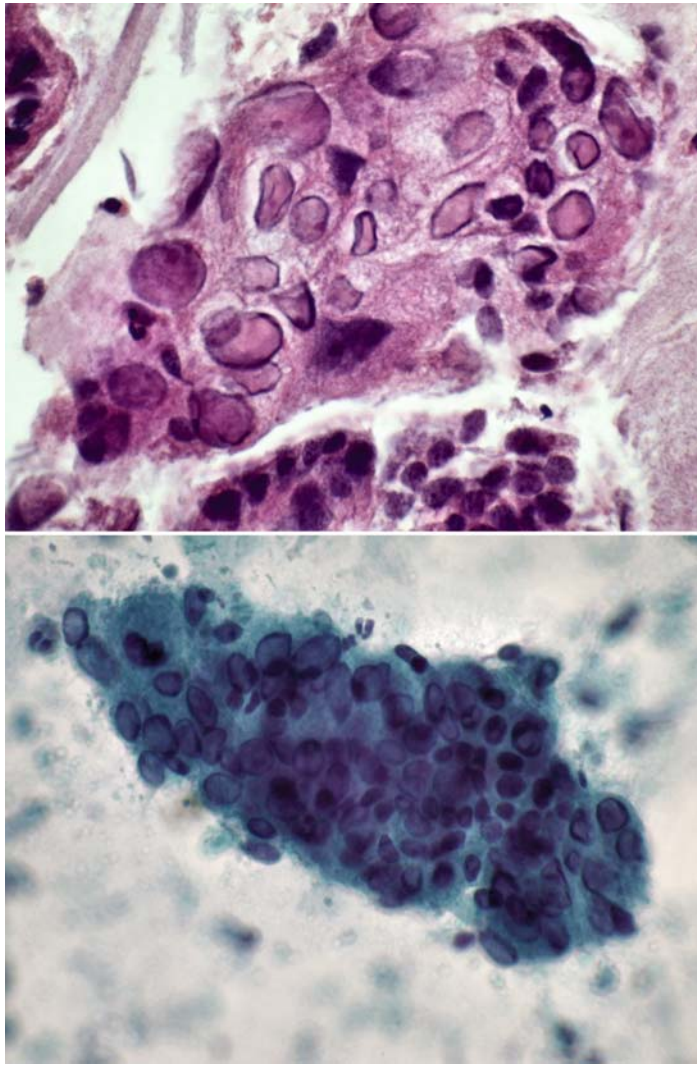

FIG. 8.9. The neoplastic tufted epithelial structures may show changes such as the intranucleoplasmic accumulation of glycogen and the appearance of frankly malignant-appearing nuclei.

Epithelial tufts with soap-bubble-like lumens are a form of mucinous metaplasia. Mucinous metaplasia within complex epithelial structures is so commonly seen in endometrioid neoplasia as to be its surrogate, especially well-differentiated endometrioid-type endometrial carcinoma (Fig. 8.10). Another event, neutrophilic emperipolesis, comprising the nondestructive insinuation of PMNs into budding epithelial structures of surface endometrium, is also commonly seen with endometrioid neoplasia (Fig. 8.11).

The compression of tubuloacinar structures, one against another in the absence of intervening stromal tissue, distorts the shape of the acini and gives them the appearance of being molded by the environment from which they were gathered (Fig. 8.12). In brush cytology preparations, frequent irregular glands with misshapen contours, even in the presence of rather bland-appearing nuclei, almost never occur in benign hyperplasia, which features smoothly contoured glands. This degree of tubuloacinar gland irregularity is also seen in adenocarcinomas (Figs. 8.13–8.15). It is the cytological counterpart of increased fractal dimension.

Another change associated with endometrioid neoplasia, and nearly signature for the presence of well-differentiated (endometrioid) type 1 endometrial carcinoma, comprises epithelial bridges without stromal support. This change appears to arise as a natural consequence of heightened epithelial stratification (Fig. 8.16) that becomes punctuated by intervening lumens. Cytologically, this form of epithelial complexity is seen as loops and channels of nuclei, frequently spanning sheets of endometrial epithelium. We believe that this change is the immediate architectural antecedent to the epithelial cribriform structure (Fig. 8.17).

In cytology preparations, cases of EIN presenting with the histological pattern previously called atypical hyperplasia may show anywhere from absent to marked cytoarchitectural alteration distributing as absent (25%), mild (25%), moderate (45%), and marked (5%); this means that 50% of cases histologically diagnosed as atypical hyperplasia show, on the cytology slide, features of gland complexity that are also typically associated with adenocarcinoma. This finding agrees with the observation that, in tissue biopsy specimens, an immediate endometrial adenocarcinoma outcome based on an initial atypical hyperplasia biopsy diagnosis occurs up to 40% of the time.

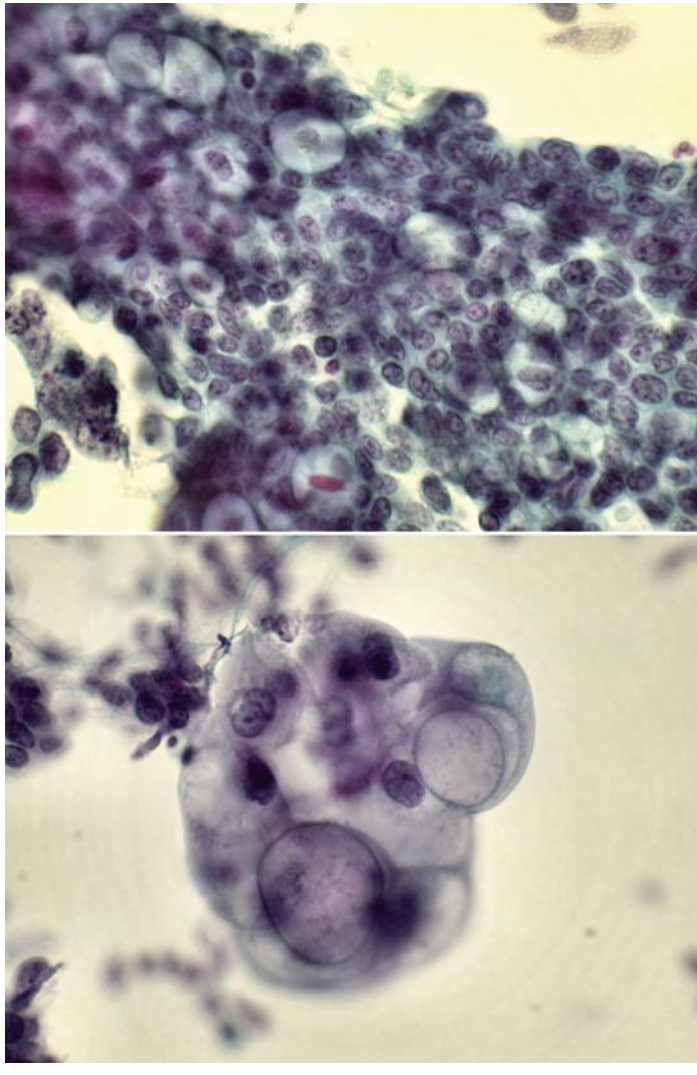

FIG. 8.10. Epithelial tufts with soap-bubble-like lumens indicate mucinous metaplasia. Type C mucinous metaplasia within complex epithelial structures is so commonly seen in endometrioid neoplasia as to be a surrogate for its diagnosis, especially the presence of well-differentiated type 1 endometrial carcinoma.

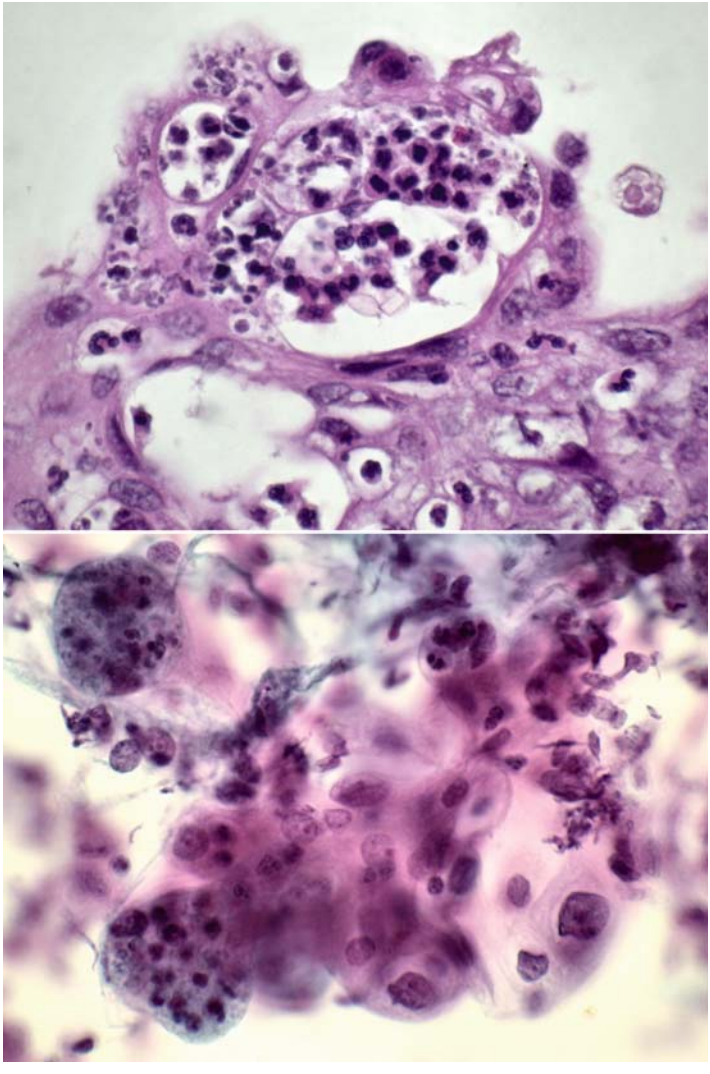

FIG. 8.11. Neutrophilic emperipolesis, comprising the nondestructive insinuation of polymorphonuclear leukocytes (PMNs) into the budding and papillary syncytial epithelial structures of surface endometrium, is also commonly seen with endometrioid neoplasia.

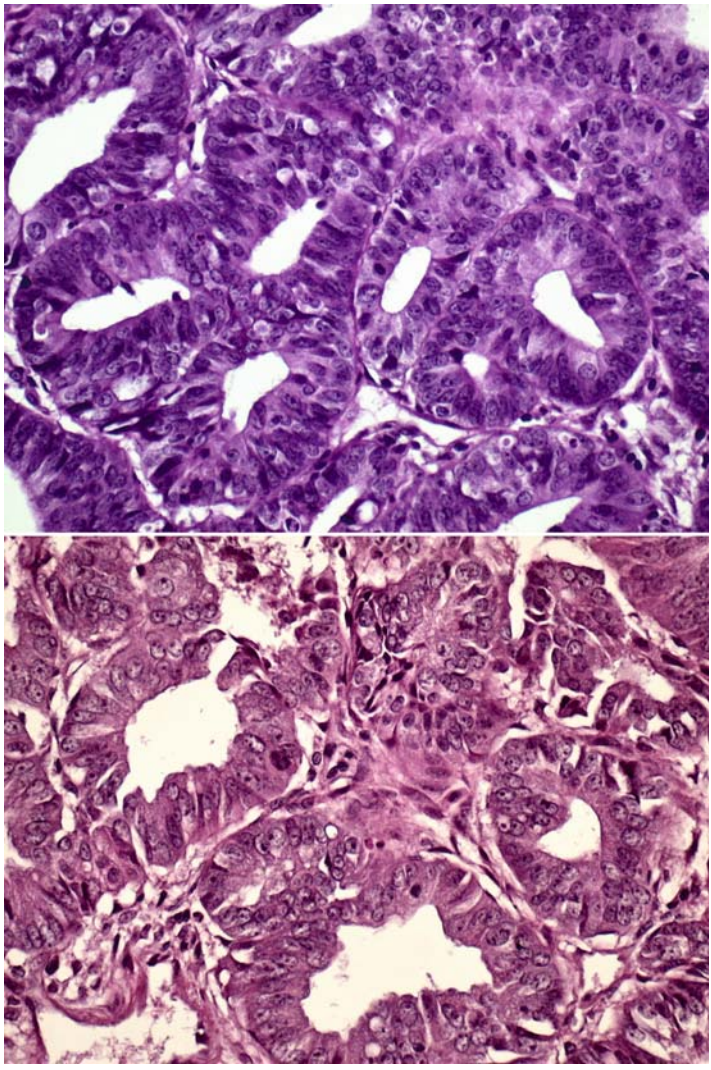

FIG. 8.12. The compression of tubuloacinar structures, one against another, causes them to appear misshapen, irregular, and gives them the appearance of being molded by the environment from which they were gathered and within which they accumulated. These images are from two similar cases of endometrioid neoplasia. The *top image* proved to be EIN and the *bottom image* proved to be well-differentiated type 1 adenocarcinoma. In small biopsy specimens, the entities are indistinguishable from one another.

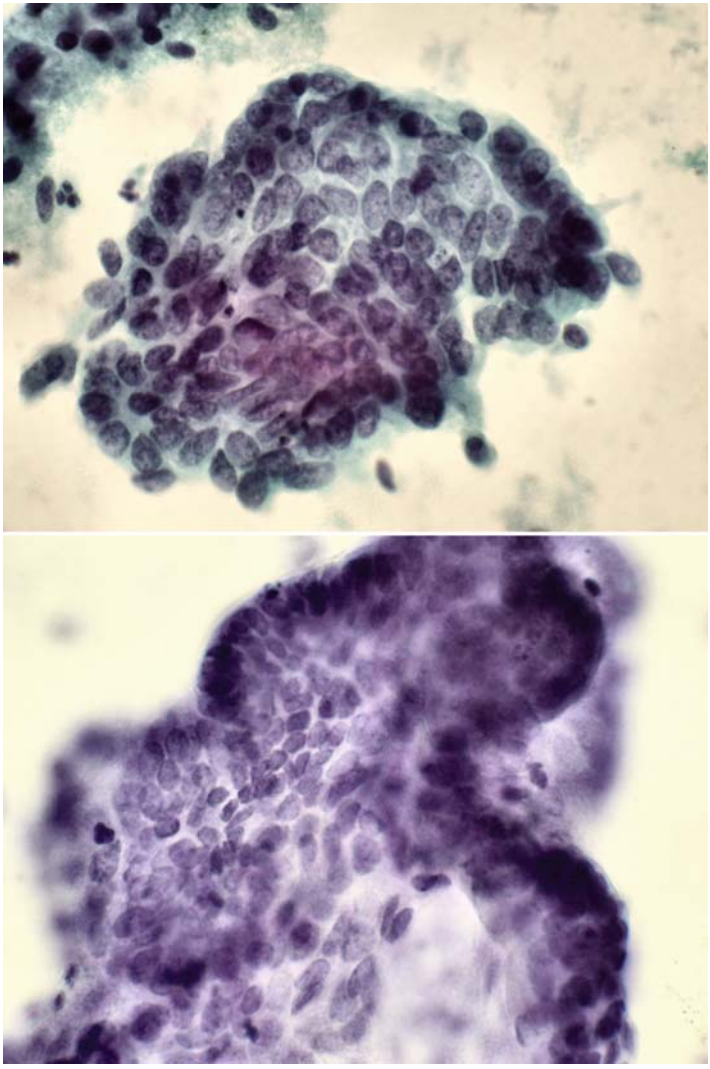

FIG. 8.13. In brush cytology preparations, frequent irregular glands with misshapen appearances, even in the presence of rather bland-appearing nuclei, never occur in benign hyperplasia. This degree of tubuloacinar gland irregularity is also seen in adenocarcinomas. The nuclear arrangement within three-dimensional cell aggregates is best defined by rinsing the slide with acetic acid and staining it with hematoxylin only, omitting counterstaining; this serves as a surrogate for Feulgen staining.

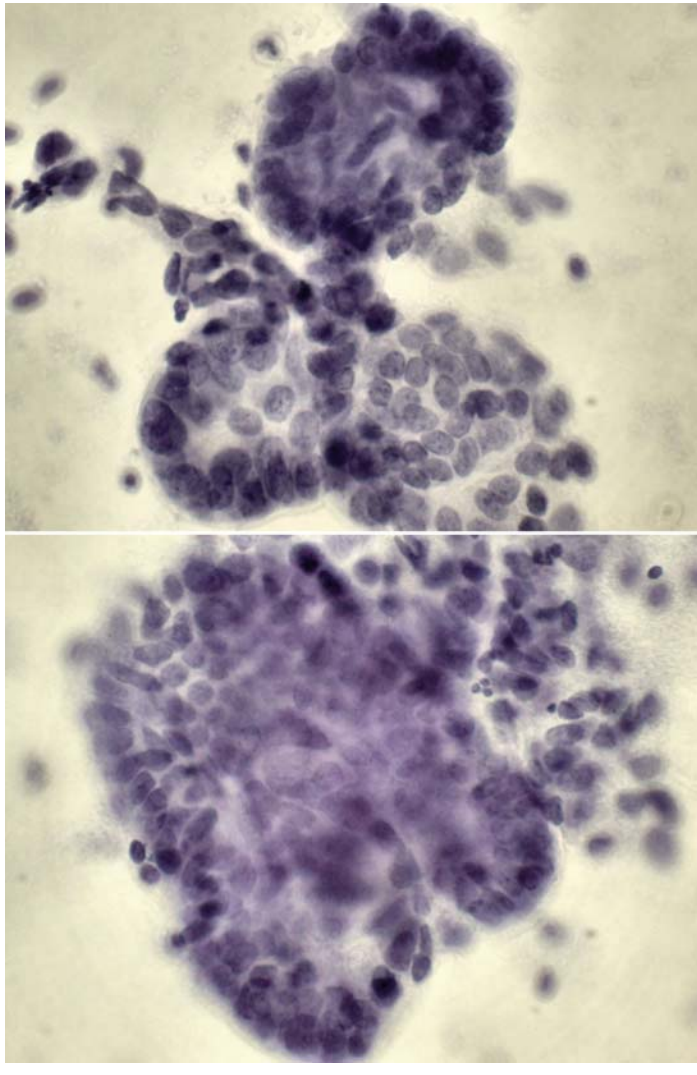

Fig. 8.14. Here, glands appear very small and distorted, but nuclei are very bland. The fractal nature of the glands by far supersedes nuclear features in the diagnosis of endometrioid neoplasia. These images were derived from a case of EIN, formerly called atypical hyperplasia.

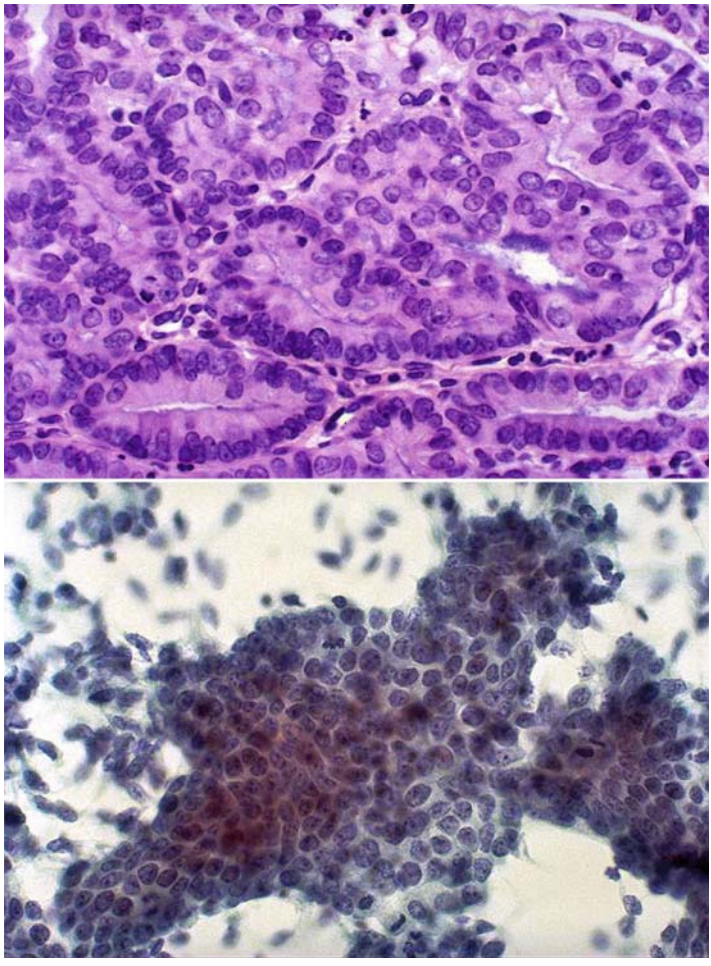

FIG. 8.15. These images were derived from a case of well-differentiated type 1 endometrial cancer. As this image shows, acinar distortion sometimes overlaps with smooth-edged budding of epithelium off a mother gland; and, in the setting of grade 1 to grade 2 nuclei, can suggest the diagnosis of complex hyperplasia. However, nuclear disorientation and associated cell changes suggest a more serious diagnosis. These images were derived from our cytobrush-hysterectomy series. In practice, we would have signed this case out as endometrioid neoplasia, giving the provision that the lesion encompasses the spectrum of atypical hyperplasia and grade 1 type 1 endometrial carcinoma.

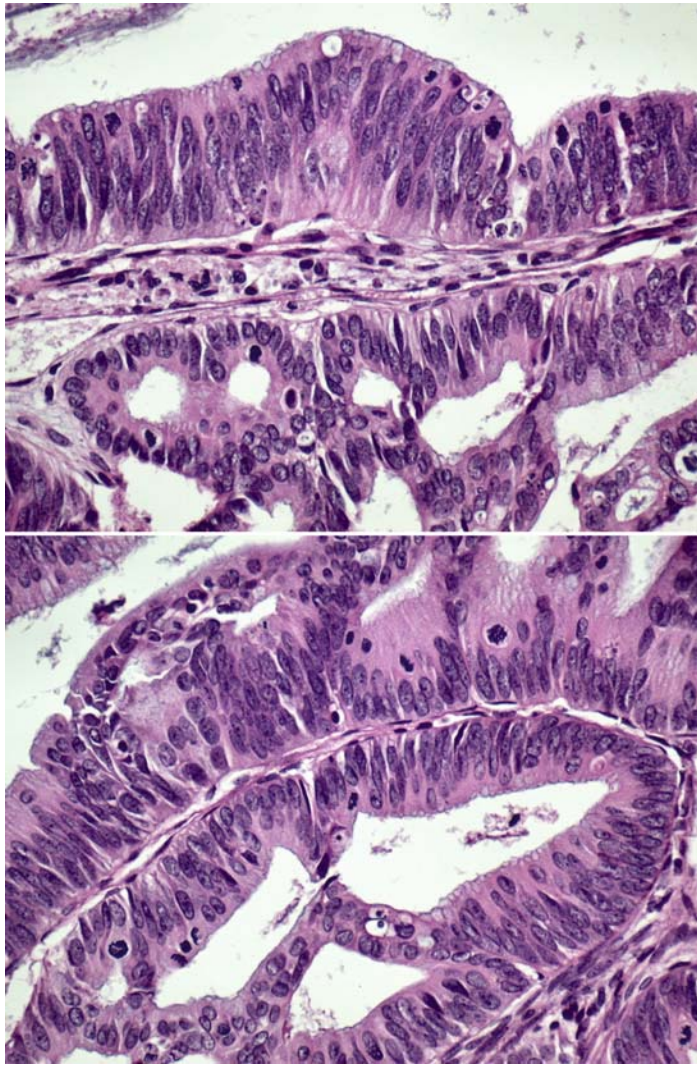

FIG. 8.16. Another change associated with endometrioid neoplasia, and nearly signature for the presence of well-differentiated type 1 endometrial carcinoma, is the appearance of epithelial bridges in the absence of stromal support. This appears to arise as a natural consequence of heightened epithelial stratification.

