Anatomy, Histology, Cytology, and Colposcopy of the Cervix

1

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Contents

Surgical pathologists should be aware of these changes to avoid misinterpretation of precursor lesions and malignant tumors of the cervix, and to understand how the various lesions develop. Being able to correlate a lesion's colposcopic appearance with cytologic and histologic changes is similarly important.

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1.1 Anatomy, Histology, and Cytology of the Cervix

In adults, the cervix is approximately 3 cm in length and 2.5 cm in diameter, but these dimensions may vary depending on many factors, including the parity of the woman; in multiparous women, the cervix is larger than in nulliparous ones. Its shape is elongated (cylindrical). The lower portion, which protrudes into the vagina, is also called the *vaginal portion* of the cervix (portio vaginalis). The upper portion is located above the vaginal vault and is called the *supravaginal portion* (portio supravaginalis). Both portions are approximately equal in length. On the anterior side, the cervix is separated from the bladder by loose connective tissue that extends into the broad ligaments laterally. Posteriorly, the cervix is covered by peritoneum and is separated from the rectum by the retrovaginal space. Also, the cervix is attached to the second to fourth sacral vertebrae through the uterosacral ligaments, the main source of fixation, support, and suspension of the organ.

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The outer surface of the vaginal portion, called the *ectocervix* or *exocervix*, is covered mostly by a stratified squamous epithelium and continues as the epithelium lining the vaginal mucosa, which is histologically almost identical. The folds formed by the reflections of the vaginal mucosa at the front, back, and sides of the cervix are known as the *vaginal fornices*. In the center of the external surface of the ectocervix, one can detect the external opening of the endocervical canal, also called the *external os*. This opening is round in nulliparous women, but in parous woman it has the shape of a transverse groove (slit-shaped), dividing the ectocervix into two portions, the anterior lip and the posterior lip (Fig. 1.1). The endocervical canal, measuring approximately 8 mm in length, connects the vagina with the uterine cavity and extends from the external os (where it opens into the vagina) to the *internal os* (thus improperly named), which marks the passage to the uterine isthmus. The internal os is basically not an opening, but rather a widening of the endocervical canal (Fig. 1.2). The junction between the cervix and the uterine corpus, located at the level of the internal os, is represented by the uterine isthmus (or lower uterine segment)—in fact, the lower portion of the uterine corpus. The endocervical canal is lined by a mucin-producing columnar, unstratified epithelium. The two types of epithelia, ectocervical and endocervical, cover the cervical stroma. The area where the ectocervical squamous epithelium continues with the endocervical glandular epithelium is called the *squamocolumnar junction* area. Normally, this area is located at the ectocervical level, but this location is highly variable during life, depending on a number of parameters, which are described below.

