6.1. Histology of the Endometrium

The uterine corpus is composed of a modified mucosa known as the endometrium, a fibromuscular wall called the myometrium, and a serosal lining. The uterine mucosa can be divided into two regions: the mucosa of the lower uterine segment (LUS) (isthmus) and the mucosa of the corpus proper (fig. 6.1). The mucosa of the LUS, located between the endocervix and endometrium, is thinner than that of the fundus and its glands respond only slightly to hormonal stimulation. There is a gradual morphologic transition from the isthmic mucosa to the endocervical mucosa.

During the reproductive years the endometrium of the corpus proper undergoes regular cyclic changes as a response to the release of the ovarian hormones, estrogen and progesterone. The endometrium consists of simple tubular glands set in a cellular vascular stroma. It is composed of a thin basal layer (basalis), which abuts on the myometrium, and a functional layer on top of the basalis. The functional layer is highly responsive to hormonal ovarian influence in contrast to the basalis. The functional endometrium consists of a superficial layer with few glands and abundant stroma (the compacta), and a deep layer that has many glands and relatively less stroma (the spongiosa) (fig. 6.2).

The structure and activity of a functional endometrium reflect the pattern of ovarian hormone secretion. The histologic types of glandular cells are columnar or cuboid. The endometrium undergoes regular growth and maturation and when the cycle ends, in the absence of pregnancy, shedding occurs followed by regeneration. The average duration of the cycle is 28 days. In a normal cycle the postovulatory phase lasts 14 days. Changes in the length of the cycle are usually due to the duration of the proliferative phase, which can vary from 8 to 21 days [47].

Fig. 6.1. Histology of endometrium. Cell block: HE, 40×.

Fig. 6.2. Functional layer of endometrium glands and stroma. Cell block: HE, 40×.
The Glands of the Endometrium. The endometrial glands are simple tubular glands lined by columnar epithelium or tall, narrow and closely packed cells with elongated and parallel nuclei (fig. 6.2). The morphology of the endometrial glands changes during the different phases of the menstrual cycle.

The Stroma of the Endometrium. The endometrial stroma consists of pluripotential mesenchymal cells, which at the beginning of the menstrual cycle are spindle-shaped, poorly differentiated and joined to one another by cytoplasmic processes (fig. 6.3). The cells lie firmly anchored within a network of reticulum fibers. At the beginning of the cycle the cytoplasm of the cells forms a narrow ring around the nuclei. Towards the end of the proliferative phase the nuclear chromatin becomes less dense. During the secretory phase vacuoles and granulocytes appear in the cytoplasm, and some of the cells differentiate into predecidual cells.

The stroma of the basalis is more cellular than that of the functional layer of the endometrium and nucleocytoplasmic ratios are high. Thick-walled arteries, lymphocytes and lymphoid aggregates are present.

6.2. The Endometrial Smear

A smear obtained by endometrial brushing techniques (EBT) of the normal endometrium is composed of large sheets of surface epithelial cells in a honeycomb pattern with gland openings, and poorly cohesive stromal cells with oval nuclei and ill-defined cytoplasm. There are histiocytes, granulocytes and red blood cells mixed with cell fragments and mucus in the smear background. Tumoral diathesis is absent (fig. 6.4).

Endometrial Epithelial Cells

The cells of endometrial glands and surface epithelium present the same morphology, both are columnar or cylindrical. Their morphology changes from the proliferative to the secretory phase. Ciliated glandular cells can be identified in a few cases, often in estrogen-stimulated endometrium.

Endometrial epithelial cells occur in sheets or cohesive groups, seldom with a honeycomb pattern. Isolated cells are common. They show little variation in size and shape with scant cytoplasm and round, ovoid or elongated nuclei with dense chromatin. Nucleoli are usually not visible. Cell borders are ill-defined (fig. 6.5). Mitotic figures are common in the proliferative phase. The morphology seen in the secretory phased is described below.
In liquid-based samples (LBP) endometrial cells may appear more hyperchromatic and more pleomorphic with somewhat more prominent nucleoli than in conventional smears (fig. 6.6). In general, the improved diagnostic yield of LBP tests may be attributed to better cell preservation and the absence of obscuring blood or inflammation that is often encountered in conventional smears. This allows the detection of even low numbers of diagnostic cells on LBP tests.

The glandular cells derived from direct sampling of the LUS include larger groups with gland openings, branched glands and nuclear palisading within the fragments accompanied by endometrial stroma [76] (fig. 6.7).