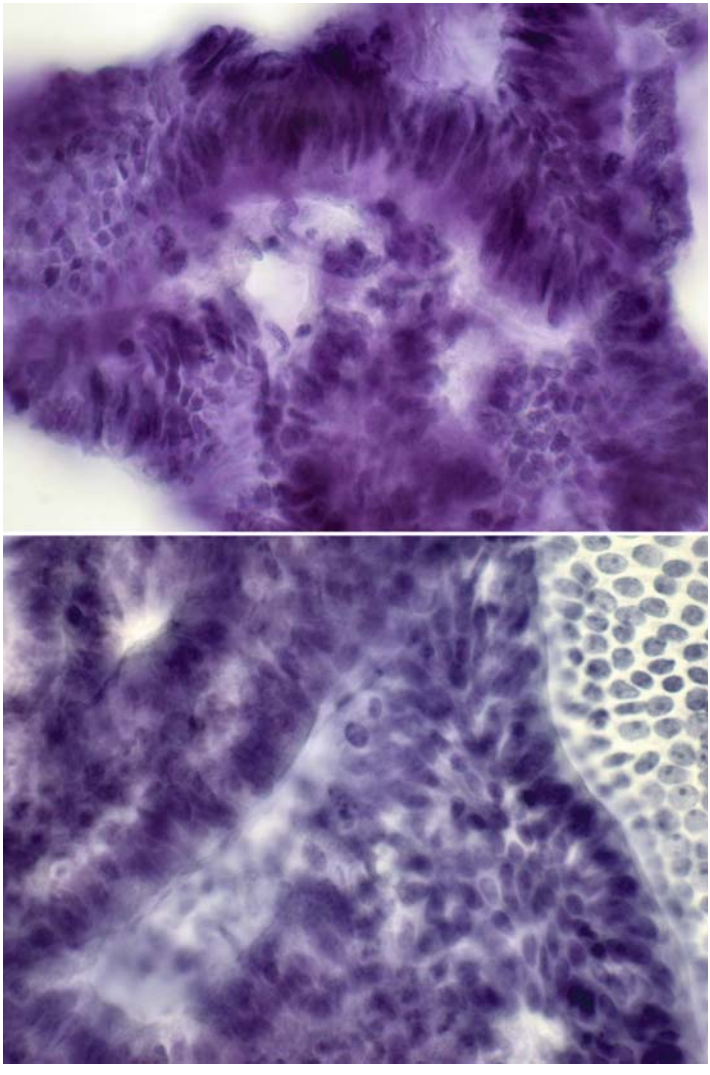

FIG. 8.17. Cytologically, epithelial bridges are seen as loops and channels of nuclei frequently spanning sheets of endometrial epithelium. We believe that this change is the immediate architectural antecedent to the epithelial cribriform structure.

Reproducibility of an atypical hyperplasia diagnosis is poor. Both underestimation and overestimation of lesion severity are common. The level of reproducibility among and between general surgical pathologists and subspecialty pathologists is also poor, as is intraobserver reproducibility of diagnoses among experts. For example, in our hysterectomy series discussed earlier in this text, 13 (18%) of 72 cases of tissue-confirmed atypical hyperplasia would have been diagnosed as benign hyperplasia on the basis of cytology alone. Not surprisingly, this same degree of discordance in tissue diagnosis exists when intraobserver agreement is assessed.

In cytology preparations the size of cell clusters may aid in the diagnosis of endometrioid neoplasia and frank adenocarcinoma. Abundant single cells and small cell groupings indicate dyshesion and gland complexity (and here is where one must be wary of not calling a menstrual endometrium an adenocarcinoma). Complex glands, those with high fractal dimensions, often fragment when the endometrium is brushed, which may have to do with structural fragility of complex glands, the tendency of multilayered epithelial structures to outgrow their blood supply and undergo necrosis, or the effect of stromal intercalation between complex glands dividing them into smaller structures that produce progressively smaller cell clusters when removed by endometrial abrasion. Regardless of the reason, benign endometrial conditions have substantial numbers of large (more than 200 cells) to medium (50–200 cells) sheets or tubules of cells. Atypia of increasing severity and malignancy show many smaller (fewer than 50 cells) clusters of cells. A frankly cancerous endometrium tends to have "fragmented" cell clusters and commonly has a necrotic/inflammatory diathesis.

Nuclear features are less important than architectural and three-dimensional cytoarchitectural features in distinguishing between endometrial hyperplasia and grade 1 adenocarcinoma. Evaluation of cell clumps is useful as they reflect the endometrial histological architecture. In one study, useful characteristics were as follows. Normal proliferative endometrium disclosed cell aggregates with a tube- or sheet-shaped pattern in 98% of cases. In endometrial hyperplasia without atypia, cell aggregates with a dilated or branched pattern were found in 35% of cases. In grade 1 adenocarcinoma, cell clumps with irregular protrusions were found in 62% of cases and a papillotubular pattern was present in 30% of cases.

Well-differentiated endometrioid adenocarcinoma has no unique markers that differ from those seen in EIN, although adenocarcinomas tend to have a higher density of genetic damage involving a broader repertoire of genes, some of which have already been abnormal in the premalignant phase.

The key cytoarchitectural features of endometrioid neoplasia (both EIN and well-differentiated endometrioid adenocarcinoma) include tufted epithelial structures (similar to those seen with syncytial metaplasia); small and often distorted acini; small sheet-like to cup-like aggregates of cells with highly pseudostratified nuclei; bridging epithelial structures that lack stromal support; and papillae and pseudopapillae. Changes generally restricted to outspoken adenocarcinoma include cribriform structures (comprising solid aggregates of cells punctuated by lumens); aggregates of cells showing soap-bubble-like expansions of mucin-filled cytoplasm with or without polymorphonuclear neutrophilic emperipolesis; abundant and widely scattered dyshesive anaplastic cells in a necroinflammatory background; highly elongated villoglandular structures; and solid cell aggregates (excluding morules or aggregates of keratinizing or nonkeratinizing squamous cells).

Another reason that what in the past has been called atypical hyperplasia cannot be reliably separated from well-differentiated adenocarcinoma in biopsy specimens is that often the "hyperplasia" is in fact a tiny bit of frank carcinoma. This is a conclusion drawn from the many cancers we have seen in the follow-up hysterectomy specimens where it was obvious that only a tiny fragment of the maize-like pattern would have been called atypical hyperplasia or EIN on biopsy. Thus, a woman diagnosed with EIN ("atypical hyperplasia") should be treated for well-differentiated endometrioid endometrial adenocarcinoma, which includes the need for informed consent about the preservation of fertility and the need for intraoperative tissue assessment if hysterectomy is contemplated.

As with EIN, treatment of cytological EIN with features of atypical hyperplasia and of well-differentiated endometrial carcinoma with progestins appears to be a safe alternative to hysterectomy in women under age 40. The median length of treatment required for regression of these lesions is about 9 months. All patients in one study were alive and well without evidence of progressive disease at a mean follow-up of 40 months. Nonetheless, when considering

management strategies for women who have a biopsy diagnosis of atypical hyperplasia, clinicians should take into account the considerable rate of concurrent carcinoma.

Endometrial Intraepithelial Carcinoma, Endometrial Gland Dysplasia, and Putative Precursor Lesions for Clear Cell Carcinoma

A dysplastic lesion composed of unquestionably malignant-appearing but noninvasive neoplastic cells has recently been described that bridges the gap between resting endometrium and serous endometrial intraepithelial carcinoma (EIC). The core study examined endometria from 32 uteri with serous carcinoma, 16 with serous EIC, and 60 with endometrioid endometrial carcinoma. The pathological features of the endometrial dysplastic lesions, designated as endometrial glandular dysplasia (EmGD), were present in 53% of uteri with serous carcinoma and 75% with EIC, as compared with 2% of uteri with endometrioid endometrial carcinoma. Most EmGDs were multifocal and involved superficial endometrial glands. However, single gland and endometrial surface epithelial involvement were also seen. Immunohistochemically, EmGD lesions exhibit p53 and MIB-1 activity intermediate to serous EIC and resting endometrium. EmGD frequently shows loss of heterozygosity (LOH) at multiple chromosomal loci, particularly at 17p and 1p, and significantly high concordant LOH frequency between EmGD and paired serous EIC or serous carcinoma.

Some believe that the terms “EIC” or “serous EIC” should be discarded as a means to describe an intraepithelial precancer because these lesions can show extrauterine spread and should thus be considered small uterine serous cancers. None of the other putative endometrial precancers have been reported to show extrauterine spread when present in isolation. The distinction between these lesions is that EmGD is the precursor of uterine serous cancer, and EIC is early serous cancer, which may invade vascular spaces and stroma or spread retrograde via the fallopian tubes, carrying with it a potential for metastasis. Our problem is that endometrial brush preparations do not reliably separate EmGD

and EIC. Therefore, we report the spectrum of these lesions as EIC/EmGD and advise our clinical colleagues as to the import of these findings.

Lesions of the EIC/ EmGD spectrum are often found in atrophic endometrium associated with serous endometrial cancers. These lesions feature grade 2 or (more commonly) grade 3 nuclei; present as isolated glands, gland clusters, or altered surface epithelium amidst otherwise unaltered endometrial stroma; and generally display intense, confluent simultaneous Ki-67 and p53 nuclear decoration.

As a surface change, EIC/EmGD may show either a flat sheet of markedly atypical nuclei or small epithelial tufts whose cells resemble those of outspoken serous carcinoma (Fig. 8.18). EIC/EmGD can be cytologically diagnosed by the triad of findings comprising marked nuclear atypia with anisopoikilonucleosis and enlarged nucleoli; confluent, strong p53 nuclear reactivity; and a concomitant marked increase in the percentage of Ki-67-reactive nuclei (Figs. 8.19, 8.20). It is exceedingly difficult, if not impossible, to distinguish EIC/EmGD one from another on a cytological basis; and, as is sometimes the case, suction biopsy may not confirm the presence of the lesion. For these reasons, an immunohistochemically confirmed diagnosis of EIC/EmGD probably warrants hysterectomy.

Cytological EIN and EIC/EmGD may be found in grossly normal postmenopausal endometrium at a rate that exceeds the discovery of symptomatic endometrial cancer, and this alone is more in keeping with a sequential cancer development scheme than is the hyperplasia-to-carcinoma sequence model. Our finding of six cases of EIC/EmGD among 237 grossly normal-appearing postmenopausal uteruses suggests that there may be a larger than expected and therefore more long lived reservoir of these changes among postmenopausal women. The detection of these lesions may be limited not because they represent metastable, short-lived, and rapidly progressive neoplastic changes, but because they are asymptomatic. There are no screening protocols for endometrial precancer, and endometrial biopsy is insensitive for identifying flat, low-volume lesions that favor the epithelial surface (sometimes as only a single layer of cells). Indeed, the absolute reliance on biopsy following a cytological diagnosis of EIC/EmGD may destroy further evidence of the lesion, and, a “negative” histology outcome may delay appropriate lifesaving therapy.

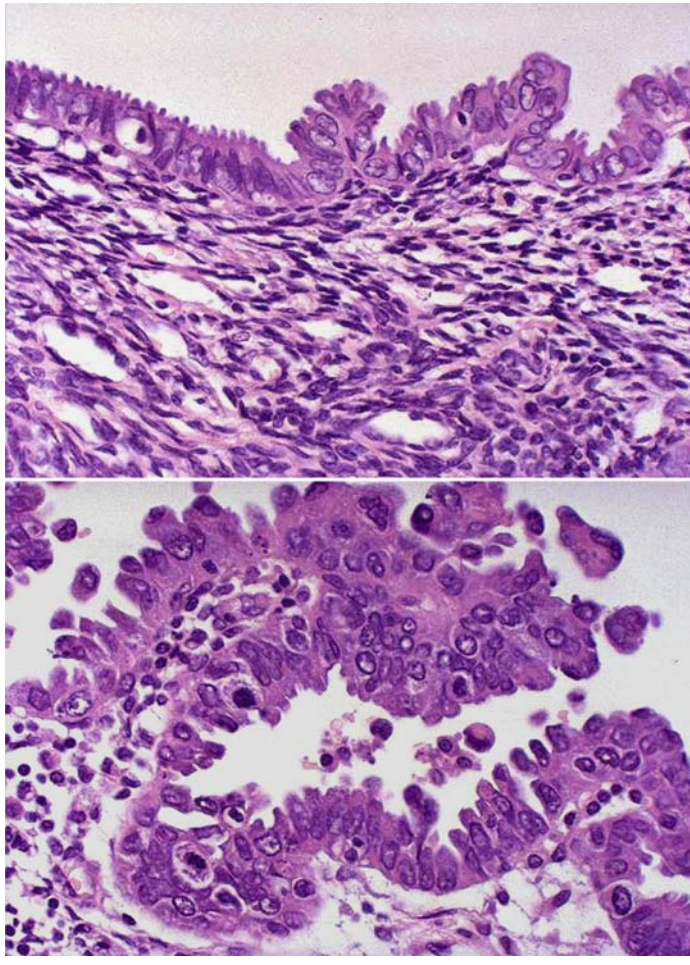

Fig. 8.18. As a surface change, endometrial intraepithelial cancer/endometrial gland dysplasia (EIC/EmGD) may show either a flat sheet of markedly atypical nuclei or small epithelial tufts whose cells resemble those of outspoken serous carcinoma. We believe that one reason such changes are only rarely reported in tissue biopsy material is that these lesions may be asymptomatic, they are generally only one cell thick, and there is no screening protocol for postmenopausal women with negative ultrasound results. A clue to the presence of EIC is watery brown discharge. EmGD is asymptomatic.

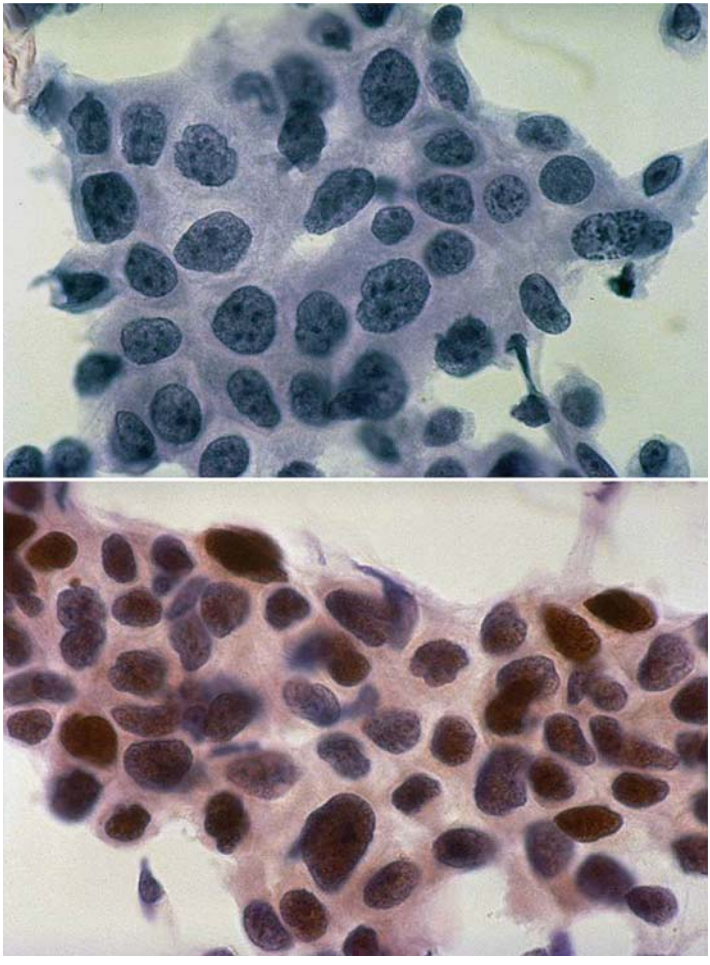

FIG. 8.19. EIC/EmGD can be cytologically diagnosed by the triad of findings comprising marked nuclear atypia with anisopoikilonucleosis and enlarged nucleoli; confluent, strong p53 nuclear reactivity; and a marked increase in the percentage of Ki-67 reactive nuclei. In these images, grade 3 nuclei (*top*) show intense p53 nuclear decoration (*bottom*).

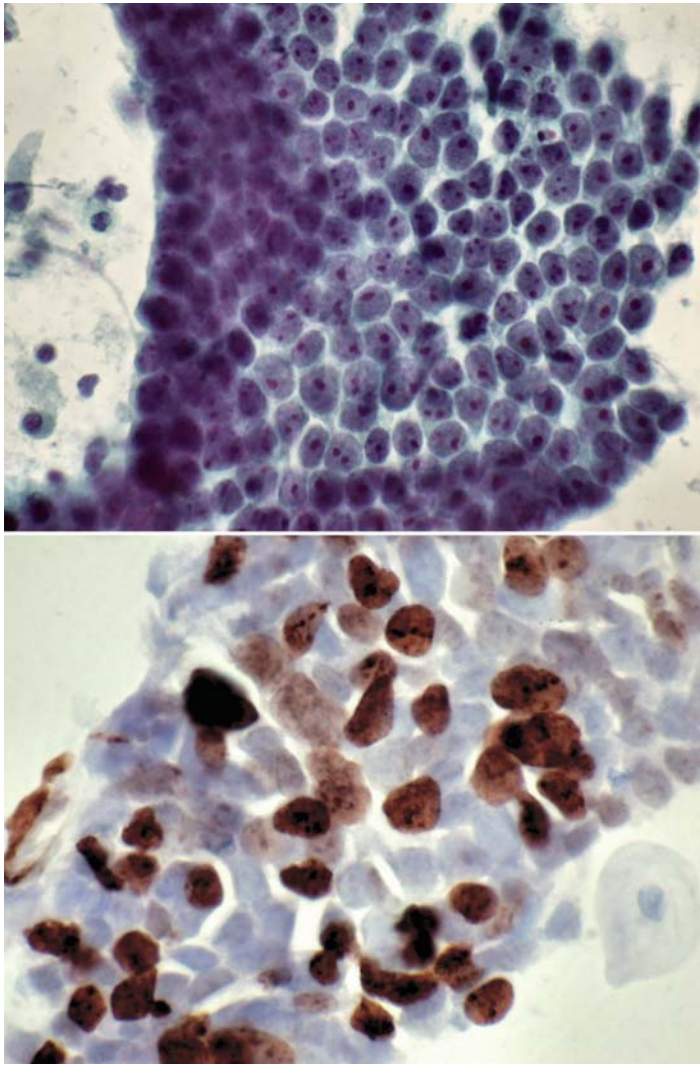

FIG. 8.20. EIC/EmGD can be cytologically diagnosed by the triad of findings comprising marked nuclear atypia with anisopoikilonucleosis and enlarged nucleoli; confluent, strong p53 nuclear reactivity; and a marked increase in the percentage of Ki-67 reactive nuclei. In these images from another case of EIC, a smooth contoured gland with grade 3 (*top*) nuclei also shows intense, confluent p53 reactivity and increased nuclear decoration with antibody to Ki-67 (*bottom*). In this case, the woman was treated with tamoxifen and had a large polyp by ultrasound.

Endometrial clear cell carcinoma, a nonendometrioid (type 2) cancer under the dualistic model, represents less than 5% of endometrial cancers. Given the significant overlap that exists between clear cell carcinoma and serous carcinoma in their morphological, clinical, and global gene expression attributes, an in situ lesion probably exists for clear cell carcinoma. The putative precursor lesions for clear cell carcinoma comprise isolated glands or surface epithelium (within an otherwise normal endometrial region) displaying cytoplasmic clarity and/or eosinophilia with varying degrees of nuclear atypia. The mean p53 and MIB-1 scores and estrogen receptor/progesterone receptor indices lie between those of benign endometria and clear cell carcinoma. The putative precursor lesions for clear cell carcinoma have a morphological and immunophenotypic profile that seems distinct from both the benign endometria in which they reside and the adjacent areas of clear cell carcinoma, although insufficient data exist to conclusively establish their precancerous nature by NCI standards.

Summary of Cytological Precancer

Because of its dichotomy with normal-appearing atrophic endometrial epithelium, cytologically atypical endometrial epithelium may be readily seen in cytology brush preparations, and, given the tendency of adenomatous epithelium to replicate in glands and migrate to the surface, it is oftentimes found among the flattened surface epithelial collections of atrophic endometria.

Cytological preparations greatly augment the pathologist's ability to distinguish between banal and atypical nuclei in atypical epithelium, a morphological dichotomy that is important to the recognition of cytological EIN and EIC/EmGD as well as the putative precursor lesion of clear cell carcinoma. Similar-appearing atypical epithelium often exists as abnormal glands within the atrophic stroma of many of these cases, but its histological demonstration generally requires sampling the entire endometrium, which is an impracticable precondition for case-finding, sometimes difficult to achieve with hysterectomy specimens, and may explain why biopsy studies of asymptomatic postmenopausal women do not commonly uncover these abnormalities.

If persistent, nonproliferating foci of EIN undergo progressive mutational change, is it possible that they may actually eventuate in EmGD/EIC or in mixed patterns of serous and endometrioid carcinoma? Although this proposal has not been tested in the literature, it is fully in keeping with the observation that in combined endometrioid and serous carcinomas, the serous component is thought to arise from a particularly aggressive clone born of the parent endometrioid lesion.

The therapeutic implication of these findings is that once EIC/EmGD, outspoken endometrioid adenocarcinoma, or benign mimics of cytological EIN are excluded from the differential diagnosis of "small quantities of atypical endometrial epithelium admixed with larger quantities of benign endometrium," progestin therapy, especially progestin-impregnated IUDs, may reduce cancer risk among these women. Further, repeat endometrial brushing with p53 and Ki-67 immunostaining to help exclude foci of EIC/EmGD may prove useful in the follow-up. The development of fluorescent in situ hybridization (FISH) testing for discovering these and related lesions is underway and shows promising preliminary results.

Suggested Reading

Archer DF, McIntyre-Seltman K, Wilborn WW Jr, Dowling EA, Cone F, Creasy GW, Kafrissen ME. Endometrial morphology in asymptomatic postmenopausal women. Am J Obstet Gynecol 1991; 165: 317–20.

Baak JP, Mutter GL, Robboy S, van Diest PJ, Uyterlinde AM, Orbo A, Palazzo J, Fiane B, Løvslett K, Burger C, Voorhorst F, Verheijen RH. The molecular genetics and morphometry-based endometrial intraepithelial neoplasia classification system predicts disease progression in endometrial hyperplasia more accurately than the 1994 World Health Organization classification system. Cancer (Phila) 2005; 103: 2304–12.

Bergeron C, Nogales FF, Masseroli M, Abeler V, Duvillard P, Müller-Holzner E, Pickartz H, Wells M. A multicentric European study testing the reproducibility of the WHO classification of endometrial hyperplasia with a proposal of a simplified working classification for biopsy and curettage specimens. Am J Surg Pathol 1999; 23: 1102–8.

Cheng L, Zhang S, Zhou Y, Zheng W. Endometrial glandular dysplasia: a putative precursor lesion of uterine papillary serous carcinoma. Part II: molecular features. Int J Surg Pathol 2004; 12: 319–31.

Dey P, Rajesh L. Fractal dimension in endometrial carcinoma. Anal Quant Cytol Histol 2004; 26: 113–6.

Effects of hormone replacement therapy on endometrial histology in postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. JAMA 1996; 275: 370–5.

Fadare O, Liang SX, Ulukus EC, Chambers SK, Zheng W. Precursors of endometrial clear cell carcinoma. Am J Surg Pathol 2006; 30: 1519–30.

Greene RR, Roddick JW Jr, Milligan M. Estrogens, endometrial hyperplasia, and endometrial carcinoma. Ann N Y Acad Sci 1959; 75: 586–600.

Hachisuga T, Sugimori H, Kaku T, Tsuneyoshi M. Microcarcinoma of the endometrium: a mapping study with special reference to cytologic atypia in the endometrium. Jpn J Clin Oncol 1992; 22: 400–5.

Hecht JL, Ince TA, Baak JP, Baker HE, Ogden MW, Mutter GL. Prediction of endometrial carcinoma by subjective endometrial intraepithelial neoplasia diagnosis. Mod Pathol 2005; 18: 324–30.

Horwitz RI, Feinstein AR, Horwitz SM, Robboy SJ. Necropsy diagnosis of endometrial cancer and detection-bias in case/control studies. Lancet 1981; 2: 66–8.