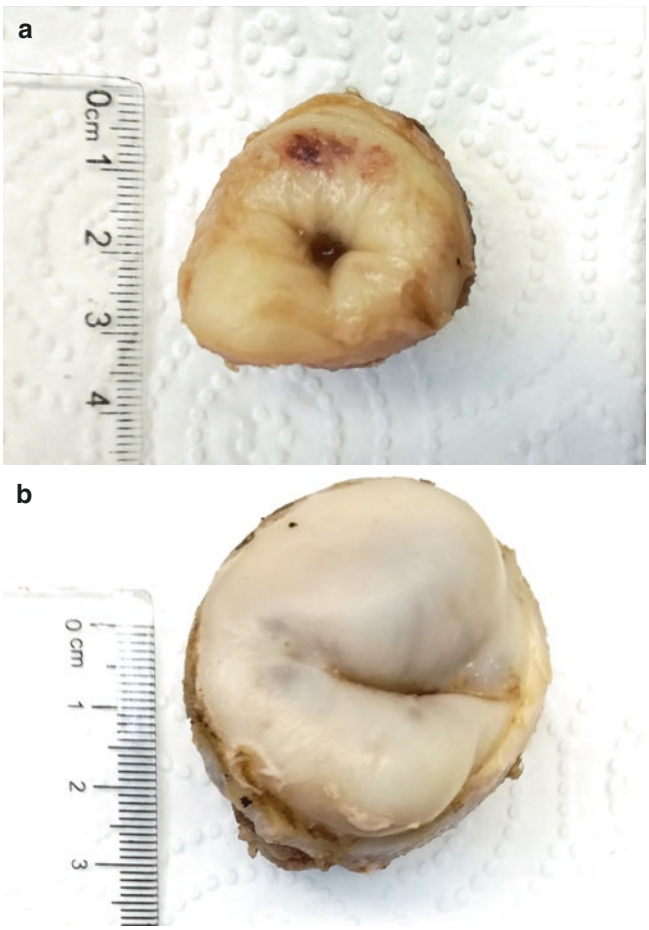

Fig. 1.1 The external opening of the endocervical canal, also called the external os, is round in nulliparous women (such as this 27-year-old patient) (**a**). In parous women, it has the shape of a transverse groove (slit-shaped), as in this 49-year-old patient (**b**)

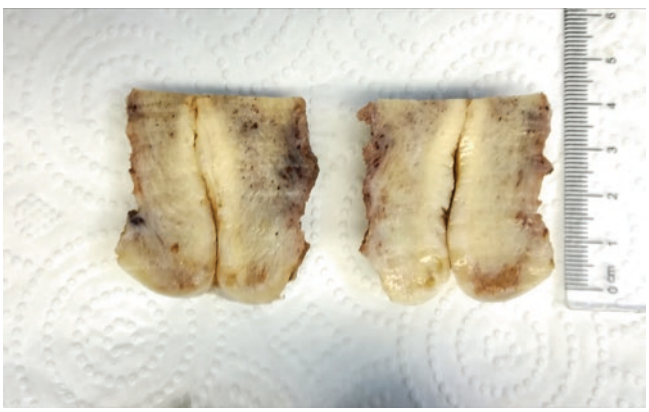

Fig. 1.2 The endocervical canal connects the vagina with the uterine cavity and extends from the external os (where it opens into the vagina) to the internal os

1.1.1 The Epithelium of the Ectocervix

Normally, the mature squamous epithelium that covers the ectocervix is stratified, non-keratinized, and glycogen-rich, related to the level of circulating estradiol (Fig. 1.3). In the first years of life and during postmenopause, when the level of circulating estrogen is low, the squamous epithelium is not mature and consequently is glycogen-free, though shortly after birth, the squamous epithelium becomes mature because of maternal estrogens (Fig. 1.4). Histologically, the cervical squamous epithelium is similar to the vaginal epithelium, but it does not show the epithelial ridges characteristic of the vaginal epithelium (Fig. 1.5).

In the mature cervical squamous epithelium, three layers can be distinguished: the basal/parabasal layer (stratum cylindricum, or the germinal cell layer), the intermediate layer (stratum spinosum), and the superficial layer (Fig. 1.6a):

- The stratum cylindricum consists of basal and parabasal cells. The *basal* layer is represented by one layer of epithelial cells of small size (10 μm in diameter) with scant cytoplasm and oval nuclei with dense chromatin, arranged perpendicularly to the basement membrane (in a picket-fence arrangement). These cells usually do not exhibit mitotic activity, and as a consequence, do not stain with Ki-67. The cells above them are called *parabasal* cells (a term used mainly in cytology and less often in histopathology), forming four to five cell layers. Parabasal cells are polyhedral in shape and larger than basal cells, with slightly more cytoplasm. The nuclei are vesicular, have less dense chromatin, and sometimes have mitotic figures; these cells are positive for Ki-67 [1].
- The intermediate layer is composed of more mature cells with more abundant cytoplasm and smaller and more vesicular nuclei than in the basal layer, arranged with the long axis parallel to the basal membrane. These cells are called *intermediate cells*. Their cytoplasm is fine, granular or clear, due to variable amounts of glycogen. The presence of glycogen is responsible for the uptake of iodine with the Schiller test performed by the gynecologist to detect abnormal areas, particularly squamous intraepithelial lesions. Normal areas containing cytoplasmic glycogen will stain brown, whereas abnormal areas devoid of glycogen will stain white on colposcopic examination.
- The superficial layer is composed of cells with a diameter of 50 μm, abundant and clear cytoplasm (as a result of glycogen accumulation), and a small, round, pyknotic nucleus that is centrally located.

