Endometriosis in Clinical Practice
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Edited by

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Preface

As a medical student in 1977, working with Dr Robert Franklin in Houston, I was first introduced to a disease called endometriosis. It seemed an odd disorder at the time, for it was being blamed for a number of different symptoms in the women I saw: pain of all sorts, infertility, fatigue, gastrointestinal upset, even psychological disorders. The frequency with which I encountered endometriosis was astounding; the variety of effects it was associated with seemed perplexing.

In those early years of my career I asked many questions about endometriosis, but few good answers were forthcoming. I began performing simple retrospective investigations at that point in an attempt to better decipher the mysteries of this disorder, and I scoured the literature to learn what others had found. Unfortunately, I discovered that when it came to endometriosis, knowledge had taken a back seat to passion and belief. Although rigorous clinical and basic research principles were finding their way into many diseases, this was not the case with endometriosis where studies were poorly constructed, analysis was simplistic and incomplete, and conclusions were frequently unsupported by data.

Fortunately, times change. A quarter of a century later the amount of quality research involving endometriosis is orders of magnitude greater than in my youth. However, all too often these ‘breakthroughs’ are lost amid the high volume of publications in today’s medical literature. Furthermore, updated knowledge often has difficulty displacing longstanding dogma in the minds of practicing clinicians. As an investigator, I had been of the opinion that providing scientific information was the key to altering practice patterns. What I have learned is that the modern researcher must do more than publish; he/she must also place the new information in context, revise existing mythologies, and publicize the new theoretical construct surrounding a disease.

This book is an attempt to do exactly that. I have tried to assemble a group of experts in their areas to provide the most current information available and to piece these bits of information together, when possible, into a coherent story. Clearly there are holes in our understanding, and surely we have on occasion drawn incorrect conclusions from available data, but overall the message is clear: we are making tremendous headway into understanding and treating this disease. Should we continue to make such progress, I believe that in short order endometriosis will be a disease that is well understood and easily treated. While that may put many of this book’s authors out of business, it will represent a major victory for the health of women. It is to this hope that I offer Endometriosis in Clinical Practice.

David L.Olive
To my parents Jerald and Leah, who dedicated a piece of their lives to make me the person I am today.

To my wife Elizabeth, whose partnership and love for me keep me focused and grounded.

To my sons Zachary, Matthew, and Alexander, and their pursuit of happiness and success.

It is to each of you that I dedicate this book.
1. Normal Cycling Endometrium: Molecular, Cellular, and Histologic Perspectives

Steven L. Young and Timothy S. Loy

CYCLIC ENDOMETRIAL CHANGES

The primary functions of the human endometrium are to allow the implantation of a normal embryo and provide mechanisms for the clearance of tissue and hemostasis at menstruation. At the same time, the endometrium must also provide a defense against invasion by potential pathogens and prevent the implantation of an abnormal embryo. In order to achieve these functions, the endometrium undergoes profound changes in structure and function during each cycle that result in defined periods of proliferation, embryo receptivity, and menstruation. The cyclic structural changes are evident on every level of examination, from gross inspection to electron microscopy. Both structural and functional changes are the result of changes in the molecular components of each cell, whereas a lack of appropriate cyclic changes is thought to underlie many common disorders, including abnormal uterine bleeding, infertility, endometriosis, and endometrial cancer. Therefore, a thorough understanding of the molecular and cellular alterations of the endometrium across the cycle should provide new approaches to the prevention, diagnosis, and treatment of endometrial disorders.

Considering that implantation of the embryo is fundamental to the survival of every human being at the earliest stage of his or her existence as an individual, it is remarkable that our current understanding of the molecular and cellular biology of the endometrium remains modest. Clearly, ethical and moral issues present significant hurdles to scientific inquiries into human embryo implantation, but an understanding of molecular and cell biology of the menstrual and immune functions of the endometrium also remains surprisingly incomplete. This chapter will provide an overview of the tissue, cellular, and molecular architecture of the endometrium, with an emphasis on changes across the cycle.

ENDOMETRIAL STRUCTURE—CELL TYPES

The endometrium is composed of multiple cell types, including epithelium, stroma, resident bone-marrow-derived immunocompetent cells, and blood vessel endothelium (Figure 1.1).

EPITHELIUM

The endometrial epithelium, embryonically derived from müllerian duct epithelium, forms a continuous layer, some of which is in direct contact with the uterine lumen.
(luminal) and some of which lines thin, glandular invaginations of the lumen (glandular). The luminal epithelium consists of both ciliated and non-ciliated cells, whose relative number and morphology change over the cycle.

Figure 1.1 Structural organization of human endometrium.