Karamursel BS, Guven S, Tulunay G, Kucukali T, Ayhan A. Which surgical procedure for patients with atypical endometrial hyperplasia? Int J Gynecol Cancer 2005; 15: 127–31.

Koss LG. Diagnosis of early endometrial cancer and precancerous states. Ann Clin Lab Sci 1979; 9: 189–94.

Koss LG. Screening for endometrial cancer. IARC Sci Publ 1986; 76: 293–300.

Koss LG. Detection of occult endometrial carcinoma. J Cell Biochem Suppl 1995; 23: 165–73.

Korhonen MO, Symons JP, Hyde BM, Rowan JP, Wilborn WH. Histologic classification and pathologic findings for endometrial biopsy specimens obtained from 2964 perimenopausal and postmenopausal women undergoing screening for continuous hormones as replacement therapy (CHART 2 Study). Am J Obstet Gynecol 1997; 176: 377–80.

Kurman RJ, Kaminski PF, Norris HJ. The behavior of endometrial hyperplasia. A long-term study of "untreated" hyperplasia in 170 patients. Cancer (Phila) 1985; 56: 403–12.

Maksem JA. Endometrial brush cytology of advanced postmenopausal endometrium: does endometrial intraepithelial neoplasia exist in the absence of hyperplasia? Diagn Cytopathol 1998; 19: 338–43.

Maksem JA, Lee SS, Roose EB, Carlson JA, Dornbier DP, Belsheim BL. Rare epithelial sheets with "cancer-like" nuclei presenting against a background of cytologically normal-appearing endometrial epithelium in endometrial brush samplings: establishing a differential diagnosis. Diagn Cytopathol 1999; 21: 378–86.

Mutter GL. Histopathology of genetically defined endometrial precancers. Int J Gynecol Pathol 2000; 19: 301–9.

Mutter GL, Duska LR, Crum CP. Endometrial intraepithelial neoplasia. In: Crum CP, Kenneth RL, eds. Diagnostic Gynecology and Obstetric Pathology. Elsevier Saunders, Philadelphia, PA, p. 500, fig. 17.4, 2006.

Mutter GL, Zaino RJ, Baak JP, Bentley RC, Robboy SJ. Benign Endometrial Hyperplasia and EIN Pathology of the Female Reproductive Tract. Elsevier, Churchill Livingstone, London, 2nd edn, p. 1045, 2009.

Norimatsu Y, Shimizu K, Kobayashi TK, Moriya T, Tsukayama C, Miyake Y, Ohno E. Cellular features of endometrial hyperplasia and well differentiated adenocarcinoma using the Endocyte sampler: diagnostic criteria based on the cytoarchitecture of tissue fragments. Cancer (Phila) 2006; 108: 77–85.

Orbo A, Rise CE, Mutter GL. Regression of latent endometrial precancers by progestin infiltrated intrauterine device. Cancer Res 2006; 66: 5613–7.

Parazzini F, La Vecchia C, Bocciolone L, Franceschi S. The epidemiology of endometrial cancer. Gynecol Oncol 1991; 41: 1–16.

Porrazzi LC, Quarto F, Maiello FM, De Falco ML, Antonucci T. The value of endometrial cytology by scraping in 1,798 cases: screening in asymptomatic women and diagnosis in symptomatic ones. Diagn Cytopathol 1987; 3: 112–20.

Randall TC, Kurman RJ. Progestin treatment of atypical hyperplasia and well-differentiated carcinoma of the endometrium in women under age 40. Obstet Gynecol 1997; 90: 434–40.

Shapiro S, Kelly JP, Rosenberg L, Kaufman DW, Helmrich SP, Rosenshein NB, Lewis JL Jr, Knapp RC, Stolley PD, Schottenfeld D. Risk of localized and widespread endometrial cancer in relation to recent and discontinued use of conjugated estrogens. N Engl J Med 1985; 313: 969–72.

Sherman ME, Ronnett BM, Ioffe OB, Richesson DA, Rush BB, Glass AG, Chatterjee N, Duggan MA, Lacey JV Jr. Reproducibility of biopsy diagnoses of endometrial hyperplasia: evidence supporting a simplified classification. Int J Gynecol Pathol 2008; 27: 318–25.

Shu YJ, Ikle FA. Cytopathology of the Endometrial Cancer. McGraw-Hill, New York, 1992.

Sivridis E, Giatromanolaki A. Proliferative activity in postmenopausal endometrium: the lurking potential for giving rise to an endometrial adenocarcinoma. J Clin Pathol 2004; 57: 840–4.

Tanaka M, Kyo S, Kanaya T, Yatabe N, Nakamura M, Maida Y, Okabe M, Inoue M. Evidence of the monoclonal composition of human endometrial epithelial glands and mosaic pattern of clonal distribution in luminal epithelium. Am J Pathol 2003; 163: 295–301.

Trimble CL, Kauderer J, Zaino R, Silverberg S, Lim PC, Burke JJ II, Alberts D, Curtin J. Concurrent endometrial carcinoma in women with a biopsy diagnosis of atypical endometrial hyperplasia: A Gynecologic Oncology Group study. Cancer (Phila) 2006; 106: 812–9.

Yi XF, Zheng WX. Endometrial glandular dysplasia and endometrial intraepithelial neoplasia. Cur Opin Obstet Gynecol 2008; 20(1): 20–5.

Zaino RJ. Endometrial hyperplasia: is it time for a quantum leap to a new classification? Int J Gynecol Pathol 2000; 19: 314–21.

Zaino RJ, Kauderer J, Trimble CL, Silverberg SG, Curtin JP, Lim PC, Gallup DG. Reproducibility of the diagnosis of atypical endometrial hyperplasia: a Gynecologic Oncology Group study. Cancer (Phila) 2006; 106: 804–11.

Zeleniuch-Jacquotte A, Akhmedkhanov A, Kato I, Koenig KL, Shore RE, Kim MY, Levitz M, Mittal KR, Raju U, Banerjee S, Toniolo P. Postmenopausal endogenous oestrogens and risk of endometrial cancer: results of a prospective study. Br J Cancer 2001; 84: 975–81.

Zheng W, Baker HE, Mutter GL. Involution of PTEN-null endometrial glands with progestin therapy. Gynecol Oncol 2004; 92: 1008–13.

Zheng W, Liang SX, Yu H, Rutherford T, Chambers SK, Schwartz PE. Endometrial glandular dysplasia: a newly defined precursor lesion of uterine papillary serous carcinoma. Part I: morphologic features. Int J Surg Pathol 2004; 12: 207–23.

Zheng WX, Schwartz PE. Serous EIC as an early form of uterine papillary serous carcinoma: recent progress in understanding its pathogenesis and current opinions regarding pathologic and clinical management. Gynecol Oncol 2005; 96(3): 579.

9
Endometrial Carcinoma

A Background to Endometrial Cancer

The successful focus of cytology on the reduction of cervical carcinoma among women through intensive prospective screening has changed the proportion of uterine cancer distribution within the United States. Endometrial cancers are now the most frequently diagnosed malignancies of the female genital tract, with 40,100 new cases of uterine corpus cancer projected for 2008. Our descriptions of endometrial neoplasia rest on our observations of 168 contemporaneously gathered hysterectomy-controlled cases and 72 office-based samplings, 127 of which represented outspoken endometrial cancer (see Table 8.1).

In 1983, a dualistic model of endometrial tumorigenesis was proposed based on clinical observations and clinicopathologic correlations. The majority of endometrial cancers (>70%), the endometrioid adenocarcinomas, were designated as type 1 carcinomas, and these follow an estrogen-related pathway. They often arise in the background of hyperplastic endometrium, show endometrioid differentiation, and are usually low grade, having a favorable clinical outcome. The tumors usually have an indolent course and frequently are preceded by endometrial intraepithelial neoplasm (EIN). In comparison with the serous and clear cell carcinomas, the type 2 tumors develop on average 10 years later. In comparison, the endometrioid tumors are better differentiated,

J.A. Maksem et al., *Endometrial Cytology with Tissue Correlations*, Essentials in Cytopathology 7, DOI: 10.1007/978-0-387-89910-7_10,

more likely to have diploid DNA content, less myometrial invasion, and low potential for lymphatic spread; frequently maintain estrogen and progesterone receptors; and carry a generally favorable prognosis. Four major genetic events are responsible for endometrioid tumorigenesis and include silencing of the PTEN tumor suppressor gene, microsatellite instability caused by altered mismatch repair genes, mutation of the K-*ras* proto-oncogene, and alterations of the beta-catenin gene.

The type 2 carcinomas account for the remaining 10%–20% of uterine cancers. They follow an estrogen-unrelated pathway and arise in atrophic endometrium. These tumors occur at an older age, are typically high-grade carcinomas of nonendometrioid differentiation, and most frequently show serous and less frequently clear cell features. They exhibit an aggressive clinical course and have a poor prognosis. p53 mutation and overexpression of Her2/neu oncogene are two major genetic alterations that characterize type 2 carcinomas.

Some endometrial carcinomas show overlapping clinical, morphological, immunohistochemical, and molecular features of types 1 and 2 endometrial cancers, and this has elicited ongoing debate about where these tumors should be assigned. The nonendometrioid component has been thought to emerge from a preexisting endometrioid carcinoma by development of genetic heterogeneity and progressive expansion of a particularly aggressive tumor subclone with genetic and behavioral features of type 2 tumors. Some unique tumor types, such as carcinosarcomas, demonstrate clinical and molecular features that do not fit cleanly into either of the proposed pathways.

A small group of women have heritable endometrial carcinoma that represents the most common extracolonic malignancy in hereditary nonpolyposis colorectal cancer (Lynch syndrome II). Women with an inherited predisposition for endometrial neoplasia have a distinctive clinical presentation of type 1 endometrial adenocarcinomas. They tend to develop disease about 15 years earlier than sporadic occurrences, and their prognosis is favorable. Most have the hereditary nonpolyposis colorectal carcinoma syndrome (HNPCC), a rare heritable genetic condition affecting approximately 1% or less of the population.

Hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome, exhibits a risk of colorectal cancer and

other cancers such as those of the endometrium, ovary, stomach, small intestine, hepatobiliary tract, upper urinary tract, brain, and skin. HNPCC is subdivided into Lynch syndrome I (familial colon cancer) and Lynch syndrome II (other cancer of the gastrointestinal system or the reproductive system). The increased risk for these cancers rests upon inherited mutations that degrade the self-repair capability of DNA.

Three genes in particular, MLH1, MSH2, and MSH6, are responsible for most HNPCC. Individuals with HNPCC have an 80% risk of colorectal cancer and a 70% risk of endometrial cancer by age 70. The endometrial tumors show many histopathological and genetic features of type 1 carcinomas, including transit through a premalignant EIN phase, endometrioid histopathology, and genetic alterations in mismatch repair genes and PTEN.

An important point to derive from this discussion is that when a woman who is less than 50 years of age presents with endometrioid endometrial cancer, she and her entire family may be at risk for colonic and other neoplasms. The gynecologist managing the woman should be advised to obtain genetic counseling for her and her family. Parenthetically, women with endometrial cancer diagnosed at younger than age 50 years in combination with a body mass index greater than 30 and no first-degree relative with a Lynch syndrome-associated cancer are unlikely to have Lynch syndrome.

Nuclear Grades and Cytoarchitectural Patterns of Cancers

Historically, cytopathologists have relied on nuclear features to diagnose malignancy. Nuclear features that separate neoplastic from benign endometrium include the major criteria applied to most cancers and include variation in nuclear size (anisonucleosis) and shape (poikilonucleosis); nuclear molding; nucleoplasmic clearing (vesiculation) with variably coarse granularity and/or hyperchromasia; and nucleolar prominence. Taken together, nuclear features comprise graded degrees of dyskaryosis, commonly called “atypia” in gynecological material. The revised FIGO histological grading system for endometrial adenocarcinoma has improved prognostic

value when specific criteria for nuclear grading are included. Among endometrial adenocarcinomas the assignment of grade based solely on nuclear features results in patient groups with statistically differing rates of disease progression and survival.

FIGO grade 1 nuclei are uniform, round to oval nuclei, with inconspicuous nucleoli, and resemble the nuclei of functional endometrium, not greatly exceeding the variability of proliferative endometrial nuclei. Grade 2 nuclei are irregular or oval with moderate-size nucleoli. Grade 3 nuclei are large and pleomorphic and with moderate to large irregular nucleoli. By benchmarking the atypia of endometrial cells to the nucleus of the proliferative gland cell, nuclear changes can be designated as progressively atypical for pathological states beyond anovulatory endometrium and simple hyperplasia.

The problem with relying on nuclear atypia for triaging cytology cases into "benign," versus "benign abnormal" versus "neoplastic" categories is that about one-quarter of endometrial cancers show pale-staining nuclei without coarse chromatin or prominent nucleoli. In those endometrial adenocarcinomas where significant dyskaryosis is absent, the tissue diagnosis of "cancer" defaults to pattern recognition, which is feasible in cytology collections, and is made easier and more accurate when the study is augmented by the examination of cell blocks.

As outlined in Table 3.1, cytological features associated with cancers comprise tufted epithelial cell cluster, pseudopapillae, and balled-up epithelial cell groups. Features suggestive of cancers include bridge-like connections composed of epithelial cells, cell clusters with intraepithelial soap-bubble-like lumens, and distorted acini. Features diagnostic of cancers are cribriform structures, solid clusters of epithelial cells (excluding morules and metaplastic squamous cells), and dyshesive epithelial cells.

Type 1 Endometrial Cancers

Low-grade type 1 estrogen-promoted endometrioid endometrial cancer occurs in younger (perimenopausal and early postmenopausal) women whose cervicovaginal smears show prominent estrogen effect. Low-grade cancer entails a low degree of nuclear

anaplasia (revised FIGO grade 1 or 2 nuclei) and supports some degree of differentiation. In isolated microscopic fields, adenocarcinoma may be indistinguishable from EIN or even some benign forms of hyperplasia.

Epithelial cribriform structures, along with villoglandular structures and solid epithelial aggregates, represent epithelial configurations that are diagnostic of endometrial malignancy. Cribriforming, meaning perforated like a sieve, actually represents solid epithelial organization where the cells are still so highly functioning that they are able throughout the tumor to form glands with lumens (Fig. 9.1). In cytology brush preparations, cribriform structures are easily recognized in microbiopsies as comprising a solid aggregate of cells through which loops upon loops of nuclei interrupt the otherwise solid nature of the cell aggregate, causing the appearance of variably shaped acinar glands that reside within the cell aggregate in the absence of a supporting stroma (Figs. 9.2, 9.3). Microacinar complexes eventuate from the fragmentation of cribriform structures.

Villoglandular structures comprise vascular structures, with or without a hyaline cuff, decorated by neoplastic cells. Villoglandular structures may be seen with either villoglandular carcinoma, as part of usual endometrioid carcinoma, or with clear cell carcinoma (Fig. 9.4). The structures are highly elongated papillae with central vascular cores. In cytology preparations, these appear as aggregates of tumor cells; and, on occasion, the central vascular core of the structure is well visualized (Fig. 9.5). In endometrioid carcinomas, villoglandular or somewhat shorter true papillary structures may arise from within tubuloacinar cisterns and may look more like tufted epithelial structures in brush cytology preparations (Fig. 9.6), yet on close inspection they have central vascular cores.

Solid adenomatous epithelial aggregates comprise a signature finding for the diagnosis of carcinoma. Histologically, their relative quantity is important in the histological grading of endometrioid endometrial carcinoma: grade 1 (<6%), grade 2 (6%–50%), and grade 3(>50%). Many of these structures may show subtle remnants of underlying glandular architecture, especially cribriform architecture (Fig. 9.7), which is especially evident in endometrial brush microbiopsies (Fig. 9.8). In other cases, no underlying adenomatous architecture is seen.

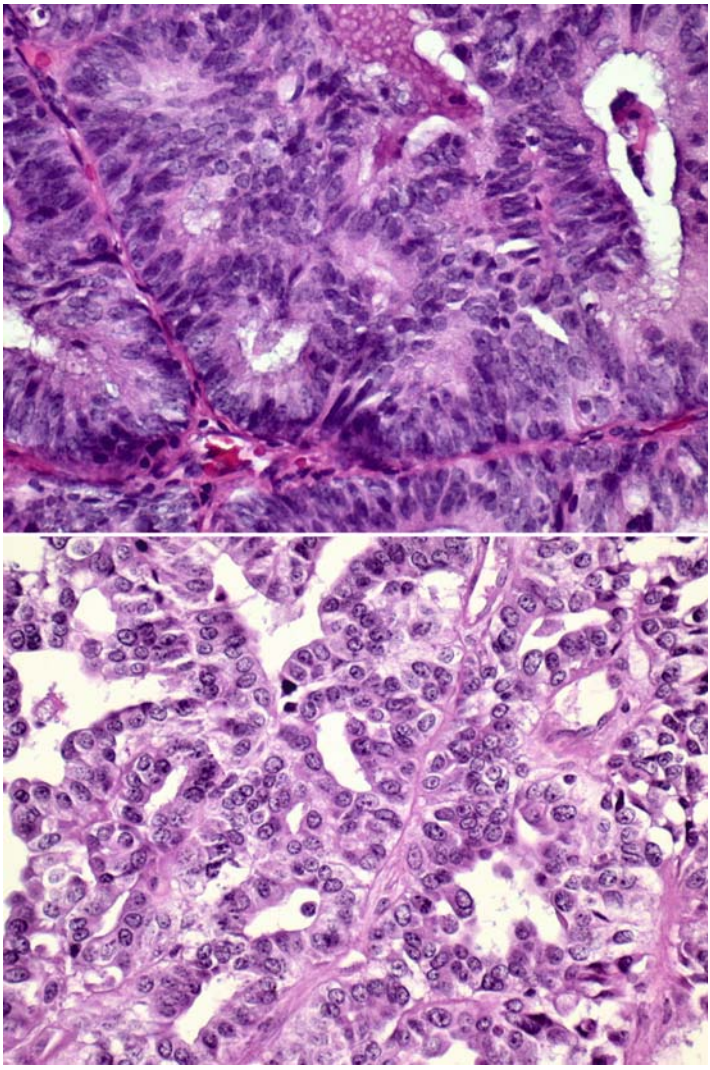

FIG. 9.1. Epithelial cribriform structures, along with villoglandular structures and solid epithelial aggregates, represent epithelial arrangements that are absolutely diagnostic of endometrial malignancy. Cribriforming actually represents solid epithelial organization that is punctuated by an anastomosing and often interconnected collection of lumens.

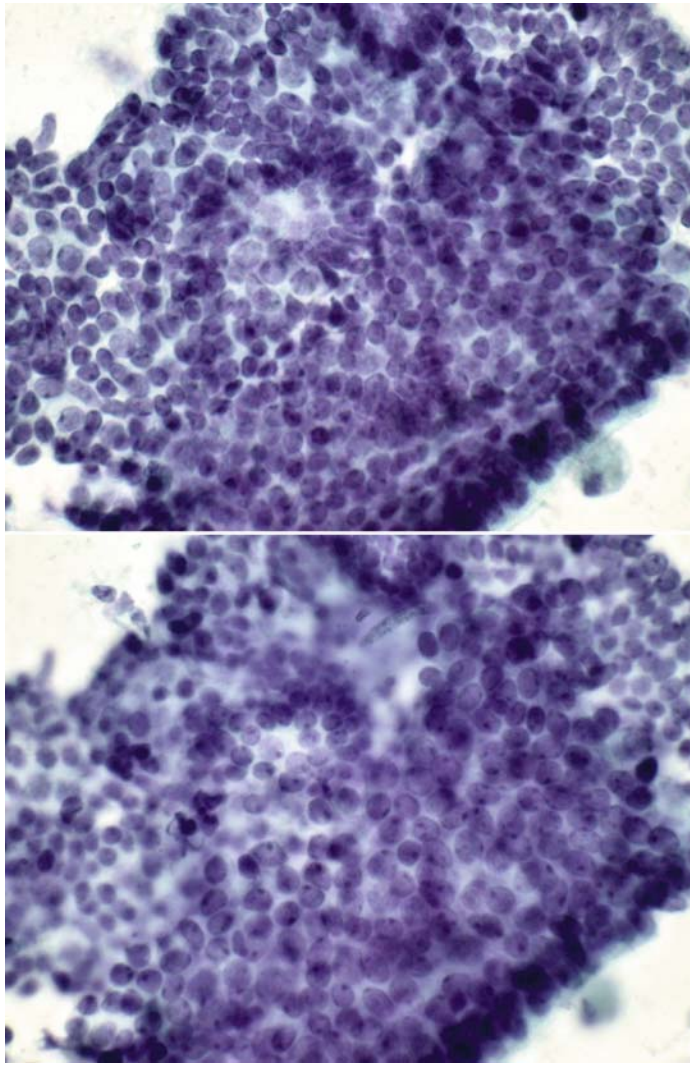

FIG. 9.2. In cytology brush preparations, cribriform structures are easily recognized as microbiopsies comprising a solid aggregate of cells through which loops upon loops of nuclei cause interruptions of the otherwise solid nature of the cell aggregate, affording the appearance of variably shaped acinar glands that reside within the cell aggregate in the absence of a supporting stroma.

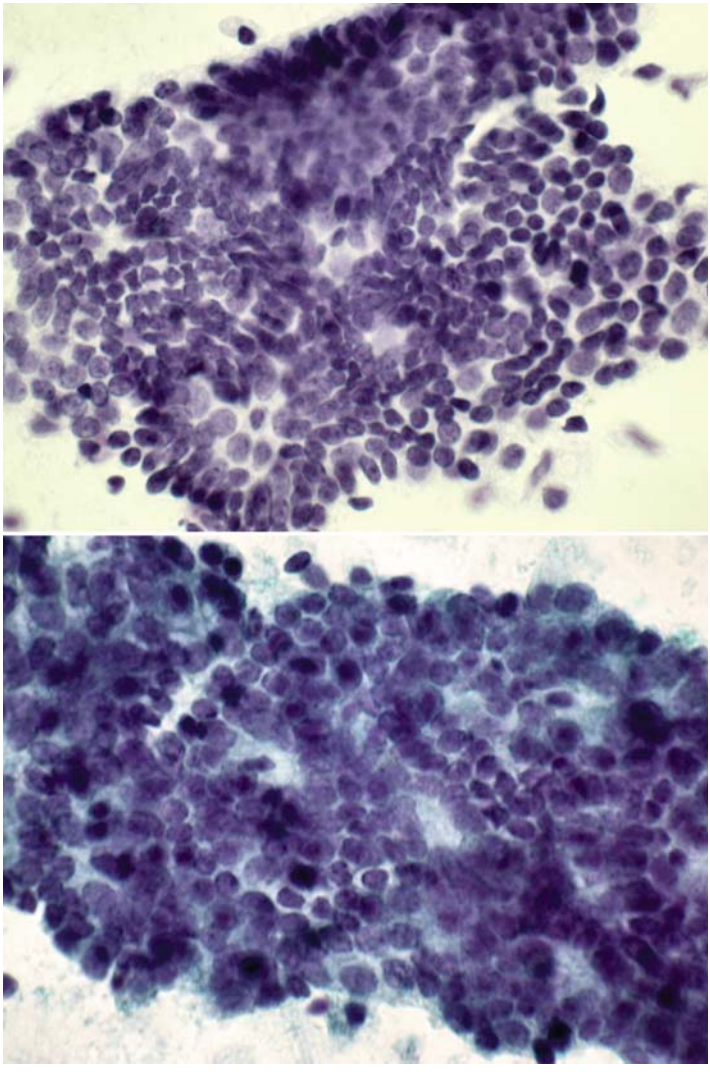

FIG. 9.3. In cytology brush preparations, cribriform structures, no matter which organ the tumor arises from, are easily recognized as microbiopsies comprising a solid aggregate of cells through which loops upon loops of nuclei cause interruptions of the otherwise solid nature of the cell aggregate, affording the appearance of variably shaped acinar glands that reside within the cell aggregate in the absence of a supporting stroma.