The accumulation of intracytoplasmic glycogen in both the superficial and intermediate layers can be localized diffusely or can be perinuclear. In the latter case, these cells may resemble koilocytes, a cellular change related to human papillomavirus (HPV) infection, but koilocytes must have large and hyperchromatic, irregular nuclei with irregular chromatin distribution and are usually associated with other changes in the epithelium (see Fig. 1.6b). Because of its PAS-positivity (deep pink), the intracytoplasmic glycogen can be highlighted by hematoxylin-PAS staining.

In both the intermediate and superficial layers, keratinization can sometimes occur. The keratinization of the intermediate and superficial cells has the role of protecting the rest of the epithelial cells and the subepithelial vascularization from various types of trauma and infections. The cells in the superficial layer become flat, have more eosinophilic cytoplasm, and exfoliate physiologically (Fig. 1.7). The squamous epithelium is completely replaced by a new population of cells every 4 to 5 days. These exfoliating cells can be sampled, constituting the Papanicolaou (Pap) smears examined for early detection of cervical cancer and its precursor lesions.

In women of reproductive age, the cervical squamous epithelium undergoes changes due to estrogen-progesterone hormone stimulation during the menstrual cycle, with a predominance of superficial cells under the influence of estrogen, and of intermediate cells with the effect of progesterone after ovulation.

In postmenopause, when estrogen levels are low, the ectocervical epithelium is thin, composed only of basal and parabasal cells that are uniform in appearance, with reduced cytoplasm and no intracytoplasmic glycogen (Fig. 1.8). The nucleus is larger, so that the nuclear/cytoplasmic ratio is slightly modified in favor of the nucleus, a change that the pathologist must be aware of, in order to avoid misdiagnosing a cervical squamous intraepithelial lesion (SIL). The atrophic epithelium has no mitotic activity and can no longer protect the subepithelial vascularization from trauma, which can lead to frequent bleeding and inflammation during this period. The same morphologic appearance of the squamous epithelium can be seen before puberty.

In contrast, thickening of the squamous epithelium may be seen in various conditions:

- *Squamous cell hyperplasia*, usually related to uterovaginal prolapse, when the epithelium is also associated with hyperkeratosis (Fig. 1.9).
- *Basal cell hyperplasia,* a rare condition with no clinical relevance, in which the basal layer and adjacent part of the parabasal layer increase in thickness, forming a well-defined stratum. Nuclear pleomorphism and hyperchromasia are absent, but the picket-fence appearance is lost.
- *Healing after trauma* due to treatment by laser ablation, laser excision, or large loop diathermy excision.

These changes may be focal or may extensively involve the ectocervix.

In about 40% of patients, the squamous epithelium may also contain other cells [2–5]:

- *Endocrine cells.* These cells are scattered and appear to be the origin of cervical tumors with neuroendocrine differentiation.

- *Langerhans cells and lymphoid-derived cells*. Various lymphocytes, plasma cells, and dendritic macrophages are found not only in the squamous epithelium but also in the endocervical epithelium and subepithelial stroma.
- *Melanocytes.* These may be located in the basal layer of the epithelium, from which nevi and malignant cervical melanomas can develop.