The luminal epithelium is the first endometrial cell type to encounter an implanting embryo and the first cell type to encounter ascending foreign cells, including sperm and microbes. Thus, luminal epithelial cells must be immunologically unresponsive to foreign sperm, allow invasion by a normal embryo, and prevent invasion by abnormal embryos and foreign microbes. The luminal barrier to invasion is multifaceted and includes tight junctions between epithelial cells as well as specific apical membrane proteins and glycocalyx. During the embryo-receptive phase, the luminal epithelium undergoes a loss of apical-basal polarity, alterations in tight junction and cytoskeletal architecture, and thinning of the basal lamina. These structural changes are probably responsible, in part for increased trophoblast adhesion and the ability of trophoblasts to invade.1–4

Although the epithelial barrier to invasion is an important facet of endometrial defense, the barrier is probably weakened during implantation and certainly by menstruation. Also, the endometrium, like other mucosal surfaces, must detect potentially pathogenic microorganisms as early as possible to allow appropriate innate and adaptive responses. The immunologic problem of tolerating the presence of foreign sperm and invasion by a semi-foreign embryo while protecting against invasion by microbes and abnormal embryos was first recognized by the transplant immunologist Medawar over 50 years ago.5 To date, however, the mechanisms by which the endometrium accomplishes this immunologic feat remain an active subject of investigation.

The glandular epithelial cells, as the name implies, line a secretory gland, which during the secretory phase produces specific products thought to be important to the implanting trophoblast.6 These products include proteins and glycoproteins (e.g. prolactin and uteroglobin) as well as sialoglycoproteins (mucins). Changes in secretory glands are a major determining feature of endometrial differentiation, and many of the characteristic changes in the microscopic anatomy of the endometrium involve changes in the glands (see below).
STROMA

Endometrial stromal cells are derived from differentiated urogenital ridge mesenchymal cells immediately surrounding the müllerian duct, and the stroma is probably induced by the developing epithelium. The stroma cells are surrounded by a complex extracellular matrix produced, in large part, by the stromal cells themselves. Intermixed with the stromal cells are blood vessels and a variety of bone marrow-derived immunocompetent cells (see below). Perhaps the most distinctive change apparent in stromal structure across the cycle is decidualization. Decidualization involves enlargement and differentiation of the stromal cells, beginning with the perivascular stroma in the mid-secretory phase and continuing toward the stromal cells adjacent to the luminal and glandular epithelium. Decidualization is maintained during pregnancy.

BONE-MARROW DERIVED IMMUNOCOMPETENT CELLS

Lymphocytes (including natural killer (NK) cells) account for about 40% of the endometrial cells in early pregnancy, but little is known about their physiologic role. By far the most prevalent endometrial leukocyte in the secretory phase and early pregnancy is a specialized NK cell with granular morphology known as a large granular lymphocyte or uterine NK (uNK) cell. uNK cells have distinct differences from peripheral NK cells. uNK cells have cytolytic potential, which is low in the early proliferative phase but comparable to that of peripheral blood NK cells in the secretory phase and pregnancy. Furthermore, whereas 85–90% of peripheral NK cells display a CD16+ CD56dim immunophenotype, >90% of endometrial NK cells display a CD16− CD56bright immunophenotype. The proportion of CD16− CD56bright uNK cells rises from about 30–50% of total endometrial leukocytes in the proliferative phase to about 50–70% in the mid and late secretory phases, whereas CD16+ CD56dim NK cells remain at about 5–10% throughout the cycle. The recruitment of this rare subset of NK cells probably arises from expression of the CXCL12 chemokine by decidual cells and its receptor CXCR4 by peripheral CD16− NK cells.

The other major type of leukocyte in the endometrium is the T lymphocyte. These cells make up approximately 5% of the total uterine cell population. Whether the numbers of T lymphocytes change during the cycle is unclear, although increased proliferation is seen in the secretory phase. Furthermore, the distribution and activity of T cells may undergo significant changes.

Although macrophages and B-lymphocytes are found in the endometrium, they represent less than 10% and 5%, respectively, of endometrial leukocytes. Interestingly, lymphoid aggregates containing a B-cell core surrounded by CD8+ T cells and a halo of macrophages have been observed in the endometrium, and the size of these aggregates increases in the secretory phase. In addition, prominent infiltrates of neutrophils are seen on histologic sections taken from the very late secretory phase.

ENDOTHELIUM

Radial arteries penetrate the myometrium and split into basal and spiral arteries. The basal arteries form a rich network of anastomoses, supplying the relatively stable endometrial basalis and, like the basalis, undergo little cyclic change. In contrast, the
spiral arteries do not anastomose and change markedly over the cycle, to supply the first proliferating and then differentiating endometrial functionalis.