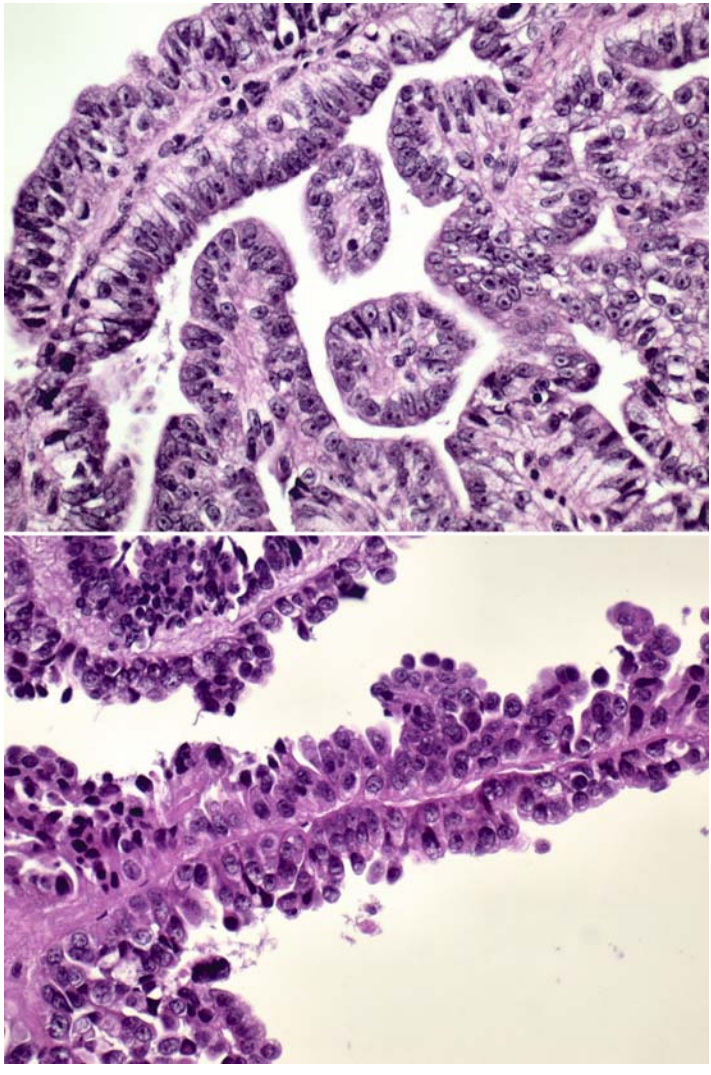

FIG. 9.4. Villoglandular structures comprise vascular structures, with or without a hyaline cuff, decorated by neoplastic cells. Villoglandular structures may be seen with either villoglandular carcinoma, as part of usual endometrioid carcinoma, or with clear cell carcinoma.

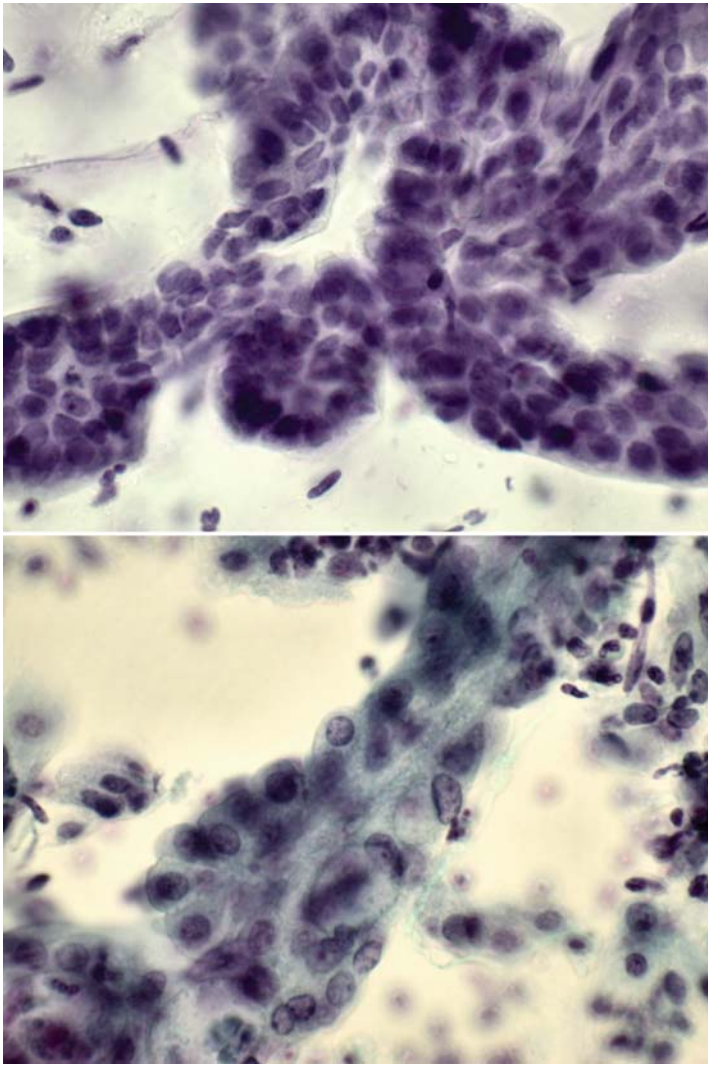

FIG. 9.5. In cytology preparations, villoglandular structures are represented by elongated aggregates of tumor cells; and, on occasion, the central vascular core of the structure may be seen.

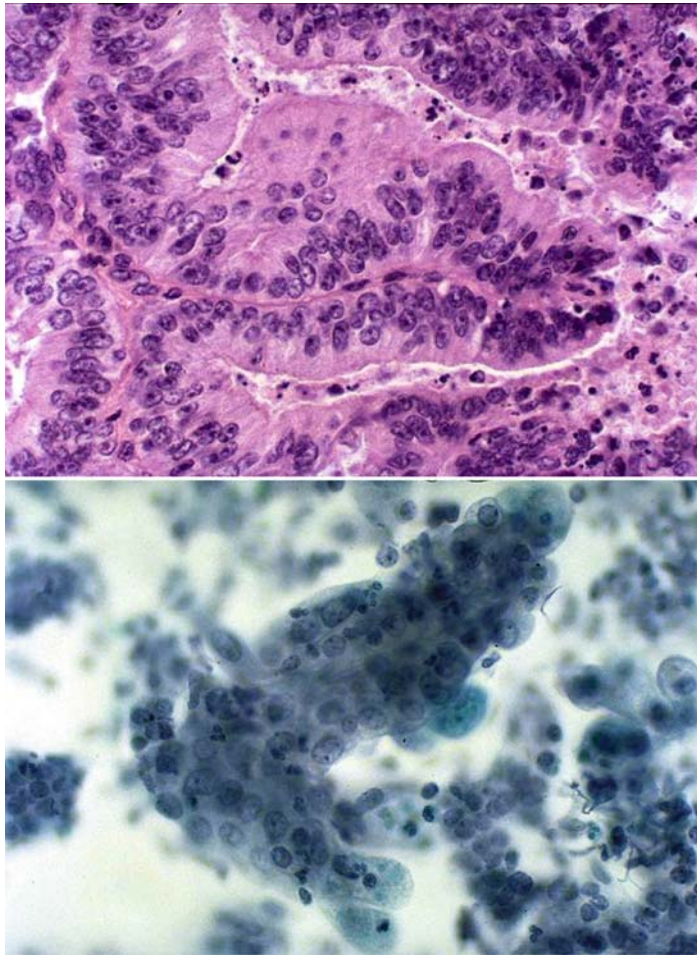

FIG. 9.6. In endometrioid carcinomas, villoglandular or somewhat shorter true papillary structures may be seen to arise from within tubuloacinar-like cisterns (*top*) and may look more like tufted epithelial structures in brush cytology preparations. Close examination of the cytology preparation (*bottom*) shows a fibrovascular core in the dominant branch.

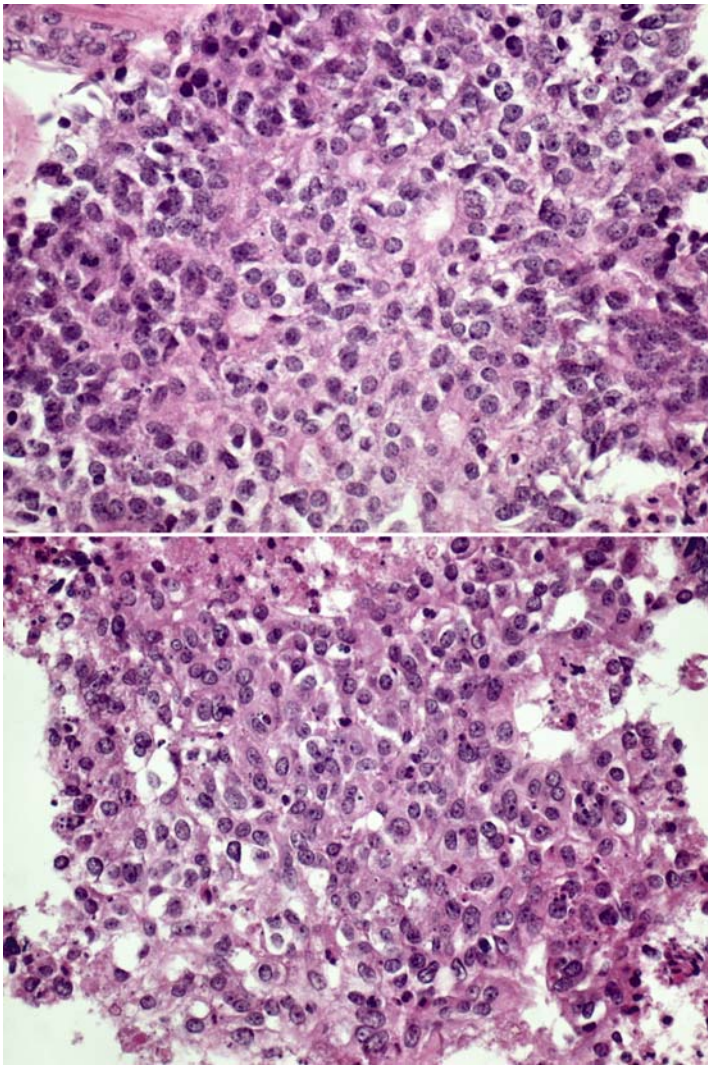

FIG. 9.7. Solid adenomatous epithelial aggregates comprise a signature finding for the diagnosis of carcinoma. Their relative quantity is important in the histological grading of type 1 endometrial carcinoma. Close inspection of the majority of these structures may show subtle remnants of underlying glandular architecture with smoothly contoured rings of nuclei (*top*) conforming to residual cribriform architecture. Sometimes, there are simply disordered back-to-back epithelial cells (*bottom*).

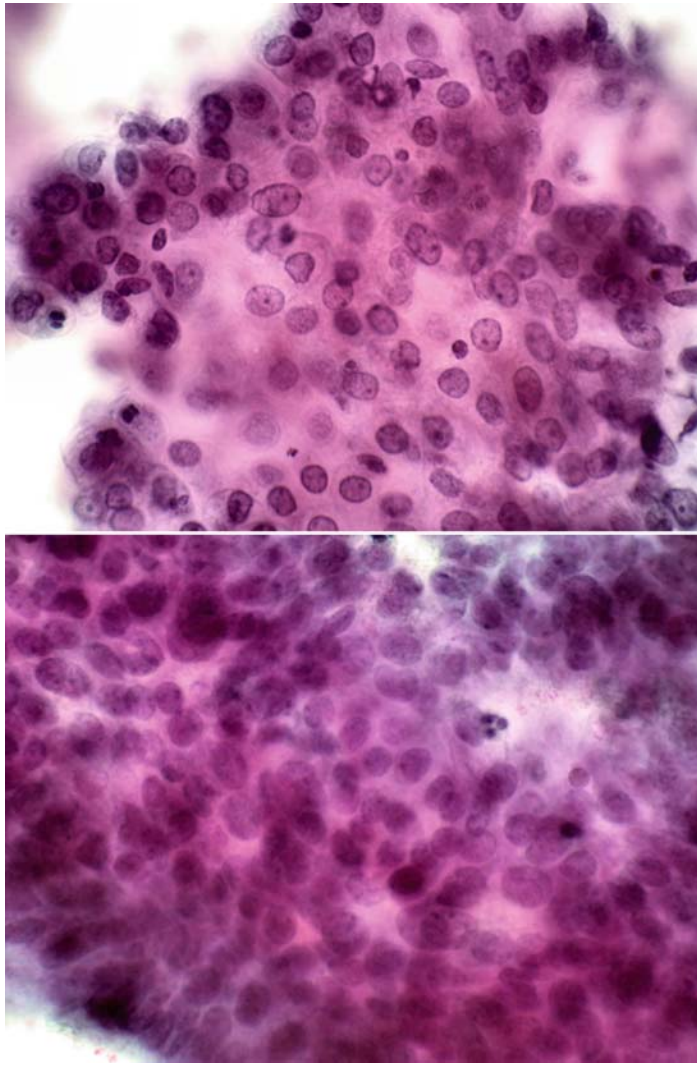

FIG. 9.8. The cribriform nature of many of these solid epithelial aggregates is evident among the cells of endometrial brush microbiopsies (*bottom*) where rings of nuclei serve to define small lumen-like structures.

In cases where epithelial cells haphazardly pack against each other, it may be difficult to decide whether the cell clusters represent epithelium or stroma. In these cases, sarcomatoid carcinoma, undifferentiated stromal sarcoma, or homologous carcinosarcoma enter into the differential diagnosis (Fig. 9.9).

In most cases of carcinoma, fragmented dyshesive epithelial aggregates appear in the cytology brush material. These dyshesive aggregates reflect the dyshesion and breakdown that accompanies malignant neoplasms. Although fragmented epithelial aggregates are not reflective of an underlying epithelial pattern, they represent a change that is nonetheless signature for the diagnosis of malignancy (Figs. 9.10, 9.11).

The individual epithelial cells of endometrial carcinoma are larger than those that would be expected in the proliferative phase. Compared with the normal endometrium, carcinoma cells have a distinctly altered cytology that varies between patients and even within areas of a single tumor, but may include rounded nuclei, clumped chromatin, and prominent nucleoli, all features that vary with the grade of the tumor.

Individual tumors frequently demonstrate patchy changes in differentiation to mucinous, squamous, tubal, or other metaplasias, and in these cases cytoplasmic as well as nuclear features stand out from the normal background. Some endometrioid adenocarcinomas secrete abundant mucin but do not have significant mucin in their cytoplasm, a feature that distinguishes them from mucinous adenocarcinomas.

Mitotic figures, while usually present, may be scanty in well-differentiated tumors, and even fewer than in benign proliferative endometrium. The Ki-67 nuclear reactivity, however, is greater in well-differentiated tumors than in residual nonneoplastic endometrial cells.

Some type 1 carcinomas show secretory changes. Secretory adenocarcinoma is an uncommon variant of endometrioid adenocarcinoma that is composed of well-differentiated glands resembling those of early or mid-secretory endometrium. Subnuclear and/or supranuclear vacuolation is therefore commonly seen (Fig. 9.12). In addition, these tumors may show solid areas consisting of small polygonal cells with clear cytoplasm. In contrast to clear cell carcinoma, the nuclei are only mildly to moderately polygonal, and

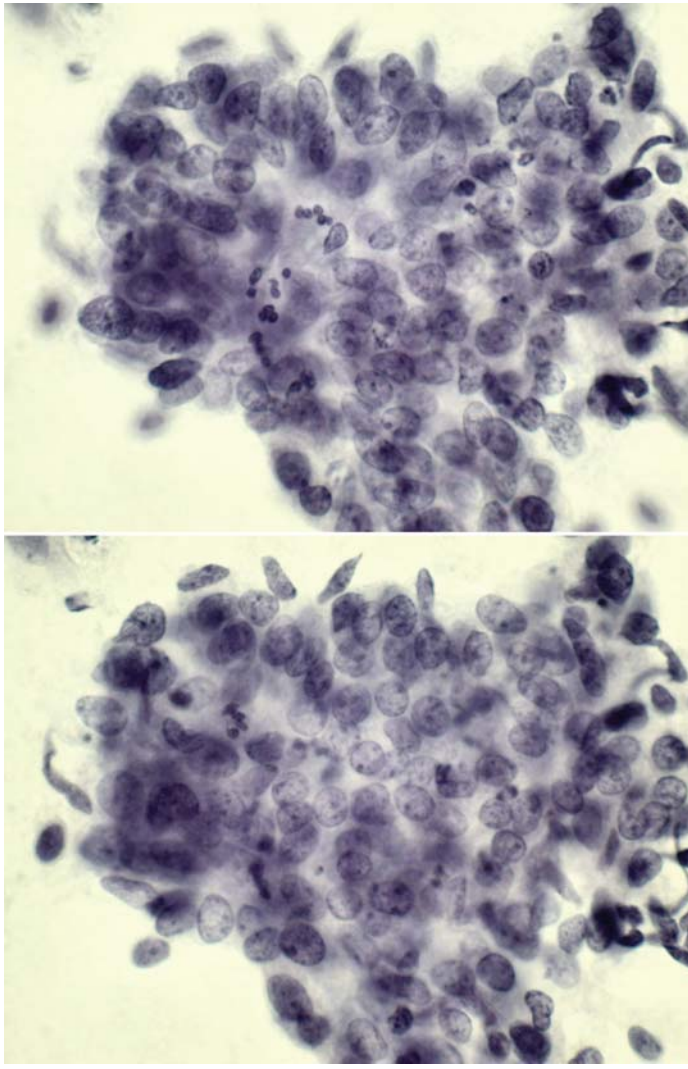

FIG. 9.9. In some adenocarcinomas with solid epithelial structures, no underlying adenomatous architecture is appreciable. In cases where epithelial cells haphazardly pack against each other, it may be difficult to decide whether the cell clusters represent epithelium or stroma; and sarcomatoid carcinoma or high-grade stromal sarcoma enter into the differential diagnosis of the cytological finding. In this microbiopsy, focusing through the tissue shows no evidence of residual glandular structure.

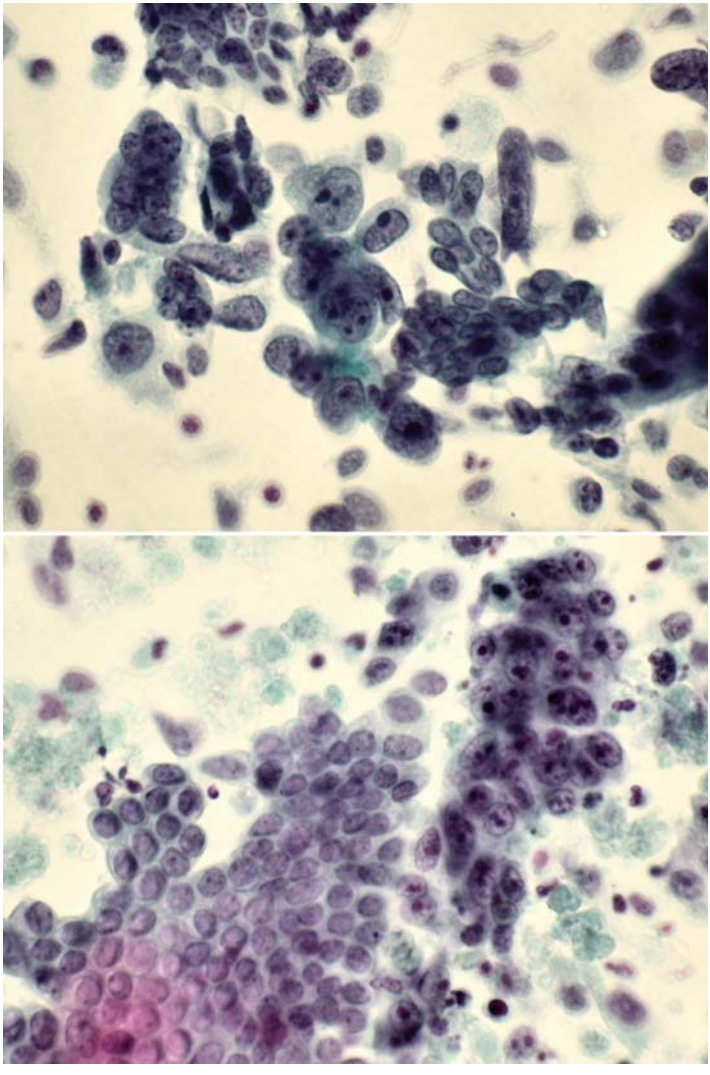

FIG. 9.10. In most cases of carcinoma, fragmented dyshesive epithelial aggregates appear in the cytology brush material. These dyshesive aggregates reflect the dyshesion that accompanies malignant neoplasms. Although fragmented epithelial aggregates are not reflective of an underlying epithelial pattern, they represent a change that is signature for the diagnosis of malignancy, especially when they exhibit high-grade nuclear atypia.

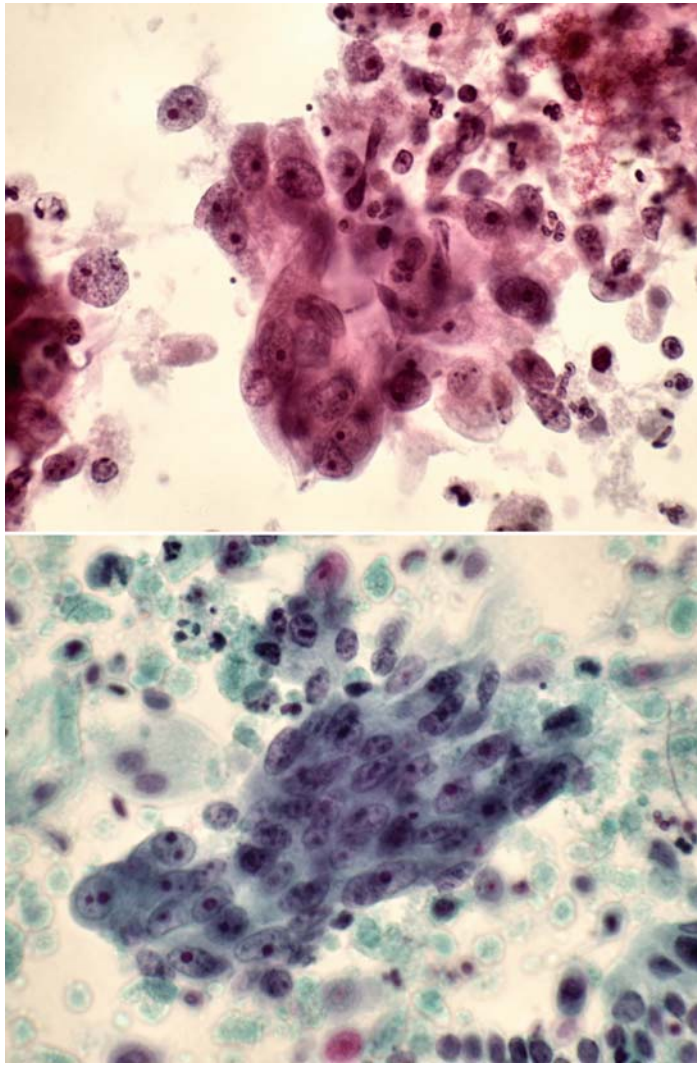

FIG. 9.11. In most cases of carcinoma, fragmented dyshesive epithelial aggregates appear in the cytology brush material. These dyshesive aggregates reflect the dyshesion that accompanies malignant neoplasms. Although fragmented epithelial aggregates are not reflective of an underlying epithelia pattern, they represent a change that is signature for the diagnosis of malignancy.