Immunohistochemically, all squamous epithelial cells are cytokeratin-positive (Fig. 1.10). Basal layer cells are positive for cytokeratin 18 and 19. Parabasal, intermediate, and superficial layer cells are positive for cytokeratin 4 and 13. Other types, such as cytokeratin 7 or 5/6, may be positive in the cervical squamous epithelium, varying by location [6–10]. The basal and parabasal cells are positive for epidermal growth factor and for HER-2/neu [11]. Also, basal cells are positive for bcl-2 [12, 13]. Cyclin D1 and CD44 are positive in both basal and parabasal cells, but cyclin D1 occurs only in parabasal cells [14–16]. The squamous epithelium is also positive for p63 and p40 (Fig. 1.11), but the squamous cells are negative for CEA. The parabasal cells are positive for Ki-67, as mentioned above, but the rest of the normal squamous epithelium is Ki-67-negative [13, 17] (Fig. 1.12). p16, a surrogate marker for high-risk HPV infection, is negative (patchy pattern) in normal squamous epithelium (Fig. 1.13), whereas it is positive (strong and diffuse block-like) in high-grade and in a significant proportion of low-grade SILs. These two markers are very helpful to differentiate between normal epithelium and an epithelium harboring an HPV-driven high-grade SIL. Estrogen receptors (ERs) have been revealed in the nuclei of the basal and parabasal cells, as well as in the nuclei of the intermediate cells (Fig. 1.14). During the menstrual cycle, the variation of ER expression in the cervix is less than in the endometrium [12, 14, 18]. During the follicular phase, ERs are slightly increased, compared with the luteal phase. In atrophic epithelium, as well as in those with inflammatory infiltrate, the expression of ERs is reduced. As for progesterone receptors (PRs), they are not detected in the ectocervical epithelium during the follicular phase, but in the luteal phase and in pregnancy, they are detected in the parabasal layer (Fig. 1.15). In the stromal cells of the cervix, ERs and PRs are evident in both phases of the menstrual cycle.