ENDOMETRIAL STRUCTURE: HISTOLOGY

It has been almost 100 years since Hitschmann and Adler first reported cyclic changes in the microscopic architecture of endometrial functionalis, and more than 50 years since Noyes et al. established the basic histologic criteria currently used by pathologists for the assessment of endometrial differentiation on endometrial biopsies stained with hematoxylin and eosin. A careful reassessment of the classic histologic dating criteria using fertile subjects, modern cyclemonitoring techniques, and modified analytic methods has confirmed that the histologic changes described in 1950 represent a good description of the usual cyclic changes in endometrial structure and composition, but has also suggested that classic histologic evaluation of the endometrium is insufficiently precise to be used for clinical evaluation of endometrial function.

Most authors describe cyclic changes in endometrial histology using an idealized 28-day cycle, with menses on day 1, lutenizing hormone (LH) surge on day 13 (d13), ovulation on d14, followed by 14 more days of secretory phase. Typical cyclic changes in endometrial morphology, demonstrated in Figure 1.2, have been extensively described and will only be outlined here.17, 18, 20, 21

The proliferative phase follows the menses and thus is initially characterized by re-epithelialization, which begins as early as d2 of the cycle.22 By the early proliferative phase (d5–7; Figure 1.2A), the endometrial epithelium covers the surface, and straight glands with a small circular crosssection are evident. The luminal and glandular epithelial cells are short, with basal nuclei, whereas the stroma is composed of oval cells with little cytoplasm. In early proliferative endometrium, mitotic figures are rare in both epithelium and stroma. Under the influence of increasing levels of estrogen, the midproliferative endometrium, (d8–10; Figure 1.2B) is characterized by increased epithelial and stromal mitosis, more columnar epithelium with pseudostratified nuclei, slightly coiled glands, and stromal edema. Estrogen continues to rise markedly in the late proliferative phase (d11–14; Figure 1.2C), causing continued glandular mitoses and increased stromal mitoses. The glands become more tightly coiled, with a wide lumen, and the glandular epithelium shows maximal pseudostratification, whereas the stromal edema lessens.

The high, sustained levels of estradiol trigger the LH surge, which in turn triggers ovulation about 34–36 hours after the onset of the surge.
The day of ovulation is defined as day 14 of the idealized 28-day cycle, and secretory days are often referred to as post-ovulatory days (POD) 1–14, instead of cycle days 15–28. After follicular rupture, the resulting corpus luteum begins producing estradiol and progesterone, resulting in secretory transformation of the endometrial glands. Interestingly, electron microscopy reveals continued secretion of products into the glandular lumen throughout the cycle, although in the ‘secretory’ phase their character changes. During the second half of the secretory phase, three zones of endometrium can be distinguished. In order from lumen to myometrium, these zones are called the zona compacta, the zona spongiosum, and the basalis. The functionalis layer, which is shed during menses, is composed of both compacta and spongiosum, and the endometrium regenerates from the basalis layer. The basalis is largely unchanged over the cycle.

In the early secretory phase (d15–18, POD 1–4, Figure 1.2D), progesterone levels are low and the endometrium continues to show mitoses; however, by d16, the first progestational effects are seen as secretory vacuoles appear beneath the glandular nuclei. Large glycogen deposits can be appreciated by electron microscopy beneath the glandular nuclei just before ovulation, at least 2 days before subnuclear vacuoles appear. As these appear at the same time as giant mitochondria, it is thought that the glycogen store may be required as an energy source in the secretory phase. The secretory vacuoles move toward the apical aspect of the glandular epithelial cell, passing the nucleus on d18. Also by d18, mitoses have virtually disappeared.

In the midsecretory phase (d19–23, POD 5–9; Figure 1.2E), the endometrial glands begin to secrete increasing amounts of material into the lumen, and maximal secretory material in the glands is seen on d20 (POD 6). Stromal edema is also prominent in this phase, with maximal edema seen on d22 (POD 8). The arterioles become more spiral in
appearance by d23 (POD 9). Apoptosis has been described in the epithelial cells of the midsecretory phase, although this is not apparent on routine histologic sections.

Embryo implantation usually occurs between d20 and d24, termed, ‘the window of implantation’. The window of implantation is characterized by ultrastructural changes including decreased density of tight junctions, alterations in basal lamina architecture, and the appearance of pinopods on the luminal surface. The emergence of pinopods correlates with uterine receptivity to implantation and represents a marked alteration in the apical cytoskeleton. An example of the appearance of pinopods is given in Figure 1.3. The pinopod-like structures persist through the implantation window and then appear to deflate in the late secretory phase.

Figure 1.3 Scanning electron micrographs of endometrial lumen. A, 2–3 days after LH surge; B, 9–10 days after LH surge.