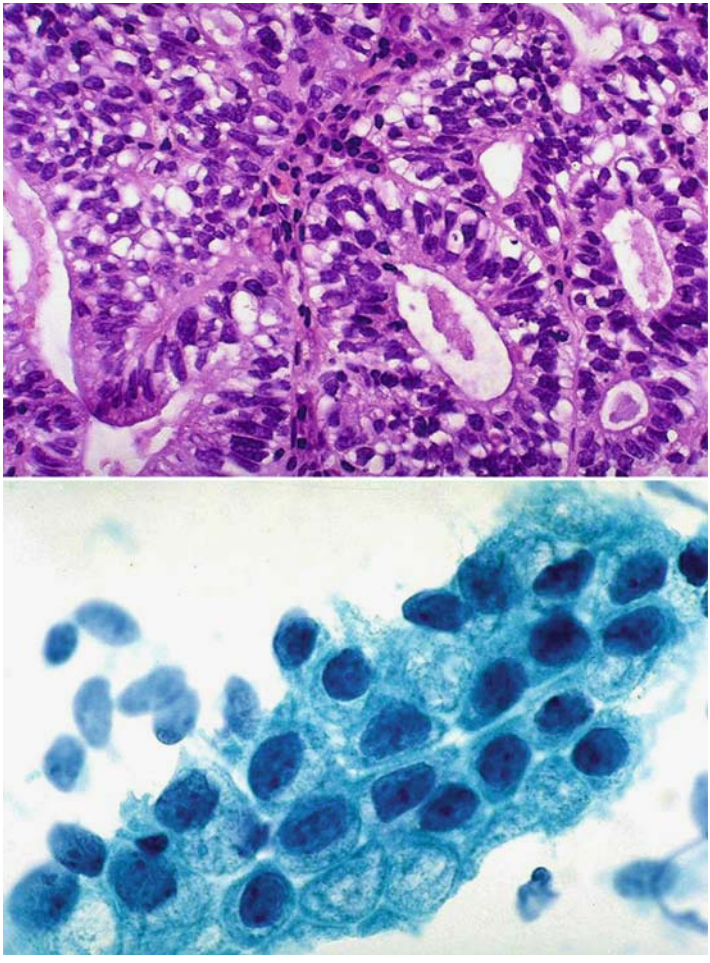

FIG. 9.12. Secretory adenocarcinoma is an uncommon variant of endometrioid adenocarcinoma that is composed of well-differentiated glands resembling those of early or mid-secretory endometrium. Solid areas of tumor may at times be seen, as illustrated in the *left upper corner of the top image*. Subnuclear and/or supranuclear vacuolation is commonly seen. Cytologically, secretory carcinoma may be mistaken for an Arias–Stella reaction, and vice versa.

hobnail features are absent. The tumor may be composed entirely of secretory glands, although it is more common to find the secretory changes focally distributed. Secretory adenocarcinomas are associated with a good prognosis.

There are two types of secretory carcinoma. One is induced by circulating progestins, while the other is an intrinsic feature independent of background hormonal state. The presence or absence of progestin-induced stromal change and the menopausal status of the woman distinguish these etiologies. It is not uncommon for a woman who has had both a biopsy and hysterectomy to have one sampling show secretory adenocarcinoma and the other typical nonsecretory adenocarcinoma, indicating that secretory effect in the tumor is a manifestation of the ovulatory cycle or the prebiopsy use of progestins, often used to control uterine bleeding or to induce withdrawal bleeding.

The cytological diagnosis of low-grade endometrial cancer is also supported by secondary features such as substantial involvement of the sample by abnormal epithelium, tumor diathesis, stromal foam cells, necrosis (even of apparently normal-appearing endometrium), neutrophilic emperipolesis, and minimal retention to absence of endometrial stroma. These features are also typical of EIN and "suggest" rather than "confirm" cancer.

Histological and cytological features of outspoken cancer are villoglandular, maze-like or cribriform architecture, and solid cell masses. Stromal desmoplasia is rarely appreciated in endometrial cytology preparations, but may be seen in corresponding cell blocks, which are generally available in more than 90% of carcinomas. In the absence of a definitive cancer diagnosis on the basis of first sampling, any of the features listed herein mandate reexamination in a timely fashion.

Type 2 Endometrial Cancers

In contrast to the endometrioid (type 1) group of endometrial carcinomas, serous carcinoma of the endometrium is an aggressive tumor with a high recurrence and low survival rate. Compared to endometrioid carcinoma, it is less common, involves older women, has no racial predilection, and is associated with p53 mutations,

abnormalities of the Her2/neu gene, and aneuploidy. It is not associated with endometrial hyperplasia as are many endometrioid endometrial carcinomas, and immunostains for estrogen and progesterone receptor protein are generally negative. These tumors may be seen in the company of endometrioid adenocarcinoma, clear cell carcinoma, or tubal-ovarian serous carcinoma.

As with EmGD/EIC, serous carcinoma is associated with endometrial polyps and is itself often polypoid; 40% of women with stage I serous carcinoma die of disease, 60% show retroperitoneal involvement, more than 60% have malignant cervicovaginal cytology smears, and 25% have primary endocervical involvement. Superficial endometrial tumor with extensive peritoneal disease is not uncommon. Serous carcinoma of the endometrium resembles tubal-ovarian serous carcinoma, spreading into the abdomen in a similar manner, often with omental nodules or cakes. Bladder metastases of serous carcinoma are notorious for simulating primary high-grade urothelial carcinoma.

Serous carcinoma is considered high grade and is not further graded using FIGO, although some pathologists append FIGO grade 3 to its diagnosis. It often shows abrupt transition from normal atrophic endometrium to tumor, and even single gland involvement portends aggressive behavior.

Serous carcinoma may be found in otherwise atrophic-appearing uteri; therefore, it is not excluded by a thin endometrial stripe on ultrasound and may be associated with an antecedent acellular suction biopsy. It shows flat epithelial surface involvement, stubby pseudopapillae, and papillae of various size or tubules with highly pleomorphic tumor cells containing prominent nucleoli, small detached buds and tufts, and frequent mitotic activity, as evidenced by Ki-67 nuclear decoration that is equal in both intensity and distribution to p53 nuclear decoration. Dirty necrosis is common, and angiolymphatic and myometrial invasion may be seen in accompanying cell block material. About 40% of tumors have psammoma bodies.

Cytologically, the tumor cells of serous carcinoma tend to shed in clusters along with a necroinflammatory tumor diathesis. Psammoma bodies are seen in some cellular samples from serous cancers but are not common with endometrioid cancers. The frequency of irregularly shaped nuclei, membrane thickness, and eccentric nuclei in serous cancer is higher than that seen in endometrioid cancer. The chromatin pattern is coarsely granular,

and both anisonucleosis and bare nuclei are prominent. Morphometrically, the maximum nuclear diameter of serous cancer significantly exceeds that of endometrioid cancer. Nucleoli are also more often seen in serous than in endometrioid cancer. The cytological diagnosis of serous cancer should be based on the findings of tumor diathesis, psammoma bodies (when present), and clusters of tumor cells with enlarged nuclei and numerous nucleoli.

Serous carcinoma may have overlapping features with clear cell carcinoma or, about 40% of the time, arise in association with clear cell carcinoma. This distinction is not important because clinical management is the same for both serous and clear cell carcinoma.

Serous carcinoma may be preceded by EIC/EmGD or it may be a component of a mixed endometrioid and serous (type 1/type 2) tumor. In its earliest stages, serous carcinoma may present with tufted epithelial structures, resembling those seen with EIC/EmGD, or it may show creeping substitution of the surface and glands of otherwise atrophic endometrium, often resulting in the juxtaposition of atrophic and neoplastic glands (Fig. 9.13) within cell blocks. The high-grade nuclear atypia of cell block sections smoothly translates into the grade 3 nuclei that typify cytology preparations derived from this malignancy (Fig. 9.14). Immunohistochemically, serous carcinoma and clear cell carcinoma show intense nuclear decoration by antibodies to p53 and Ki-67 (Fig. 9.15). Sometimes, serous and clear cell carcinoma admix with one another, whereby the high-grade nuclear features of serous carcinoma and the typical solid clear cell areas of clear cell carcinoma may be found in different areas of the same tumor (Fig. 9.16).

Similar to serous carcinoma, clear cell carcinoma shows a distinctive immunoprofile, lacking immunoreactivity for estrogen and progesterone receptor proteins (ER and PR). It has a lower immunoreactivity for p53 than serous cancers, but a high Ki-67 proliferation index. Findings suggest that the molecular events that underlie the development of clear cell carcinoma differ from those of endometrioid and serous carcinoma. When compared to endometrioid endometrial carcinoma, ER and PR expression is significantly lower, p53 expression is somewhat higher, and the Ki-67 proliferation index is significantly higher. On a practical note, immunohistochemical staining for Ki-67 and p53 helps in distinguishing endometrial hobnail or clear cell metaplasias and the Arias–Stella reaction from clear cell carcinoma.

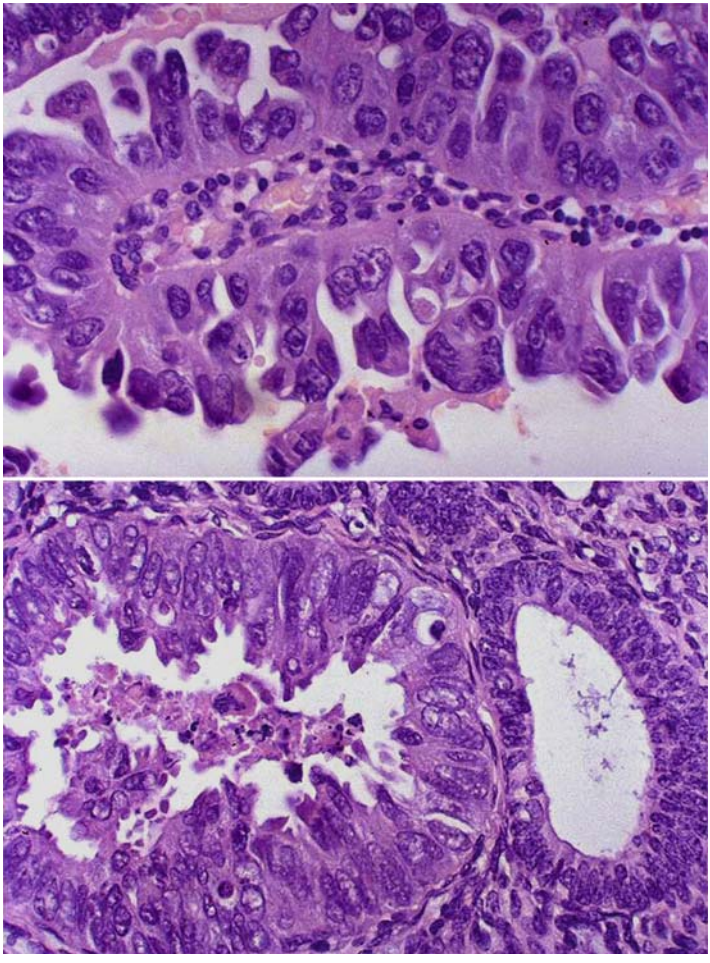

FIG. 9.13. In its earliest stages, serous carcinoma may present with tufted epithelial structures, resembling those seen with endometrial intraepithelial cancer/endometrial gland dysplasia (EIC/EmGD); or, it may show creeping substitution of the surface and glands of otherwise atrophic endometrium, often resulting in the juxtaposition of atrophic and neoplastic glands.

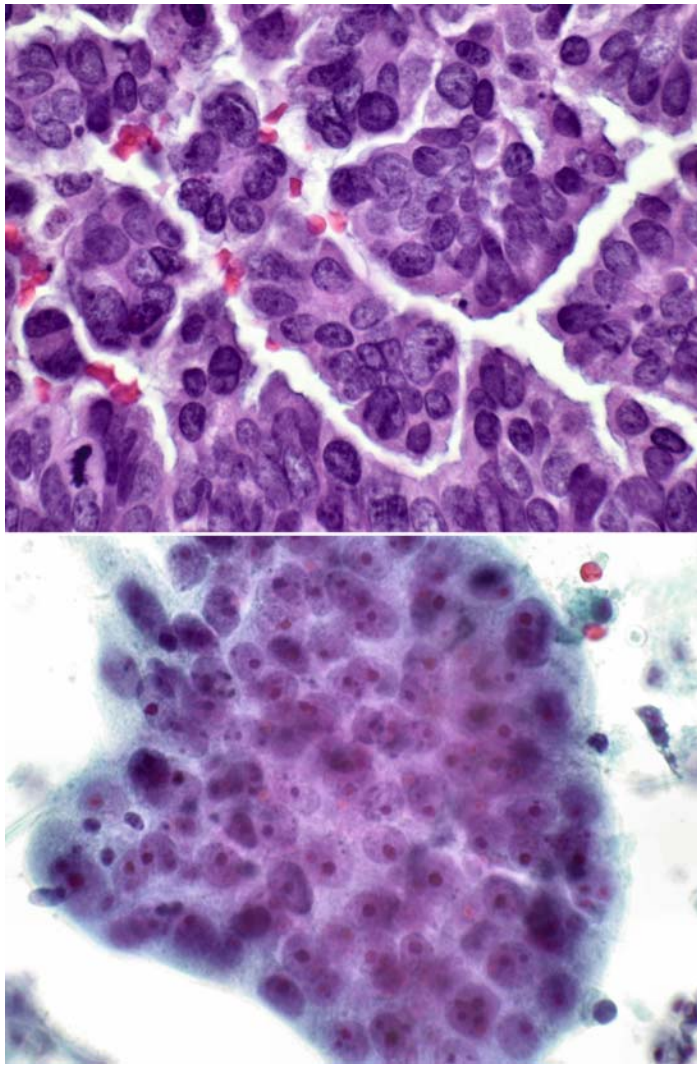

FIG. 9.14. The high-grade nuclear atypia of cell block sections of serous carcinoma (*top*) smoothly translates into the grade 3 nuclei that typify cytology preparations derived from this malignancy (*bottom*).

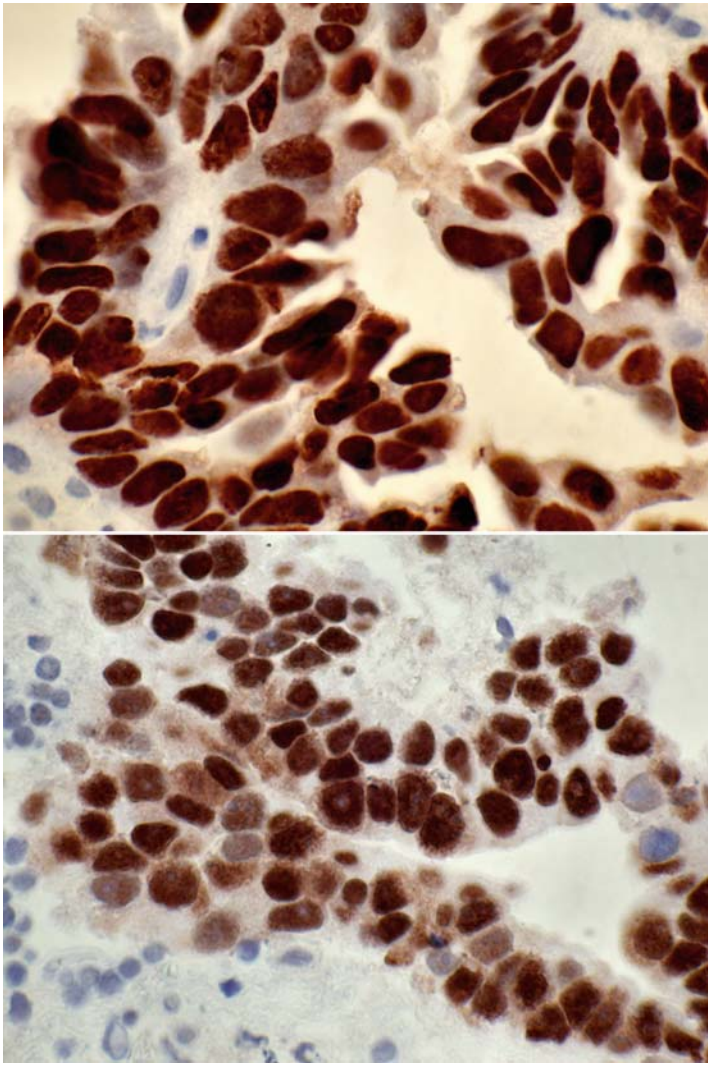

FIG. 9.15. Immunohistochemically, serous carcinoma and clear cell carcinoma show intense nuclear decoration by antibodies to p53 (*top*) and Ki-67 (*bottom*).

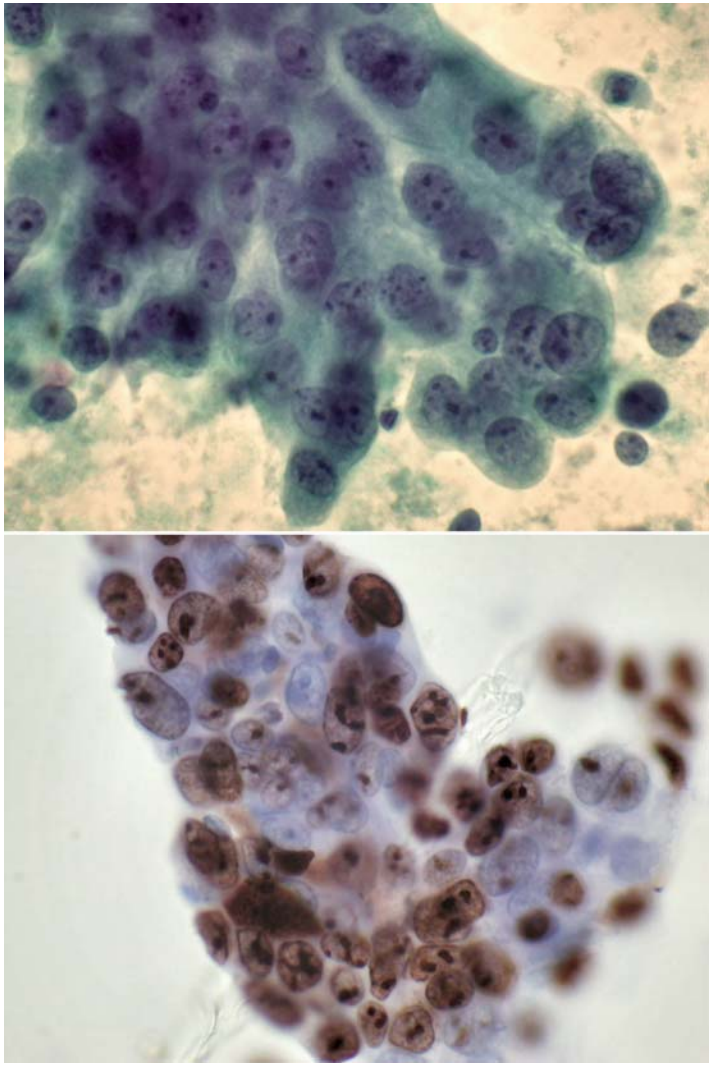

FIG. 9.16. Immunohistochemically, serous carcinoma and clear cell carcinoma show intense nuclear decoration by antibodies Ki-67 (*bottom*). This case, which appeared to show both serous and clear cell features, was signed out as type 2 carcinoma, NOS (not otherwise specified).

Structurally, serous cancers appear far more dyshesive than endometrioid or the other so-called type 1 cancers. For example, in a study of cervical smears, serous carcinomas were hypercellular with a background tumor diathesis, papillae, bare nuclei, and cells with large pleomorphic nuclei and bulky dense cytoplasm. In contrast, endometrioid endometrial carcinoma showed a relatively monomorphic population of cells with moderately enlarged oval nuclei and delicate cytoplasm. In the case of mixed tumors, the features were similar to those of serous carcinoma, suggesting preferential exfoliation or structural instability of the serous carcinoma component of the tumor.

The hallmark change of clear cell carcinoma that is seen in cell block preparations is the solid aggregation of highly malignant-appearing cells with central nuclei and significant amounts of peripherally cleared cytoplasm (Fig. 9.17). In some cell blocks obtained from clear cell carcinomas that we have seen among our material, malignant papillae showed subtle amounts of perivascular hyaline material; and corresponding cytological material showed epithelial tufts with grade 3 nuclei (Fig. 9.18). In other cases, the high-grade cells of type 2 carcinoma were admixed with malignant papillae whose perivascular cores contained amorphous material (Fig. 9.19). We observed at least two cases of type 2 carcinoma with isolated dyshesive cells with such a degree of anaplasia and cytoplasmic smudginess as to raise a suspicion for an accompanying rhabdoid-like malignant "stromal" component (Fig. 9.20).

Endometrial Carcinosarcoma

Endometrial carcinosarcomas are epithelial malignancies with a malignant mesenchymal component that may include homologous or heterologous sarcomatous elements. They were long known as malignant mixed Müllerian tumors, but were redesignated as endometrial carcinosarcomas by the World Health Organization (WHO) in 2003 to reflect current understanding that they are primarily epithelial tumors that have developed a mesenchymal component. Another less popular idea is that endometrial carcinosarcoma is an endometrial stem cell tumor, akin, for example, to chronic myelogenous leukemia, where heterologous elements represent the gamut of tissues that might develop from endometrial

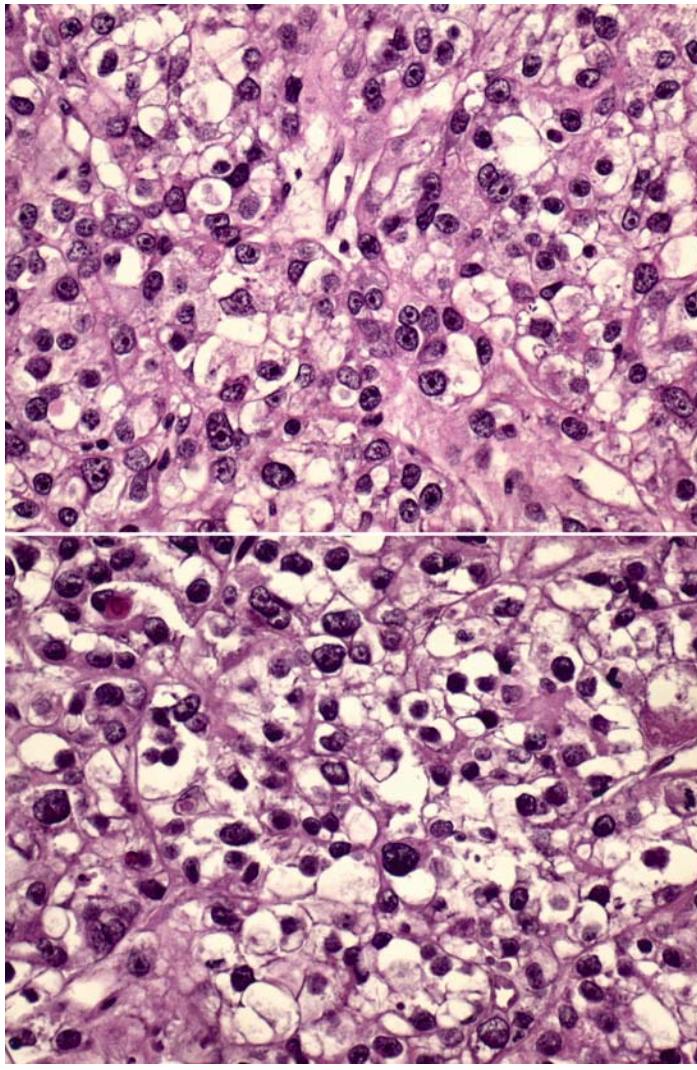

FIG. 9.17. The hallmark change of clear cell carcinoma that is seen in cell block preparations is the solid aggregation of highly malignant-appearing cells with often significant amounts of peripherally cleared cytoplasm. Although the degree of cytoplasmic clearing may resemble solid areas of secretory carcinoma, the nuclear anaplasia is that of a type 2 carcinoma.