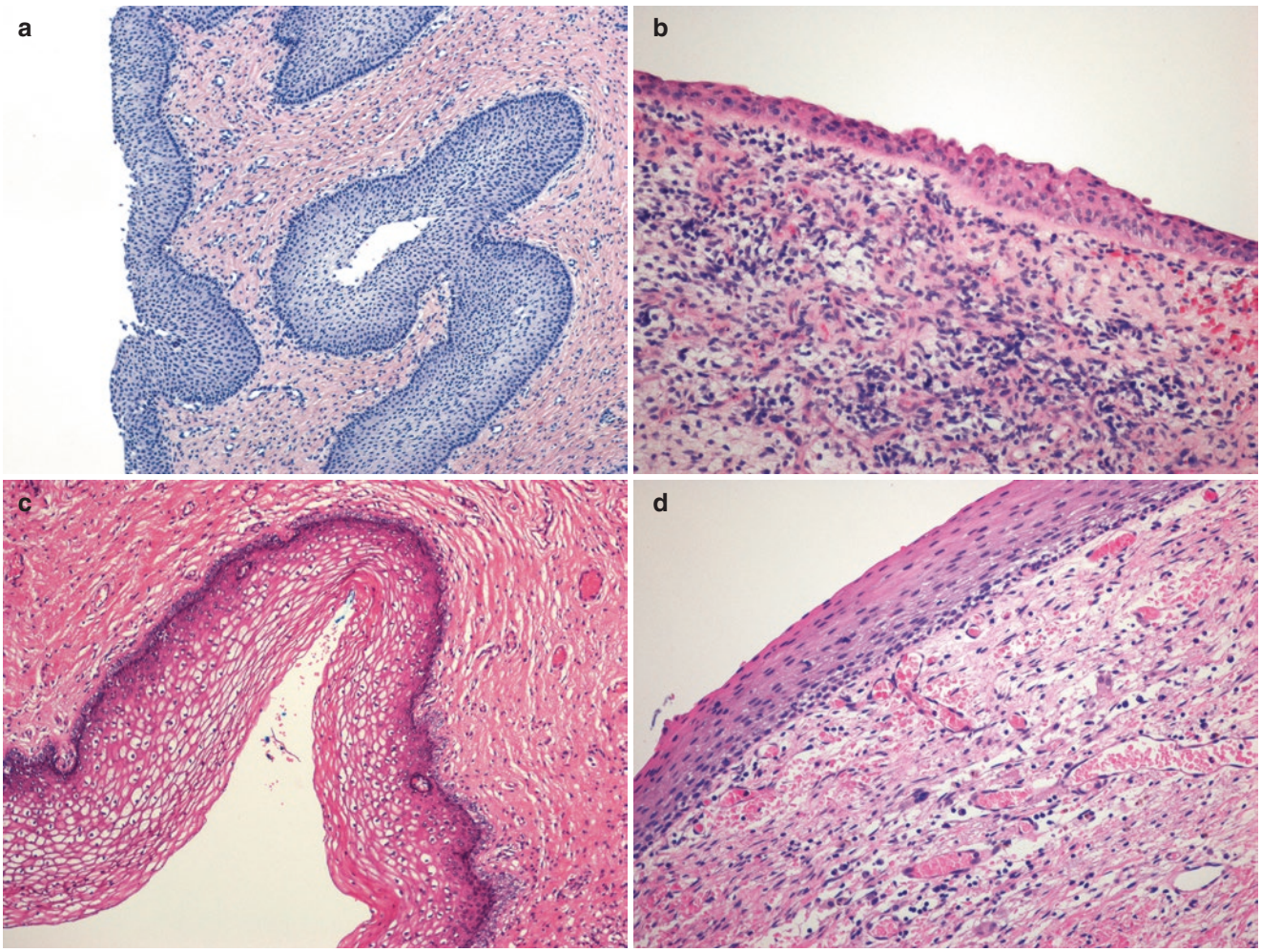

Fig. 1.3 Squamous epithelium of the exocervix is immature and glycogen-free in the first year of life (**a**) and at 15 years (**b**). In women of reproductive age, the mature squamous epithelium that covers the exocervix is stratified, non-keratinized, and glycogen-rich (**c**), whereas in postmenopause, it is glycogen-free (**d**)

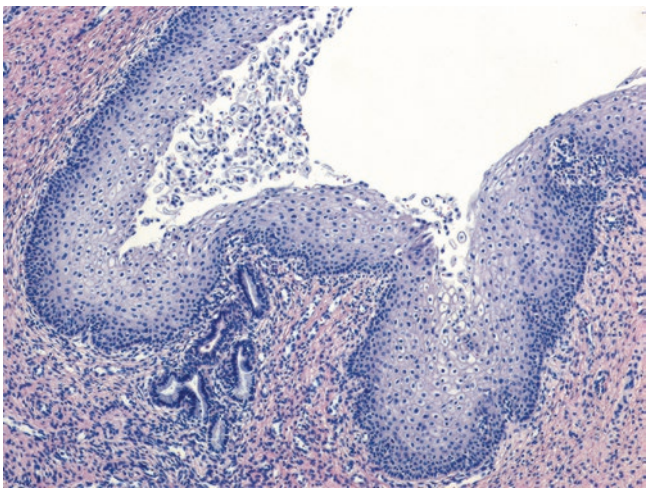

Fig. 1.4 Squamous epithelium of the exocervix shortly after birth, with glycogen due to maternal estrogens

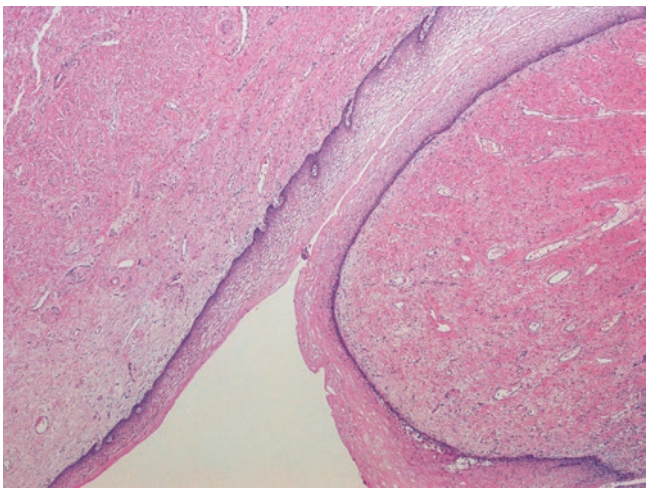

Fig. 1.5 Histologically, the cervical squamous epithelium is similar to the vaginal epithelium, but it does not show the epithelial ridges characteristic of the vaginal epithelium