**Stromal Cells of the Endometrium**

The endometrial stromal cells are mesodermal cells, mainly of fibroblastic, seldom of histiocytic type. In the endometrial brush smears the morphology of the endometrial stromal cells varies with the clinical status of the patient and with the phase of the menstrual cycle. In the early proliferative phase the stromal cells occur singly or in loose groupings, they have scant cytoplasm and ovoid or fusiform nuclei (fig. 6.8). In the late proliferative and early secretory phases the stromal cells appear as more cohesive groups of spindle cells (fig. 6.9). Variable degrees of predecidualization, form small isolated stromal cells to large predecidual cells with abundant cytoplasm and ovoid or vesicular nuclei and seldom with obvious nucleoli, can be seen during the later secretory phase.
6.3. Patterns of Normal Endometrial Smears

6.3.1. Endometrial Smear during the Menstrual Cycle

Proliferative Phase

Histology. Estrogenic stimulation causes the endometrium to regenerate and proliferate. In the early proliferative phase, the glands are straight and narrow and the glandular epithelium is cubo-columnar. Nuclear chromatin appears dispersed and mitotic figures are present. The stromal cells also show mitotic activity and have ill-defined borders (fig. 6.10). In the late proliferative phase the glands increase in size and appear tortuous with pseudostratification of the epithelium showing nuclei at different levels. The stromal cells are small and spindle-shaped similar to predecidual cells.

Cytology. In the early proliferative phase, EBT show glandular cells in cohesive monolayered sheets. They sometimes appear as straight or twisted tubular structures resembling glove fingers irregularly sheared at the ends, the so-called glove-finger pattern (fig. 6.11) [40]. Almost all the tubular fragments are open at both ends (open type), but a few are closed at one end (closed type) and have a cup-like, a half moon, or spherical shape [160]. The nuclei are of uniform size and shape and have granular chromatin and distinct micronucleoli. Loose aggregates of stromal cells, which have oval nuclei and poorly defined cytoplasm, can be identified. In the late proliferative phase, sheets of endometrial cells are highly cellular with nuclear crowding, denser nuclear chromatin and frequent mitotic figures. Tubular structures are small and a glove-finger pattern is frequently seen (fig. 6.12).
Secretory Phase

**Histology.** During the early secretory phase the endometrium shows the effects of both progesterone and estrogen influence. The endometrial glands undergo progressive distension, appear plumper and more tortuous and are lined by low columnar cells. Subnuclear cytoplasmic glycogen vacuoles may discharge into the gland lumina. In the late secretory phase, stromal cells increase in size and volume and they acquire an epitheloid appearance called predecidual cells. The finding of spiral arteries surrounded by a cuff of predecidual stromal cells is useful in diagnosis (fig. 6.13).

**Cytology.** In the early secretory phase, the glandular cells become larger with a well-defined clear cytoplasm and a honeycomb pattern. The spindled stromal cells occur in loose or cohesive aggregates. The secretory glandular cells and the stromal predecidual changes are more evident in the late secretory phase (fig. 6.14). The predecidual cells are arranged in small irregular sheets and have abundant dense cytoplasm without vacuolization. Their nuclei have an irregular chromatin structure and visible micronucleoli.

Menstrual Phase

**Histology.** In the menstrual phase, a plane of separation appears between the superficial endometrium and the basal layer. A variable amount of functional endometrium remains attached to the basalis.

**Cytology.** EBT produces a bloody smear with many ball-like tissue fragments (menstrual cell balls) consisting of degenerate glandular cells surrounded by predecidual cells [205]. The stromal cells have ovoid, small, pyknotic nuclei and may appear as aggregates. Isolated predecidual cells, neutrophils and nuclear debris are present in the background.

Cervicovaginal smears have a bloodier and dirtier background with typical exodus, menstrual cell balls and dispersed epithelial and stromal cells (fig. 6.15).

**Summary of Endometrial Cytology during the Menstrual Cycle.** Based on a review of current knowledge and on more than 35 years’ experience using adequate diagnostic criteria, a cytological diagnosis of normal cyclic endometrium is possible with acceptable accuracy (Coscia 92%) [40]. True endometrial dating is more difficult, in our experience impossible. Of greater practical importance is the identification of an early secretory phase in EBT to confirm ovulation. Endometrial cytology has specific problems. A proper cytological training and a good knowledge of the endometrial histopathology in various clinical conditions is necessary to achieve acceptable accuracy.
6.3.2. Endometrial Cytology in the Menopause

Histology. Following the physiological decline of ovarian function with a fall in the secretion of both progesterone and estrogen, the postmenopausal non-functional endometrium usually changes progressively over a few years into an atrophic endometrium (fig. 6.16). But in 20–30% of women this transformation may take several years. We found signs of proliferative endometrial activity in such cases sometimes persisting for many years. It is very important to bear this in mind before prescribing hormone replacement therapy.