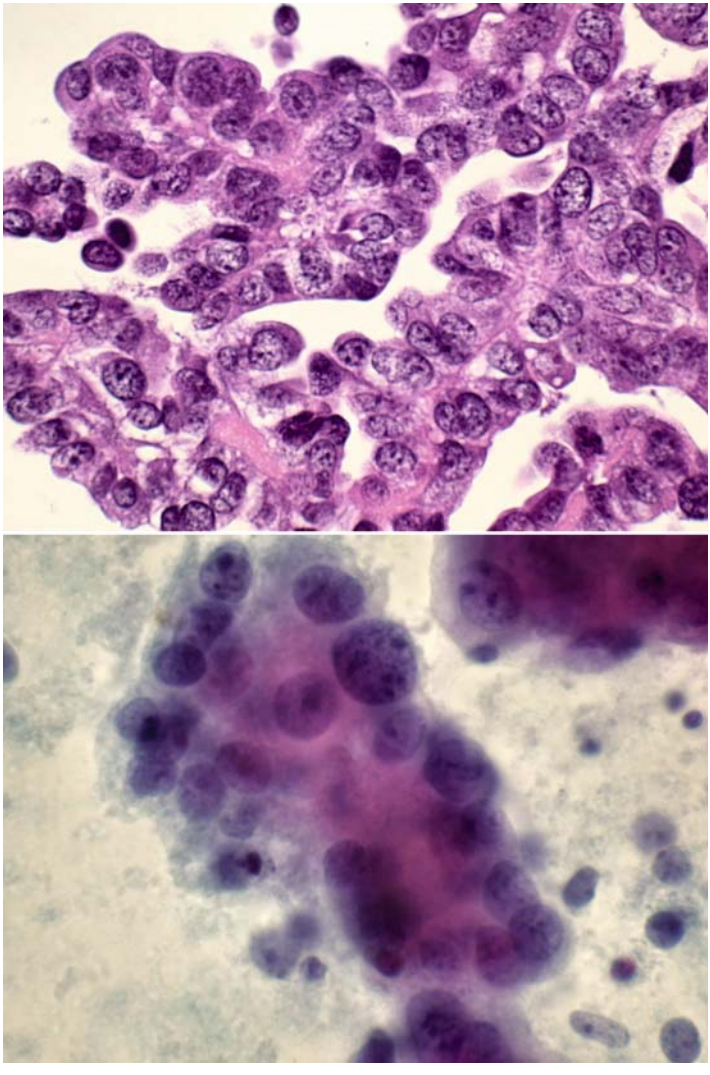

FIG. 9.18. In some cell blocks obtained from clear cell carcinomas that we have seen among our material, malignant papillae showed subtle amounts of perivascular hyaline material; corresponding cytological material showed epithelial tufts with grade 3 nuclei, sometimes surrounding an amorphous core.

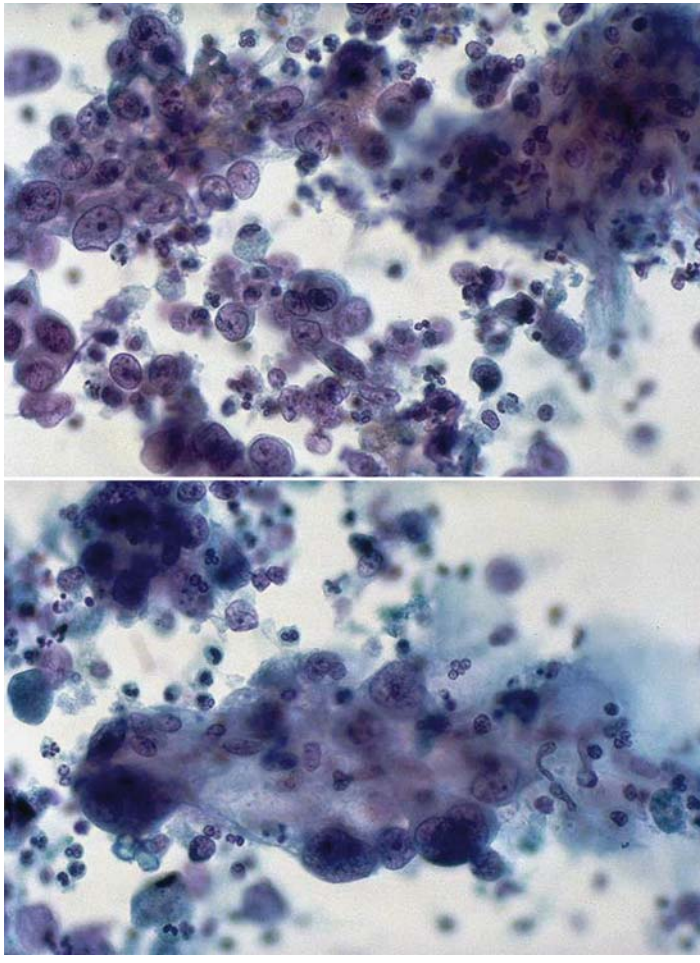

FIG. 9.19. In other cases, the high-grade cells of type 2 carcinoma could be seen admixed with malignant papillae whose perivascular core contained more obvious amorphous material (*bottom*), which led us to render a cytological diagnosis of type 2 carcinoma with (at least) a clear cell component. Once type 2 carcinoma is diagnosed, typing is irrelevant: although these tumors probably arise along divergent pathways, serous and clear cell carcinoma are treated in the same way and have the same abysmal prognosis.

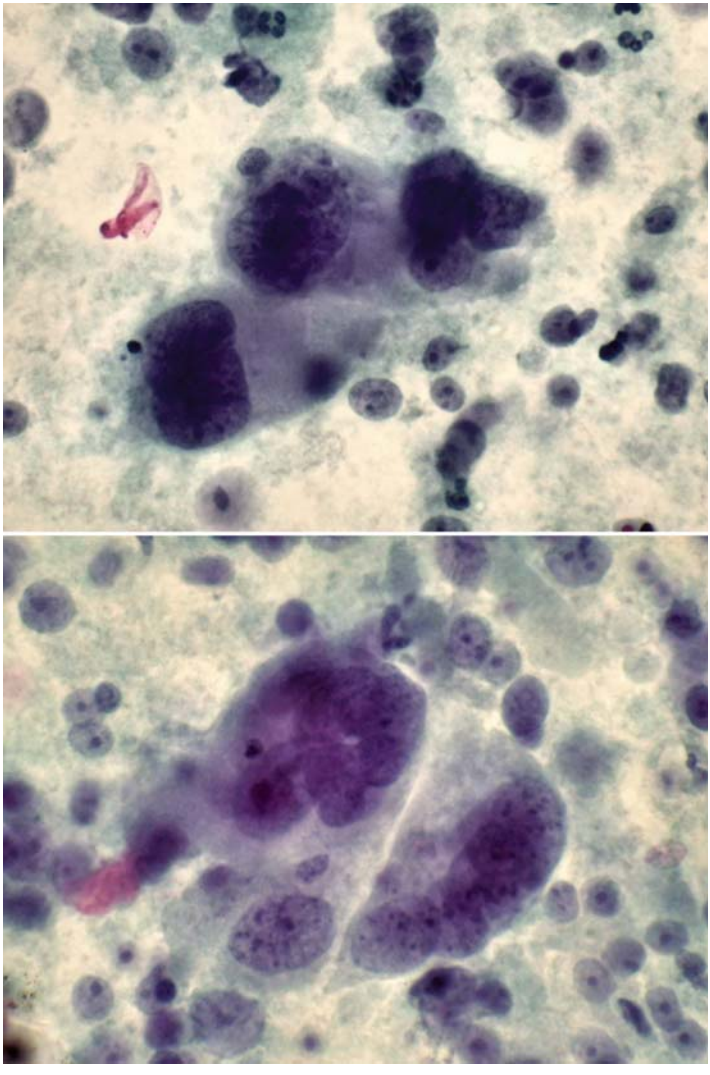

FIG. 9.20. We observed at least two cases of type 2 carcinoma with isolated dyshesive cells with such a degree of anaplasia and cytoplasmic smudginess as to raise a suspicion for an accompanying rhabdoid-like malignant stromal component.

stem cells. The stem cell protein Piwil2 that is widely expressed in tumors has been identified in uterine carcinosarcomas.

Carcinosarcoma is rare, normally occurs in postmenopausal women, presenting with bleeding, uterine enlargement, and sometimes a large, protruding polypoid mass. Many women have a history of radiation therapy or chronic estrogen stimulation. Sometimes tumor metastases may resemble serous carcinoma metastases. Stromal differentiation may be homologous (resembling normal stromal components) or heterologous (representing other forms of soft tissue sarcoma). Heterologous differentiation is of no prognostic importance, and the prognosis of this highly aggressive tumor defaults to stage grouping, being very poor if the tumor extends to the uterine serosa.

Carcinosarcomas are large, fleshy, often polypoid, hemorrhagic, highly invasive and friable tumors that may protrude through the cervical os. The most common epithelial component is glandular (endometrioid, clear cell, serous), and it is most commonly poorly differentiated. The sarcomatous components are either homologous (endometrial stromal sarcoma, leiomyosarcoma) or heterologous (skeletal muscle, cartilage, osteoid, fat). As with other poorly differentiated tumors, angiolymphatic invasion is common. Because the progenitor cell of the tumor is common to both the carcinomatous and sarcomatous elements of the tumor, both elements may stain positively for keratin, epithelial membrane antigen (EMA), p53, vimentin, myoglobin, etc.; indeed, ultrastructural examination may reveal hybrid epithelial/stromal cells.

In studies of the pattern and frequency of defective DNA mismatch repair and p53 alterations in the epithelial and mesenchymal components of uterine carcinosarcomas, defective DNA mismatch repair and p53 defects were commonly found early events in carcinosarcoma tumorigenesis. The high rate of concordance for these molecular defects between the carcinoma and sarcoma components provides molecular evidence that carcinosarcomas are clonal malignancies.

Carcinosarcomas share some molecular and epidemiological risk factors with endometrioid endometrial carcinoma, including PTEN mutation, microsatellite instability, obesity, use of exogenous hormones, and nulliparity. However, carcinosarcomas have a worse prognosis than other high-grade endometrial carcinomas.

Carcinosarcoma is sufficiently distinctive that it cannot be thought of as a subset of another endometrial tumor type; rather, it is an entity onto itself. Features of the stromal component of the primary tumors, including grade, mitotic index, and the presence and types of heterologous elements, show no relationship to the presence of metastases. High-grade, serous, and clear cell carcinomatous components, on the other hand, are associated with a higher frequency of metastases, as are deep myometrial invasion, lymphatic or vascular space invasion, and involvement of the uterine isthmus or cervix.

We diagnosed two uterine carcinosarcomas, both of which showed serous carcinoma as their epithelial component. In addition to the carcinomatous elements, both cases showed a combination of rhabdoid-like cells and spindled malignant cells (Figs. 9.21–9.24). In one of two cases, cell block preparations showed significant Ki-67 nuclear decoration in the "stromal" as well as in the epithelial cells (Fig. 9.24).

The Cytology of High-Grade Endometrial Malignancy

The cytological diagnosis of high-grade endometrial carcinoma is usually straightforward. High-grade cancers may include special variants of endometrioid cancers such as those with trophoblastic differentiation or giant cells; serous or clear cell cancers admixed with typical endometrioid adenocarcinoma; squamous cell cancer; glassy cell cancer; large and small cell undifferentiated cancer; and carcinosarcomas with homologous or heterologous stromal elements. Rather than featuring recognizably differentiated structures, high-grade cancers show solid cell aggregates, cellular dyshesion, and sarcomatoid changes.

Mitoses are frequent and oftentimes bizarre; therefore, the degree of dysdifferentiation is reflected by both the mitotic index and Ki-67 staining. Normal endometrial stroma is essentially absent. Tumor diathesis is usually present, and collagen and elastic fibers and isolated smooth muscle cells, all indicative of myometrial invasion, may be seen. Because of their collective appearance of extreme anaplasia and dysdifferentiation, these tumors may be difficult to subclassify, cytologically, but they are easily recognized as highly malignant and warranting extended operative staging from the outset.

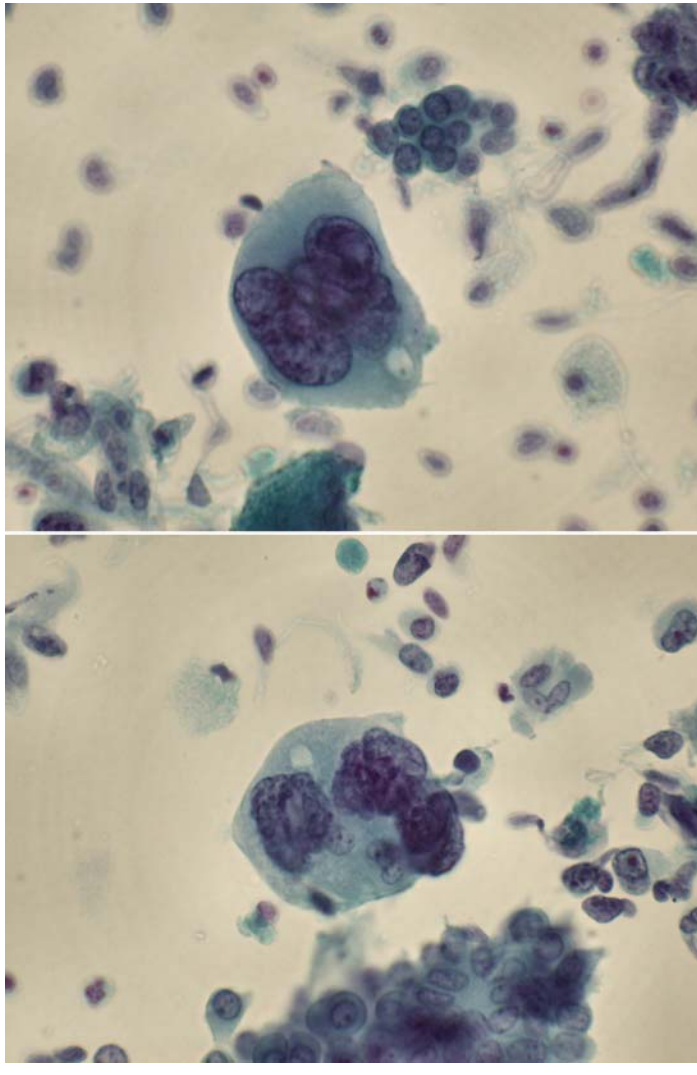

Fig. 9.21. We diagnosed two uterine carcinosarcomas, both of which showed malignant type 2 (serous) carcinoma elements. In addition to the carcinomatous elements, both cases showed a combination of rhabdoid-like cells and spindled malignant stromal-like cells (sarcomatoid carcinomatous elements). These images show tumor giant cells.

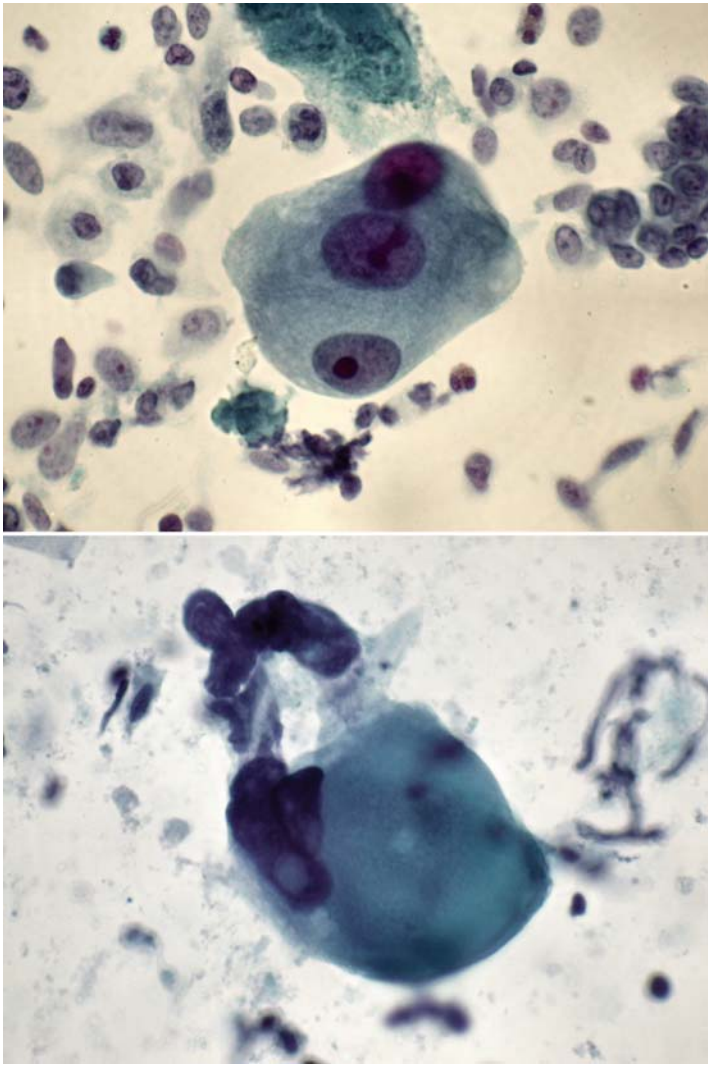

FIG. 9.22. These images show the coexistence of epithelioid cells (*top*) and rhabdoid cells (*bottom*).

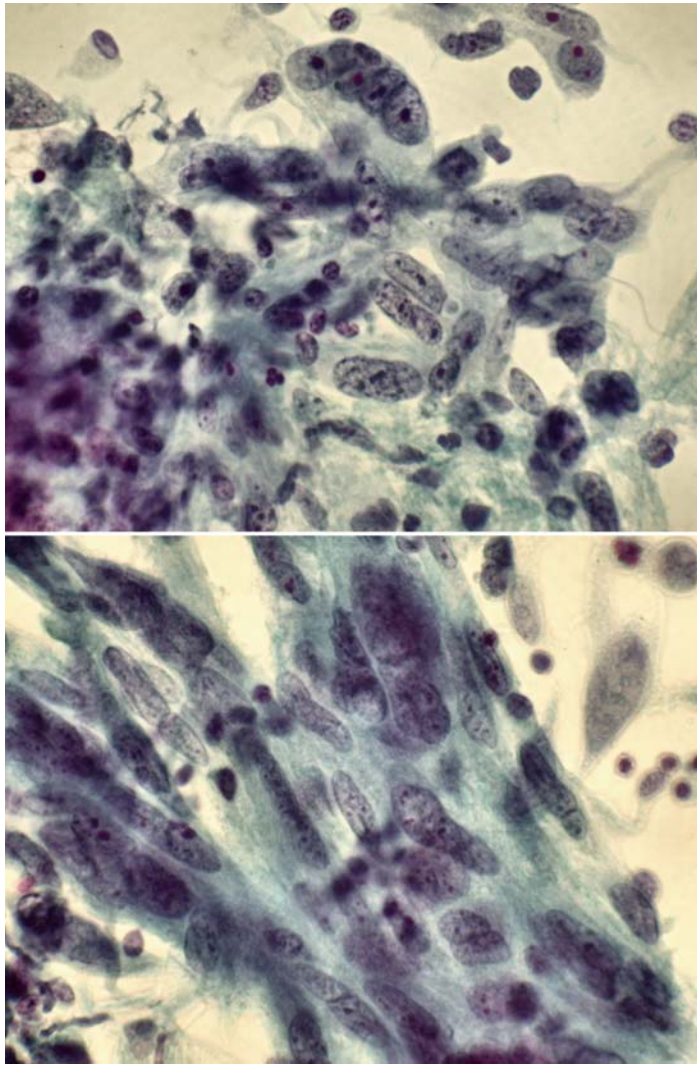

FIG. 9.23. When the spindled cells of a carcinosarcoma represent the only element that is harvested, the differential diagnosis includes uterine sarcoma. Immunostains for epithelial markers are useful for diagnosis.

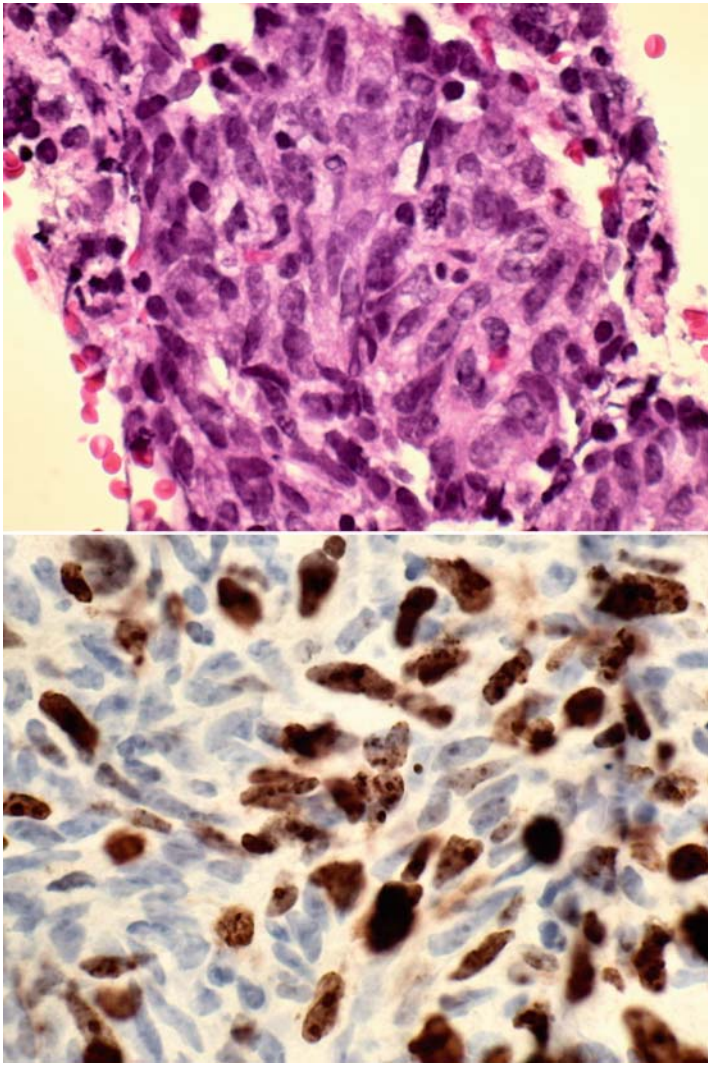

FIG. 9.24. In one of two cases of uterine carcinosarcoma, cell block preparations showed significant Ki-67 nuclear decoration in the "stromal" as well as in the epithelial cells. In contrast to the case with squamous elements in adenoacanthoma, the metaplastic elements of carcinosarcoma participate in the malignant process.

Stromal Sarcoma of the Endometrium

Endometrial stromal sarcomas are rare uterine mesenchymal neoplasms. Low-grade lesions resemble normal proliferative phase endometrium and lack significant cytological atypia; but, in tissue preparations, they show infiltrative borders with or without vascular space invasion. Endometrial stromal tumors resembling normal-appearing endometrial stroma have been traditionally classified into endometrial stromal nodule and endometrial stromal sarcoma.

Endometrial stromal sarcoma comprises less than 10% of the uterine mesenchymal neoplasms, and approximately one-half of women affected are premenopausal. Low-grade endometrial stromal sarcomas are indolent tumors with local recurrences and distant metastasis occurring even 20 years after initial diagnosis. Several morphological variants include those with fibromyxoid features and those showing smooth muscle, fibroblastic, and epithelial differentiation, which sometimes resembles sex cord elements. One unifying feature among cases is a prominent arborizing and pericytomatous vasculature.

Until recently, stromal sarcomas of the endometrium were classified as low grade or high grade, but today they are simply stromal sarcomas. As opposed to being well circumscribed, stromal sarcomas comprise irregular, jagged islands, or tongues of neoplastic stromal cells that infiltrate between smooth muscle bundles of the surrounding normal myometrium. Angiolymphatic invasion is common. Low-grade endometrial stromal sarcoma shows slow clinical progression with repeated local and late systemic recurrence. Cases have occurred in association with tamoxifen therapy.

As originally defined, high-grade stromal sarcoma is much less common than low-grade endometrial stromal sarcoma, having a 5-year survival of less than 50% with recurrences in the pelvis and frequent metastases to lung. This tumor diffusely involves most of the endometrial surface and frequently extends beyond the uterus. Vascular invasion not as evident as with low-grade stromal sarcomas, and it usually shows extensive myometrial invasion, marked nuclear atypia, cellular enlargement, and pleomorphism. Simply put, the cells of high-grade stromal sarcoma do not resemble those of the endometrial stroma. No arborizing or hemangiopericytomatous vasculature is seen. The tumor resembles the stromal

component of endometrial carcinosarcoma. These tumors are now classified as undifferentiated stromal sarcoma.