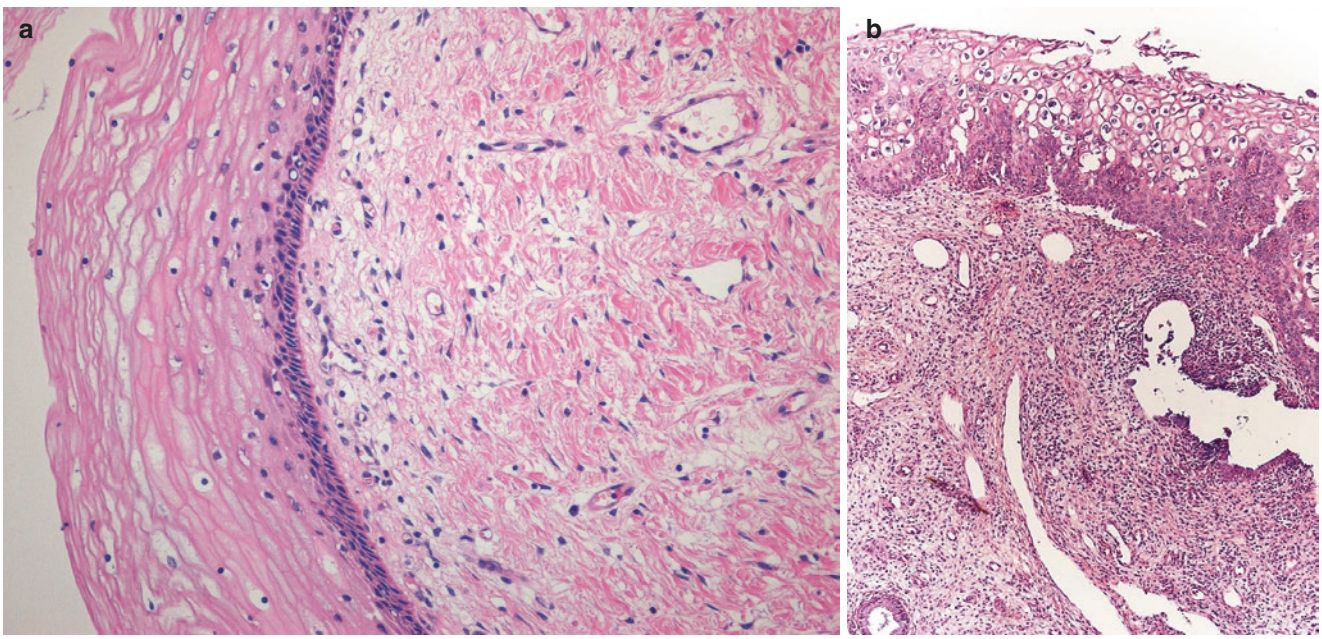

Fig. 1.6 Mature cervical squamous epithelium, in which three layers can be distinguished: the basal/parabasal layer, the intermediate layer, and the superficial layer (**a**). In contrast, koilocytes present large, hyperchromatic, irregular nuclei with irregular chromatin distribution and are usually associated with other changes in the epithelium (**b**)