Atrophic Endometrium. Since the endometrium is thin, endometrial biopsy samples are often scanty if not inadequate. In more adequate samples there are sparse remnants of narrow glands lined by a low epithelium with small inactive nuclei, supported by a dense fibrous stroma of spindle cells. The functional layer is difficult or impossible to separate from the basalis. More commonly than atrophy, we find signs of weak proliferative activity of the endometrium. In such women, the menopause seems to develop gradually over a few or many years. A third common pattern of menopausal endometrium is seen when the last cycles were anovulatory or had irregular proliferative phases, which results in a senile cystic atrophy (fig. 6.17). The stroma becomes fibrous and the glands vary in size, some of them are narrow and tubular, but many are dilated and cystic. The glandular epithelium is cuboidal and inactive but has a tendency to become polygonal. This histological pattern may be mistaken for glandular-cystic hyperplasia.

Cytology. EBT from atrophic endometrium are sparsely cellular containing straight tubular glands and few surface epithelial cells. The epithelial cells are smaller and their appearance is less characteristic and they have a low columnar or cuboidal shape. There are scattered, shrunk stromal cells with scanty cytoplasm. Mitotic figures are absent (fig. 6.18).

In the weakly proliferative endometrium, EBT contain glandular cells analogous to those seen in the early proliferative phase of the menstrual cycle, except that there are fewer mitotic figures and the nuclei seem less crowded (fig. 6.19). The disordered proliferative endometrium of postmenopausal women usually yields endometrial smears similar to those from the endometrium of the proliferative phase in the reproductive years [205]. In senile cystic atrophy EBT produces a moderately cellular smear with few tubular glands and proliferative surface endometrial cells. Scattered stromal cells complete the pattern and distinguish it from cystic hyperplasia.
6.4. Differential Diagnosis of Normal Endometrial Cells

In table 6.1 we present the main cytological features distinguishing normal endometrial epithelial cells from stromal cells. In table 6.2 we compare the arrangement, size, nuclear features and cytoplasm of normal endometrial cells, endocervical cells and histiocytes (figs. 6.20, 6.21). It is not difficult to distinguish cells of secretory endometrium from endocervical cells.

**Table 6.1.** Differential diagnosis of normal endometrial cells

<table>
<thead>
<tr>
<th>Epithelial cells</th>
<th>Stromal cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shedding</td>
<td>Large sheets</td>
</tr>
<tr>
<td>Cell pattern</td>
<td>Honeycomb. Tube</td>
</tr>
<tr>
<td>Type of cell</td>
<td>Columnar. Cylindrical</td>
</tr>
<tr>
<td>Shape</td>
<td>Round. Oval</td>
</tr>
<tr>
<td>Nuclei</td>
<td>Round</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>Dense. Relatively scanty</td>
</tr>
</tbody>
</table>

**Table 6.2.** Differential diagnosis of endometrial and endocervical cells and histiocytes

<table>
<thead>
<tr>
<th>Endometrial</th>
<th>Endocervical</th>
<th>Histiocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell pattern</td>
<td>Dense sheets</td>
<td>Honeycomb. Palisading</td>
</tr>
<tr>
<td>Size</td>
<td>Double that of granulocyte</td>
<td>Larger than endometrial</td>
</tr>
<tr>
<td>Nuclei</td>
<td>Vary in shape not in size</td>
<td>No variation of shape and size</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>Ill-defined</td>
<td>Well-preserved</td>
</tr>
</tbody>
</table>

Fig. 6.18. Atrophic endometrium. Pap, 60×.

Fig. 6.19. Weakly proliferative endometrium. a Cell block: HE, 20× and b endometrial brushing: Pap, 20×.

Fig. 6.20. Differential diagnosis with endocervical cells. Cytology: Pap, 40×.
We consider it useful to comment on some specific situations, which frequently present diagnostic problems in the routine examination of endometrial smears. To differentiate between proliferative and inactive endometrium, we must look carefully for mitotic figures, which are more frequent in a proliferative endometrium. It is more problematic to distinguish between late proliferative endometrium and cystic hyperplasia (fig. 6.22). Prominent overlapping of cells within sheets and increased number of mitoses is more evident in hyperplasia. Finally, pseudodecidual cells could be confused with cells of hyperplasia due to the irregular arrangement and overlapping of cells in the sheets. A careful evaluation of the cytoplasmic features and confirming the absence of mitoses will be useful (fig. 6.23).