CD10 is a reliable and sensitive immunohistochemical marker of normal endometrial stroma. Strong and diffuse immunoreactivity occurs in both endometrial stromal nodules and low-grade endometrial stromal sarcomas. Positive staining with CD10, when strong and diffuse, may be useful in distinguishing these tumors from histological mimics, especially cellular leiomyoma. In this situation, CD10 should be part of a panel including desmin, actin, or h-caldesmon and alpha-inhibin. A caveat is that the entity known as stromomyoma, which shows immunoreactivity akin to uterine smooth muscle, may actually fall within the spectrum of changes seen with low-grade stromal sarcoma.

CD10 expression is not restricted to endometrial stromal sarcoma but can show reactivity in endometrial carcinosarcoma and Müllerian adenosarcoma as well as in a variety of uterine tumors including high-grade leiomyosarcoma and rhabdomyosarcoma. CD10 expression might be one characteristic of Müllerian system-derived neoplastic mesenchymal cells. Furthermore, evaluation for CD10 immunoreactivity alone is frequently not informative about differentiating endometrial stromal sarcomas from mimics with pericytomatous vascular patterns. Although ER best discriminates between these entities, an immunohistochemical panel of anti-CD10, antiestrogen receptor protein (anti-ER), and anti-CD34 may help in this situation. Progesterone receptor protein (PR) evaluation may have therapeutic relevance in endometrial stromal sarcoma, but the results do not distinguish endometrial stromal sarcoma from solitary fibrous tumor or hemangiopericytoma. Furthermore, endometrial stromal sarcoma variants can lose CD10 immunoreactivity and may also react with epithelial and myoid markers depending on differentiation.

We diagnosed two uterine stromal sarcomas, both low-grade types, using cell block preparations and correlative ultrasonography; these presented with overwhelming proliferative-like endometrial stroma that lacked intervening proliferative glands. Cell blocks showed essentially normal to only mildly abnormal-appearing stromal cells (Fig. 9.25). Brush cytology preparations showed normal to only mildly abnormal-appearing compact stromal fragments (Figs. 9.26, 9.27). Thin-walled vasculature permeated the cell blocks of both cases, and vessels were especially noticeable as negatively stained

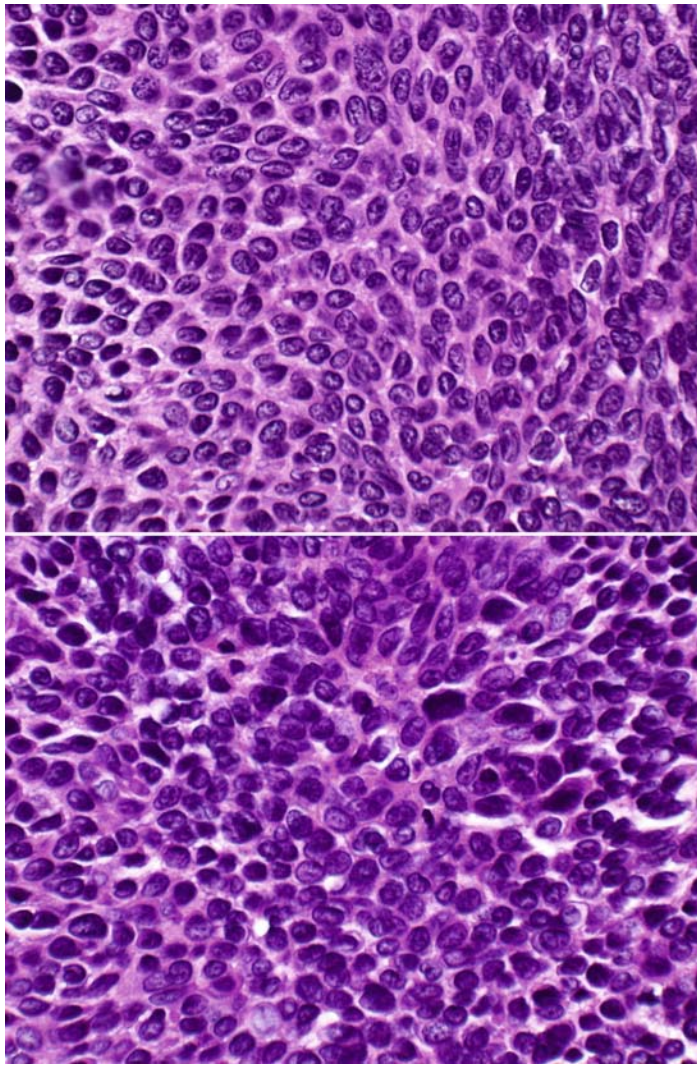

FIG. 9.25. Cell blocks prepared from two cases of low-grade stromal sarcoma showed essentially normal (*top*) to only mildly abnormal-appearing (*bottom*) stromal cells with proliferative-like features.

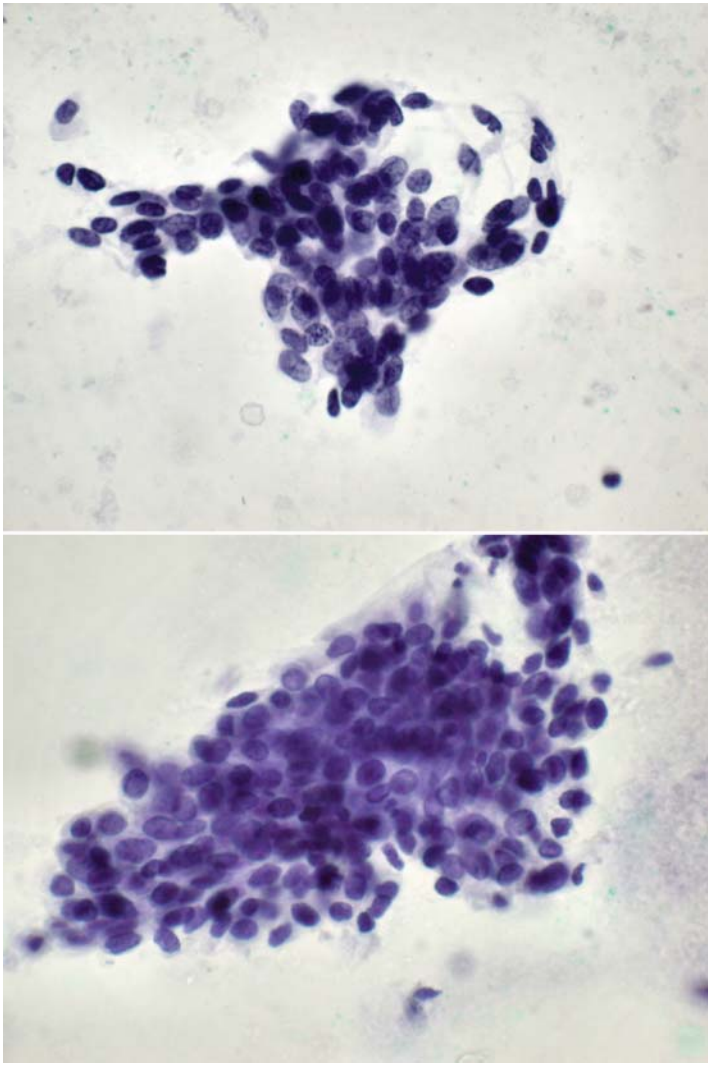

FIG. 9.26. These brush cytology preparations are derived from the case illustrated in Fig. 9.25 (*top*), which shows normal-appearing compact stromal fragments.

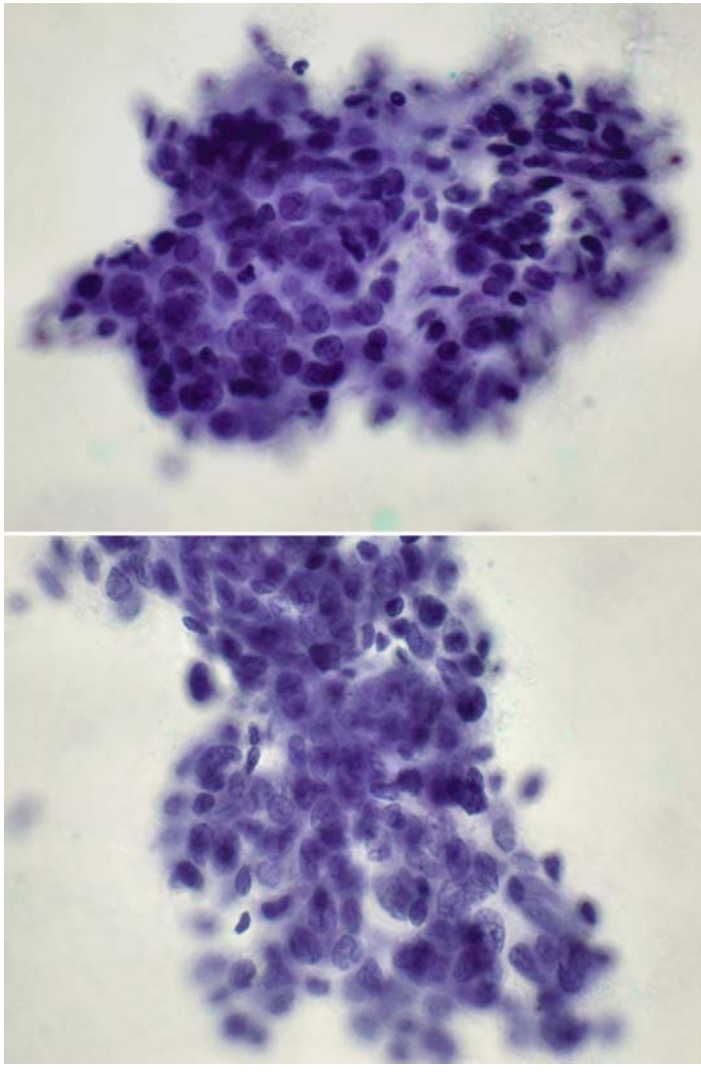

FIG. 9.27. These brush cytology preparations are derived from the case illustrated in Fig. 9.25 (*bottom*), which shows slightly abnormal-appearing compact stromal fragments with minimal variation in nuclear size and polarity.

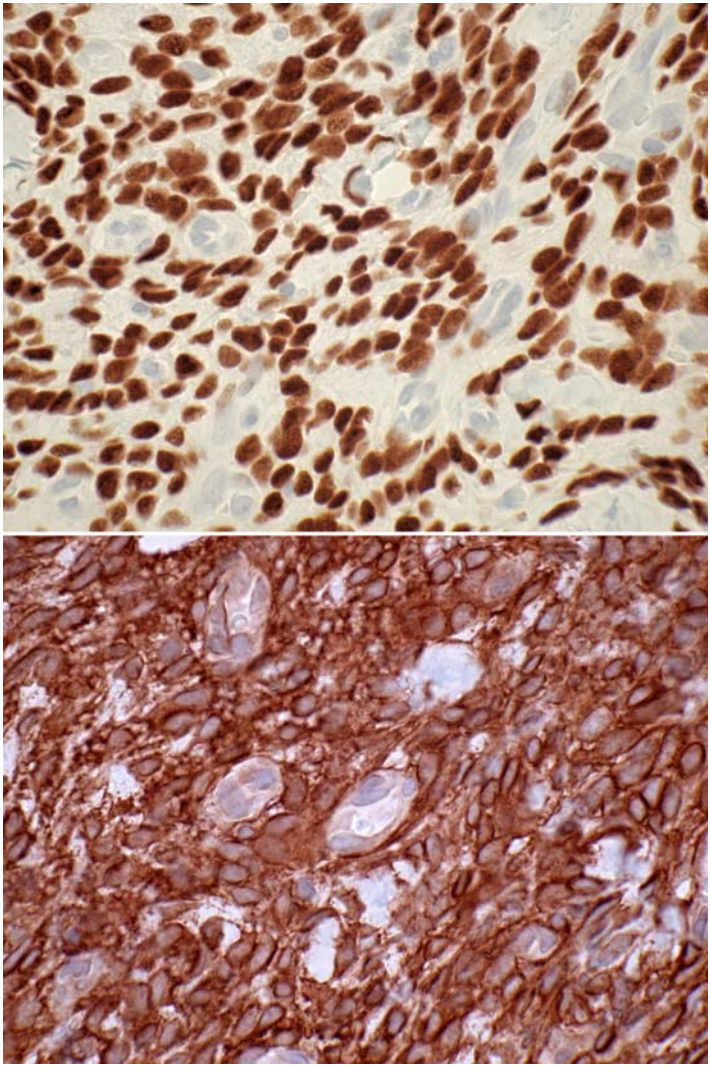

FIG. 9.28. Thin-walled vasculature permeated the cell blocks of both cases illustrated in Fig. 9.25, and vessels are especially noticeable as negatively stained structures in a background of positive immunostaining of the tumor cells for inhibin (*top*) and CD10 (*bottom*).

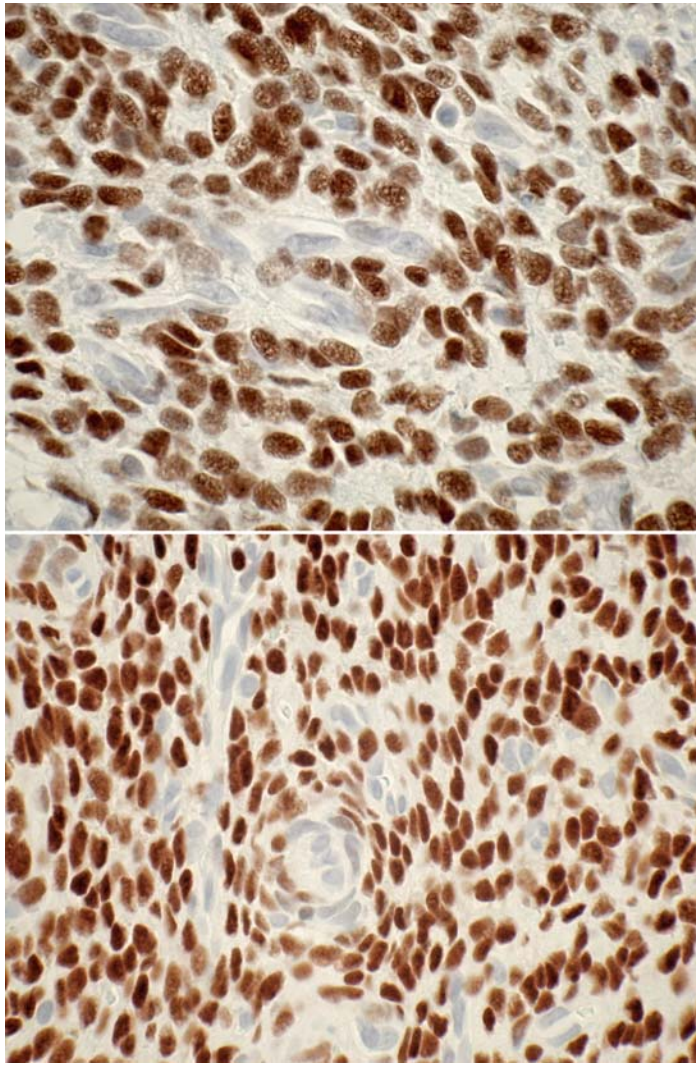

FIG. 9.29. Both cases of low-grade stromal sarcoma illustrated in Fig. 9.25 show strong nuclear decoration for immunostains directed against estrogen (*top*) and progesterone (*bottom*) receptor proteins. CD10 and estrogen receptor protein staining is especially important in separating low-grade stromal sarcoma from other tumors with a hemangiopericytomatous pattern. Positive staining for progesterone receptor protein appears to correlate with good prognosis.

structures in a background of positive immunostaining of the tumor cells for inhibin and CD10 (Fig. 9.28). Both cases showed strong nuclear decoration for immunostains directed against estrogen and progesterone receptor proteins (Fig. 9.29).

Suggested Reading

Amant F, de la Rey M, Dorfling CM, van der Walt L, Dreyer G, Dreyer L, Vergote I, Lindeque BG, Van Rensburg EJ. PTEN mutations in uterine sarcomas. Gynecol Oncol 2002; 85: 165–9.

Bandera CA, Boyd J. The molecular genetics of endometrial carcinoma. Prog Clin Biol Res 1997; 396: 185–203.

Bhargava R, Shia J, Hummer AJ, Thaler HT, Tornos C, Soslow RA. Distinction of endometrial stromal sarcomas from 'hemangiopericytomatous' tumors using a panel of immunohistochemical stains. Mod Pathol 2005; 18: 40–7.

Brunisholz Y, Miller J, Scurry J, Proietto A. Endometrial stromal sarcoma resembling adenomyosis and menstrual-phase endometrium. Gynecol Oncol 2004; 95: 256–9.

Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol 1983; 15: 10–7.

Gargett CE. Terrine stem cells: what is the evidence? Hum Reprod Update 2007; 13: 87–101.

Goldblum JR, Clement PB, Hart WR. Adenomyosis with sparse glands. A potential mimic of low-grade endometrial stromal sarcoma. Am J Clin Pathol 1995; 103: 218–23.

Hayasaka K, Morita K, Saitoh T, Tanaka Y. Uterine adenofibroma and endometrial stromal sarcoma associated with tamoxifen therapy: MR findings. Comput Med Imaging Graph 2006; 300: 315–8.

Hagiwara T, Kaku T, Kobayashi H, Hirakawa T, Nakano H. Clinico-cytological study of uterine papillary serous carcinoma. Cytopathology 2005; 16: 125–31.

Hutter P, Couturier A, Membrez V, Joris F, Sappino AP, Chappuis PO. Excess of hMLH1 germline mutations in Swiss families with hereditary non-polyposis colorectal cancer. Int J Cancer 1998; 78: 680–4.

Ichikawa Y, Tsunoda H, Takano K, Oki A, Yoshikawa H. Microsatellite instability and immunohistochemical analysis of MLH1 and MSH2 in normal endometrium, endometrial hyperplasia and endometrial cancer from a hereditary nonpolyposis colorectal cancer patient. Jpn J Clin Oncol 2002; 32: 110–2.

Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007; 57: 43–66.

Kauff ND. How should women with early-onset endometrial cancer be evaluated for Lynch syndrome? J Clin Oncol 2007; 25: 5143–6.

Lax SF, Pizer ES, Ronnett BM, Kurman RJ. Clear cell carcinoma of the endometrium is characterized by a distinctive profile of p53, Ki-67, estrogen, and progesterone receptor expression. Hum Pathol 1998; 29: 551–8.

Lee JH, Schütte D, Wulf G, Füzesi L, Radzun HJ, Schweyer S, Engel W, Nayernia K. Stem-cell protein Piwil2 is widely expressed in tumors and inhibits apoptosis through activation of Stat3/Bcl-XL pathway. Hum Mol Genet 2006; 15: 201–11.

Liu FS. Molecular carcinogenesis of endometrial cancer. Taiwan J Obstet Gynecol 2007; 46: 26–32.

Matias-Guiu X, Catasus L, Bussaglia E, Lagarda H, Garcia A, Pons C, Muñoz J, Argüelles R, Machin P, Prat J. Molecular pathology of endometrial hyperplasia and carcinoma. Hum Pathol 2001; 32: 569–77.

Maxwell GL, Chandramouli GV, Dainty L, Litzi TJ, Berchuck A, Barrett JC, Risinger JI. Microarray analysis of endometrial carcinomas and mixed mullerian tumors reveals distinct gene expression profiles associated with different histologic types of uterine cancer. Clin Cancer Res 2005; 11: 4056–66.

McCluggage WG, Sumathi VP, Maxwell P. CD10 is a sensitive and diagnostically useful immunohistochemical marker of normal endometrial stroma and of endometrial stromal neoplasms. Histopathology (Oxf) 2001; 39: 273–8.

Mikami Y, Hata S, Kiyokawa T, Manabe T. Expression of CD10 in malignant müllerian mixed tumors and adenosarcomas: an immunohistochemical study. Mod Pathol 2002; 15: 923–30.

Palermo VG. Interpretation of endometrium obtained by the Endo-pap sampler and a clinical study of its use. Diagn Cytopathol 1985; 1: 5–12.

Peel DJ, Ziogas A, Fox EA, Gildea M, Laham B, Clements E, Kolodner RD, Anton-Culver H. Characterization of hereditary nonpolyposis colorectal cancer families from a population-based series of cases. J Natl Cancer Inst 2000; 92: 1517–22.

Renkonen-Sinisalo L, Butzow R, Leminen A, Lehtovirta P, Mecklin JP, Jarvinen HJ. Surveillance for endometrial cancer in hereditary nonpolyposis colorectal cancer syndrome. Int J Cancer 2007; 120: 821–4.

Robboy SJ, Merino M, Mutter GL. The female reproductive system. In: Rubin RSD, ed. Pathology, ed., 5th edn., Lippincott, Philadelphia, PA, pp 781–840, 2007.

Sesti F, Patrizi L, Ermini B, Palmieri G, Orlandi A, Piccione E. High-grade endometrial stromal sarcoma after tamoxifen therapy for breast cancer. Gynecol Obstet Invest 2005; 60: 117–20.

Silverberg SG, Major FJ, Blessing JA, Fetter B, Askin FB, Liao SY, Miller A. Carcinosarcoma (malignant mixed mesodermal tumor) of the uterus.

A Gynecologic Oncology Group pathologic study of 203 cases. Int J Gynecol Pathol 1990; 9: 1–19.

Silverberg SG, Mutter GL, Kurman RJ, Kubik-Huch RA, Nogales F, Tavassoli FA. Tumors of the uterine corpus: epithelial tumors and related lesions. In: Tavassoli FA, Stratton MR, eds. WHO Classification of Tumors: Pathology and Genetics of Tumors of the Breast and Female Genital Organs. IARC Press, Lyon, France, pp 221–232, 2003.

Tashiro H, Blazes MS, Wu R, Cho KR, Bose S, Wang SI, Li J, Parsons R, Ellenson LH. Mutations in PTEN are frequent in endometrial carcinoma but rare in other common gynecological malignancies. Cancer Res 1997; 57: 3935–40.

Taylor HS. Endometrial cells derived from donor stem cells in bone marrow transplant recipients. JAMA 2004; 292: 81–5.

Taylor NP, Zighelboim I, Huettner PC, Powell MA, Gibb RK, Rader JS, Mutch DG, Edmonston TB, Goodfellow PJ. DNA mismatch repair and TP53 defects are early events in uterine carcinosarcoma tumorigenesis. Mod Pathol 2006; 19: 1333–8.

Tobon H, Watkins GJ. Secretory adenocarcinoma of the endometrium. Int J Gynecol Pathol 1985; 4: 328–35.

Vaidya AP, Horowitz NS, Oliva E, Halpern EF, Duska LR. Uterine malignant mixed mullerian tumors should not be included in studies of endometrial carcinoma. Gynecol Oncol 2006; 103: 684–7.

Vang R, Barner R, Wheeler DT, Strauss BL. Immunohistochemical staining for Ki-67 and p53 helps distinguish endometrial Arias-Stella reaction from high-grade carcinoma, including clear cell carcinoma. Int J Gynecol Pathol 2004; 23: 223–33.

Wang J, Wieslander C, Hansen G, Cass I, Vasilev S, Holschneider CH. Thin endometrial echo complex on ultrasound does not reliably exclude type 2 endometrial cancers. Gynecol Oncol 2006; 101: 120–5.

Watson P, Vasen HFA, Mecklin JP, Järvinen H, Lynch HT. The risk of endometrial cancer in hereditary nonpolyposis colorectal cancer. Am J Med 1994; 96: 516–20.

Wright CA, Leiman G, Burgess SM. The cytomorphology of papillary serous carcinoma of the endometrium in cervical smears. Cancer (Phila) 1999; 87: 12–8.

Zaino RJ, Kurman RJ, Diana KL, Morrow CP. The utility of the revised International Federation of Gynecology and Obstetrics histologic grading of endometrial adenocarcinoma using a defined nuclear grading system. A Gynecologic Oncology Group study. Cancer (Phila) 1995; 75: 81–6.