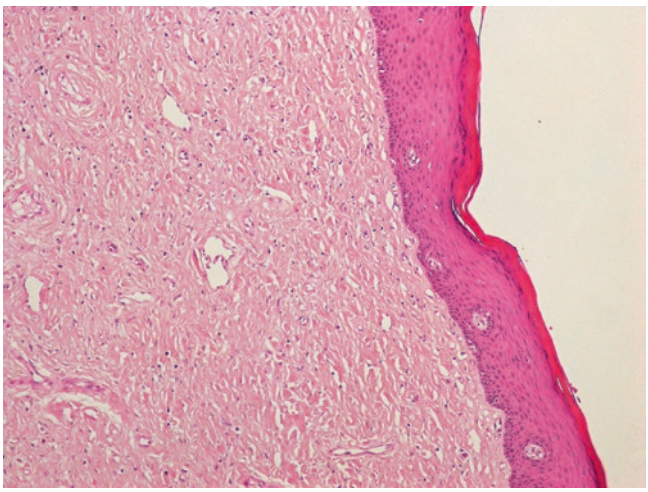

Fig. 1.7 Keratinization in superficial layers in a postmenopausal patient with genital prolapse; the cells in the superficial layer are flat and have a more eosinophilic cytoplasm

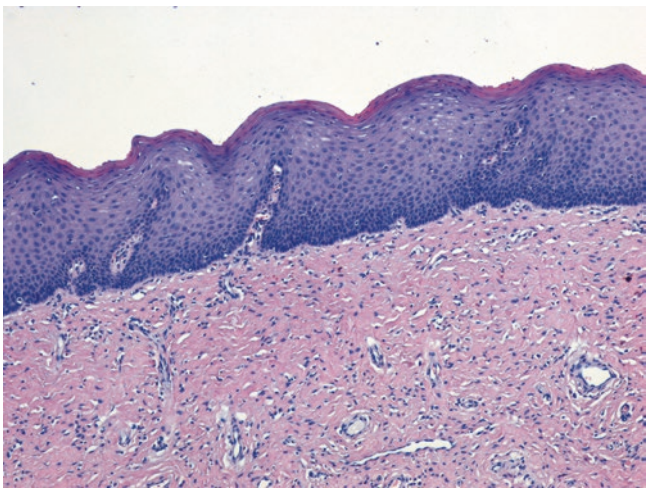

Fig. 1.9 Squamous cell hyperplasia associated with hyperkeratosis, usually related to uterovaginal prolapse

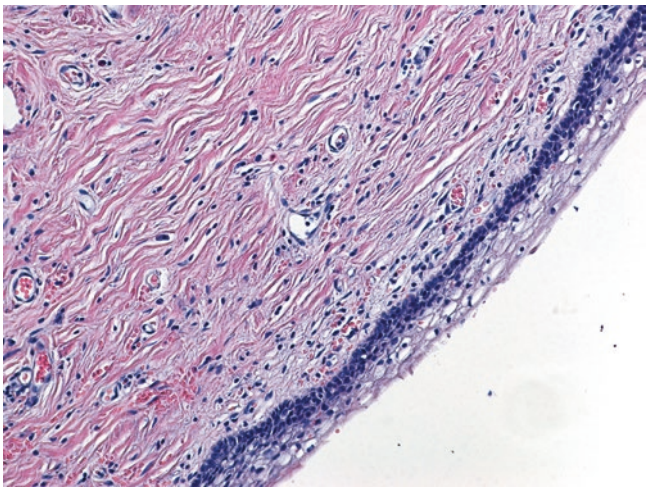

Fig. 1.8 Squamous epithelium in postmenopause is thin

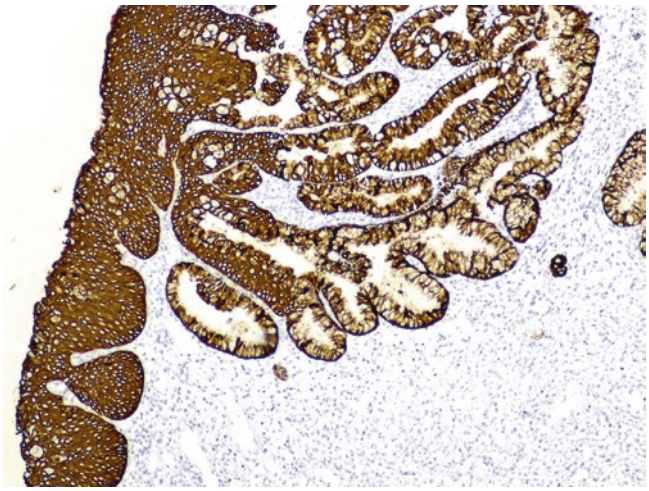

Fig. 1.10 Both squamous and glandular epithelium are positive for pan-cytokeratin