Zelmanowicz A, Hildesheim A, Sherman ME, Sturgeon SR, Kurman RJ, Barrett RJ, Berman ML, Mortel R, Twiggs LB, Wilbanks GD, Brinton LA. Evidence for a common etiology for endometrial carcinomas and malignant mixed mullerian tumors. Gynecol Oncol 1998; 69: 253–7.

Zhou XP, Kuismanen S, Nystrom-Lahti M, Peltomaki P, Eng C. Distinct PTEN mutational spectra in hereditary non-polyposis colon cancer syndrome-related endometrial carcinomas compared to sporadic microsatellite unstable tumors. Hum Mol Genet 2002; 11: 445–50.

10
Technical Appendix

The following comprises a technical disclosure of the reagents and methods used in preparing the cytology and cell block histology slides of endometrial brushings. The methods are based on cumulative experience with processing liquid-based cytology without specialized preparative instruments.

The reagents and methods disclosed herein have evolved over several years and are generally applicable to cytopathology. We have tested them with more than 125,000 cervicovaginal specimens, 10,000 endometrial specimens, and 25,000 nongynecological specimens, including brushings and fine-needle aspiration biopsies. The same methods may be applied to fluid cytology after the naturally occurring cell suspension has been concentrated to a cell button, decanted, and fixed.

Primary Fixative

The constituents of the primary cytology and histology fixative are shown in Table 10.1. The fixative is blended by mixing 1 gal 10% neutral buffered formalin with 5 gal water and adding the indicated amounts of sodium citrate and sodium chloride. The solution is mixed until all its components thoroughly dissolve, which is best done by dissolving the salts in 1 gal water before blending together all the aforementioned ingredients and adding the remaining water.

J.A. Maksem et al., *Endometrial Cytology with Tissue Correlations*, Essentials in Cytopathology 7, DOI: 10.1007/978-0-387-89910-7_11,

TABLE 10.1. Primary fixative

10% neutral buffered formalin	1 gallon
Sodium citrate (tribasic dihydrate)	40 grams
Sodium chloride	80 grams
Water	5 gallons
Methanol	4 gallons

Then, 4 gal methanol is added to this solution, which has a final formulation of approximately 1% formalin and 40% methanol. The fixative has a shelf-life of at least 2 years at ambient temperature and a hazard profile identical to Saccomanno solution.

Attributes of the fixative solution include its universal application to the simultaneous fixation of tissue and cells, its low formalin content, its ability to maintain proteins in a soluble state, and its ability to completely lyse and clarify about 250 ml whole blood per 10 ml fixative. Cells appear as if they had been fixed in alcohol, and cells and dissolved protein products remain stable in solution for at least 2 years.

Polymer Encapsulation Solution

The constituents of the polymer encapsulation solution are shown in Table 10.2. The polymer encapsulation solution is not difficult to blend, but its formulation is tedious. The agarose VI is a critically specific reagent because it ensures high gelling temperature. The polyethylene glycol (PEG) 1000 may be of industrial grade. PEG 1000 is generally used for softening wood, especially gunstocks, and may be obtained as a relatively inexpensive reagent from hobby outlets (as opposed to purchasing it from a chemical supply house).

The polymer encapsulation solution is formulated at high temperature with continuous, uninterrupted mixing. The specified type of agarose is dispersed by adding it to a tornadic cone of room temperature water formed by vigorous mixing of 2,000 ml room temperature water, and then it is completely dissolved by slowly bringing the water to boiling with sustained vigorous mixing. The PEG is dissolved in the remaining 1,500 ml water and is added to the boiling agarose blend. The solution is brought back to boiling and then allowed to slowly cool to room temperature

Table 10.2. Polymer encapsulation solution

Water	3500 mL
Agarose VI	3.5-to-4 grams
Carbowax (PEG 1000)	100 grams
Reagent alcohol (or ethanol)	500 mL
Poly-L-lysine concentrate (0.1% w/v in water; Sigma)	4 mL
Igepal CA-630	1 mL
5% Thymol in reagent alcohol	2.5 mL

while uninterrupted mixing is continued. The alcohol is then slowly added to the room temperature agarose/PEG blend, followed by the other components with continuous, sustained mixing, which is maintained for another 48 h.

The polymer encapsulation solution may be transferred to bottles that are capable of receiving a pump dispenser. Four liters of polymer encapsulation solution serve to process about 4,000 specimens. The polymer encapsulation solution is shaken before each use and is dispensed as aliquots of 0.75–1.0 ml using the pump dispenser. The polymer encapsulation solution has a shelf-life of about 2 years at room temperature.

Attributes of the polymer encapsulation solution include the extreme slowing of Brownian motion that keeps cells and particles from aggregating with one another, which affords a highly stable and uniform suspension of cells and particles. This property allows for the manual manufacturing of "circle slides" without specialized instruments; and, on creation of these circle slides, the polymer encapsulation solution hermetically seals the cell and particle sample. The process affords an insoluble agarose membrane with an extractable PEG portion in which cells and particles remain trapped after water/alcohol extraction of the PEG portion of the circle is completed before, or as the first step of, tissue staining.

Slide-Coating Reagent

It must be understood that cells and particles do not, themselves, stick to the glass slide; rather, the agarose membrane entraps cells and particles, and it is the membrane that sticks to the glass slide. To assure adherence of the agarose/PEG membrane to the glass microscope slide, a slide-coating reagent is used.

The stock solution for the slide-coating reagent is made by adding 100 ml Igepal CA-630 to 500 ml Poly-L-lysine concentrate (0.1% w/v in water; Sigma). This mixture must be set to vortex in a sealed bottle for at least 24 h, creating the slide-coating stock solution. The working solution that is used to actually coat the slides is made by dissolving 20 ml stock solution in 1 l distilled water and letting it set until the bubbles caused by the non-ionic detergent, Igepal CA-630, settle out. The working solution is good for about 1 week.

Slides are racked in staining carriers and coated by soaking the slides for about 30 min and then letting them air dry in their racks. Several hundred slides may be coated with the working solution, and the slides may be stored for up to 6 months in closed boxes without showing significant degradation.

Specimen Processing

The specimen, generally comprising the brush portion of the Tao brush along with its adherent material, is collected into a 30-ml-capacity Evergreen conical test tube that contains about 15 ml fixative. After vigorously mixing the specimen—and vigorous shaking followed by vortexing seems to be the most effective mixing strategy—a cut portion of nylon ribbon-filter is placed over the mouth of the Evergreen test tube. The nylon ribbon is of a gauze type with interfiber interstices of about 200–250 mm, and it is available as a bulk product from most bridal shops or fabric stores. A 20-ml specimen bottle (Surgipath®) is fitted to tightly seal over the Evergreen test tube/filter assembly (Fig. 10.1, top). The specimen is filtered through the nylon ribbon-filter. Tissue fragments larger than 250 mm are trapped on the filter. Cells and tissue microbiopsies are collected in the bottle (Fig. 10.1, bottom).

Tissue material that is deposited on the nylon ribbon-filter is scraped together into a tight microbiopsy pellet (Fig. 10.2). A drop of eosin is applied over the microbiopsy pellet so that it can be visualized in the cell block. The nylon ribbon-filter is tri-folded over the microbiopsy pellet and snapped closed within a tissue-embedding block. Excess nylon ribbon material is cut off the edges of the tissue-embedding cassette with pinking shears (Fig. 10.3).

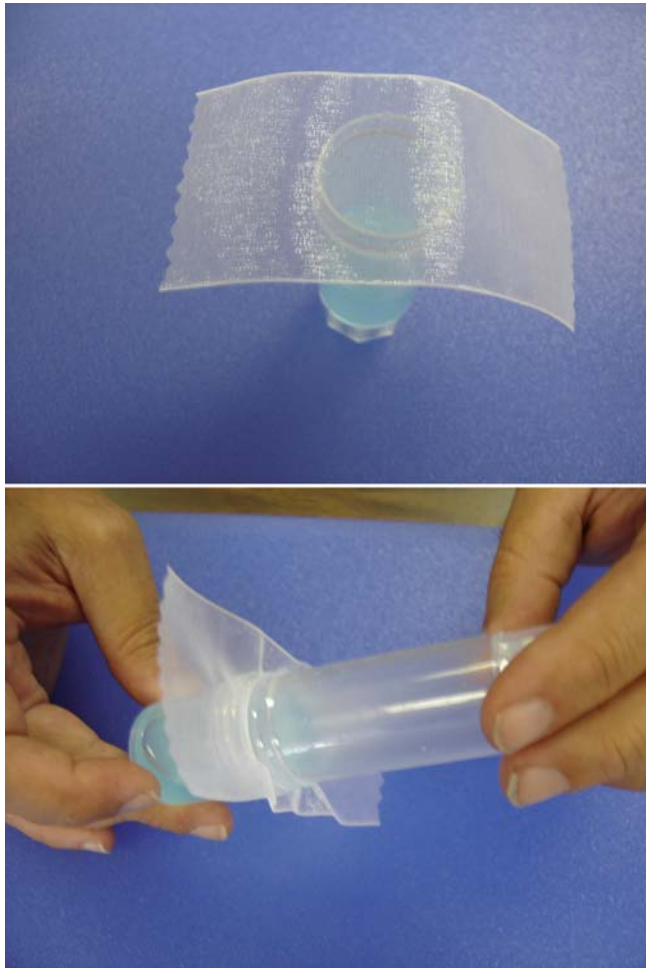

FIG. 10.1. A cut portion of nylon ribbon-filter is placed over the mouth of the Evergreen test tube. A 20-ml specimen bottle (Surgipath®) is fitted to tightly seat over the Evergreen testtube/ filter assembly. The specimen is filtered through the nylon ribbon-filter by gently inverting the assembly.

The cell and microbiopsy material that was collected in the bottle is poured back into the conical test tube, in which the brush may be kept. The test tube is centrifuged at low velocity, and the cell and microbiopsy material collects in the Evergreen test tube's

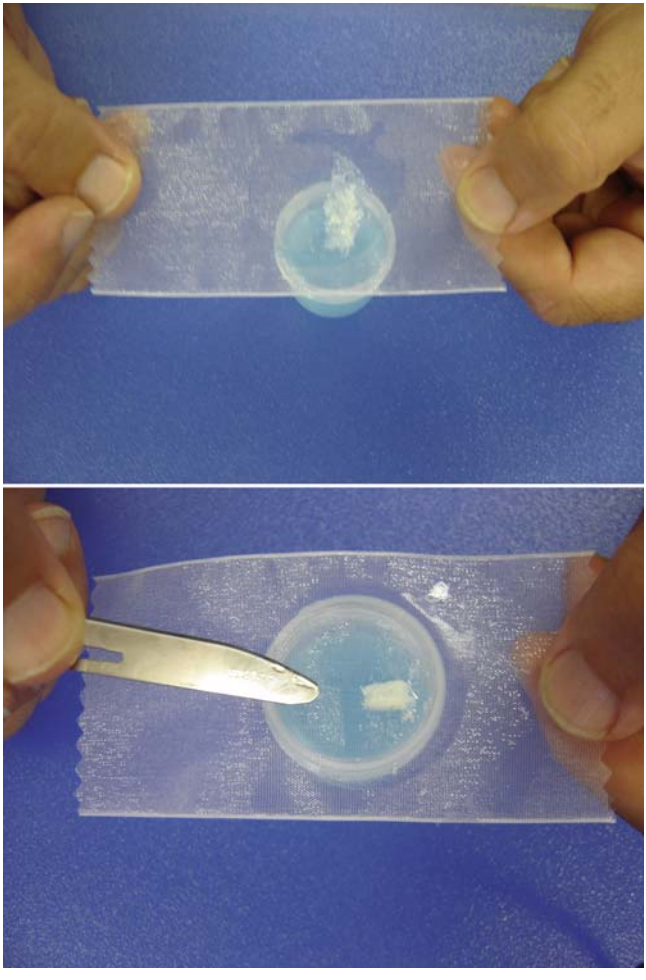

FIG. 10.2. Tissue material that is deposited on the nylon ribbon-filter is scraped together into a tight microbiopsy pellet. To facilitate identifying the pellet after processing, a drop of eosin may be applied to the pellet before the ribbon is folded around it.

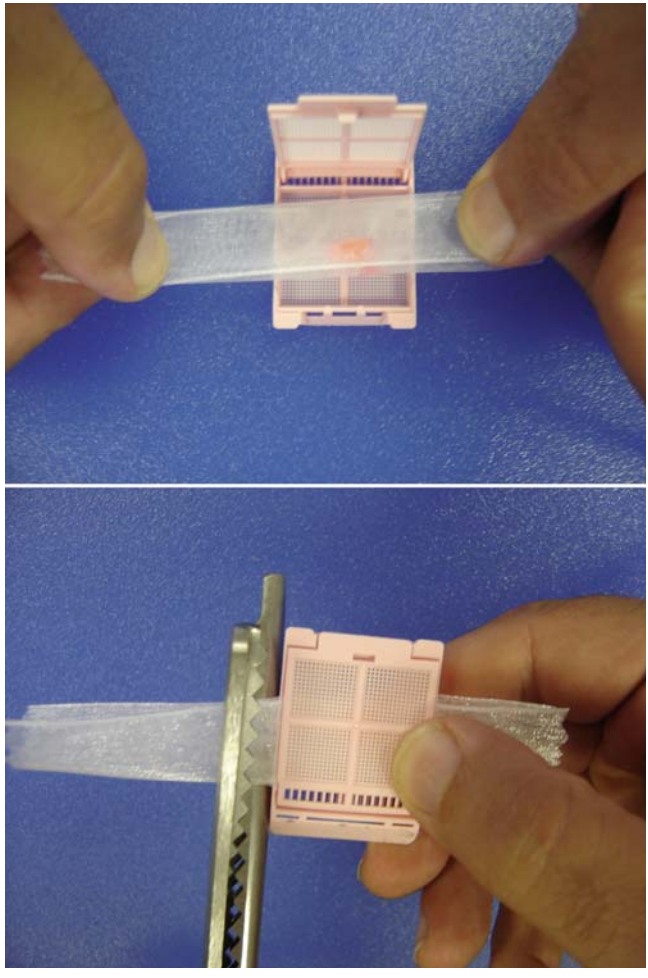

FIG. 10.3. A drop of eosin has been applied over the microbiopsy pellet so that it can be visualized in the cell block. The nylon ribbon-filter is tri-folded over the microbiopsy pellet and snapped closed within a tissue-embedding block. The nylon ribbon easily survives standard or microwave tissue processing. Excess nylon ribbon material is cut off the edges of the tissue-embedding cassette with pinking shears to make it more manageable for processing.

conical base. The cell and microbiopsy material are removed from the bottom of the conical test tube into a sealed vacutainer™ test tube using a Becton-Dickenson (BD) vacutainer™ urine transfer straw (Becton, Dickenson), whose tip is placed into the cone of the Evergreen test tube and gently swirled as vacuum is applied by engaging the vacutainer™ test tube (Fig. 10.4).

The cell and microbiopsy material are centrifuged at high velocity into a cell button. The supernatant solution is decanted off the cell button, and the mouth of the vacutainer test tube is dried with a cotton-tipped applicator, so that as much fixative solution can be removed from the cell and particle button as possible. Immediately following this, 0.75–1 ml agarose/PEG polymer encapsulation solution is added to the cell button with the aid of a calibrated pump dispenser; and, the mixture is thoroughly blended by vortex-mixing into a highly stable cell and particle suspension (Fig. 10.5, top).

Because of the high molecular weights of both the agarose and PEG, Brownian motion becomes so slowed that no recognizable cell or particle settling occurs over the next 8–12 h. About 3 drops of the particle suspension are applied, dropwise, to a dry, previously coated glass slide (Fig. 10.5, bottom). Because of the properties of a properly coated glass slide, the drops spontaneously spread out into a liquid circle of 18–20 mm diameter; stable gelation of the liquid circle occurs in about 15 min at room temperature.

The products of specimen processing comprise a cell block and several cytology circle-slides. The cell block resides within the nylon filter, about which has been snap-closed an embedding cassette; and, the cell block is processed as a tissue specimen (Fig. 10.6, top). The cytology circle-slides first gel, which takes about 15 min, and are then baked at 80°C for at least 15 min to firmly anneal the agarose/PEG membrane-circle onto the glass slide; this does not damage the cell morphology (Fig. 10.6, bottom). Although the process seems tedious, we typically manufacture about 300 circle-slides derived from 100 specimen vials, along with submitting their resulting cell blocks, with the aid of one technician in one 8-h period.

Cytology slides are stained using an automated stainer, as if they were spray-fixed sides, with stains such as Papanicolaou, hematoxylin and eosin, or acid-washed hematoxylin. We generally examine six levels of histology material on two slides and

FIG. 10.4. The cell and microbiopsy material that was collected in the bottle is poured back into the conical test tube. The test tube is centrifuged at low velocity, and the cell and microbiopsy material collects in the Evergreen test tube's conical base. The cell and microbiopsy material are removed from the bottom of the conical test tube into a sealed vacutainer™ test tube using a BD vacutainer™ urine transfer straw, whose tip is placed into the cone of the Evergreen test tube and whirled about its base while cell material is gathered into the test tube.

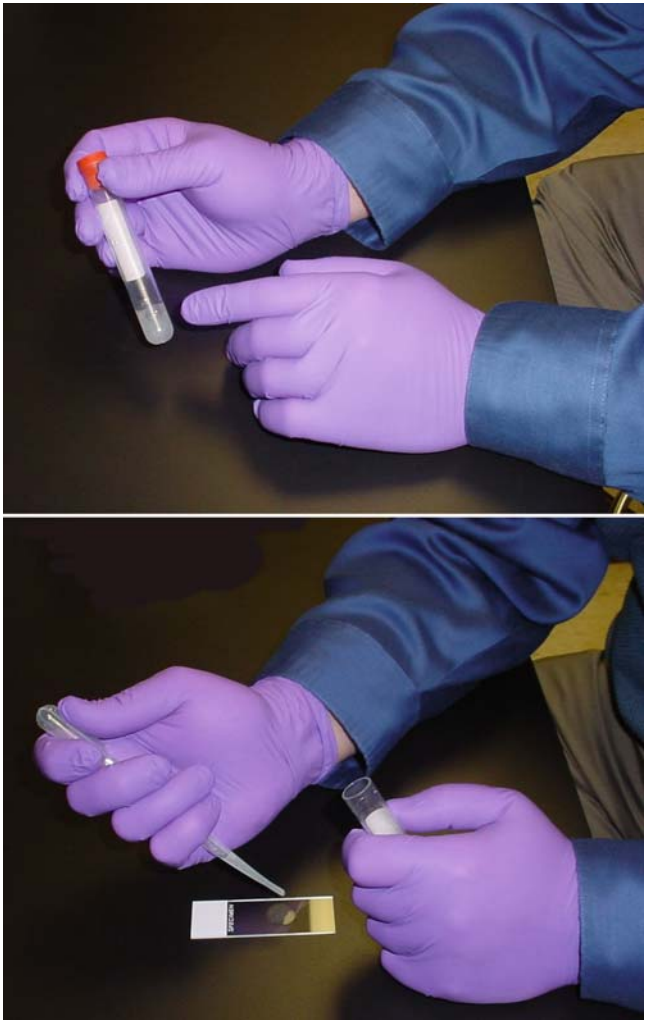

FIG. 10.5. The cell and microbiopsy material are centrifuged at high velocity into a cell button. Then, 0.75–1 ml agar/PEG polymer encapsulation solution is added to the cell button; and the mixture is thoroughly blended by vortex-mixing into a highly stable cell and particle suspension. About 3 drops of the particle suspension are applied dropwise to a dry, previously coated glass slide. In general, we make three such slides per case.

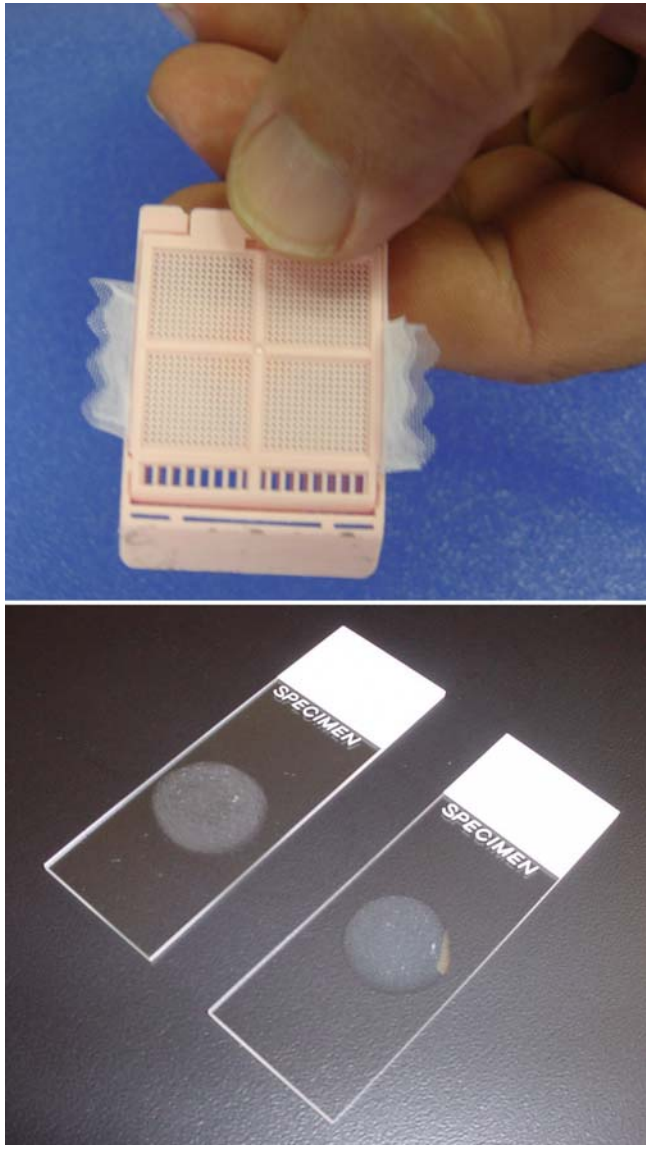

FIG. 10.6. The cell block resides within the nylon filter, about which has been snap-closed an embedding cassette; and the cell block is processed as a tissue specimen. The cytology circle-slides first gel, which takes about 15 min, and are then baked at 80°–90°C for at least 15 min to firmly anneal the agar/PEG circle onto the glass slide. The agar chosen for the specified formulation has a high gelling temperature, meaning that the agar membrane circles set up quickly even at high ambient temperatures.

three cytology slides, comprising one Papanicolaou and two acid-washed hematoxylin slides, per specimen vial.

Suggested Reading

Maksem JA, Weidmann J. Specialized preparative devices are not needed for liquid-based, thin-layer cytology: an alternate manual method using a metastable alcoholic gel. Diagn Cytopathol 2001; 25: 262–4.

Maksem JA, Finnemore M, Belsheim BL, Roose EB, Makkapati SR, Eatwell L, Weidmann J. Manual method for liquid-based cytology: a demonstration using 1,000 gynecological cytologies collected directly to vial and prepared by a smear-slide technique. Diagn Cytopathol 2001; 25: 334–8.

Maksem JA, Bedrossian CW, Kurtycz D, Sewall S, Shalkham J, Dhanwada V, Lind H, Bibbo M, Weidmann J, Kane B, Shi Fu Y. Resolving ASCUS without recourse to HPV testing: manual reprocessing of residual automated liquid-based cytology (ALBC) material using manual liquid-based cytology (MLBC). Diagn Cytopathol 2005; 33(6): 434–40.

Maksem JA, Dhanwada V, Trueblood JE, Weidmann J, Kane B, Bolick DR, Bedrossian CW, Kurtycz DF, Stewart J III. Testing automated liquid-based cytology samples with a manual liquid-based cytology method using residual cell suspensions from 500 ThinPrep cases. Diagn Cytopathol 2006; 34: 391–6.

Lee JM, Kelly D, Gravitt PE, Fansler Z, Maksem JA, Clark DP. Validation of a low-cost, liquid-based screening method for cervical intraepithelial neoplasia. Am J Obstet Gynecol 2006; 195: 965–70.

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