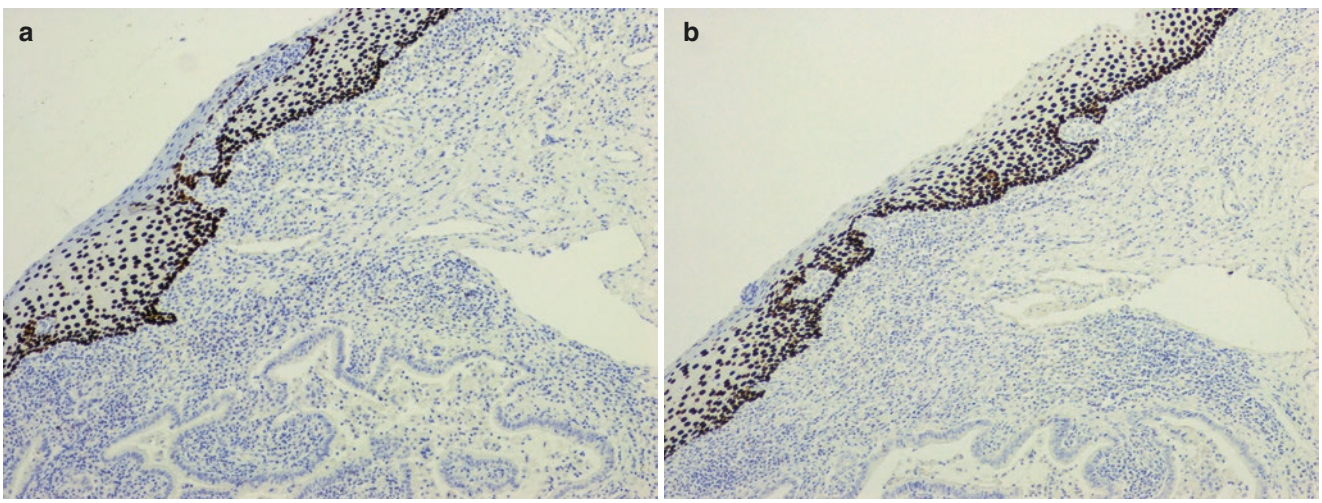

Fig. 1.11 The squamous epithelium is positive for both p63 (**a**) and p40 (**b**)

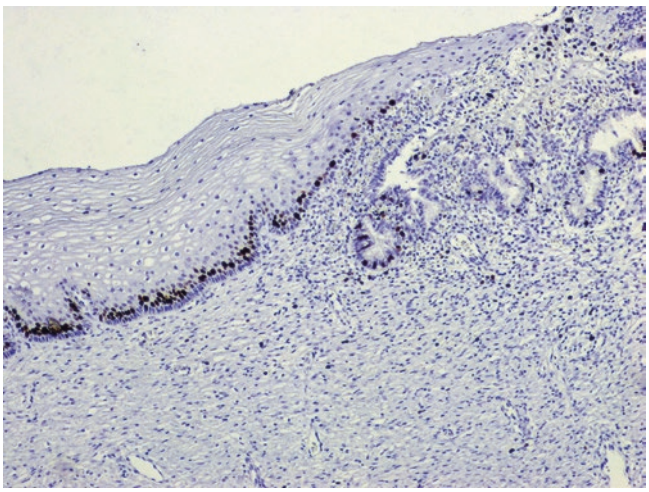

Fig. 1.12 The parabasal cells are positive for Ki-67, but the rest of the normal squamous epithelium is Ki-67-negative

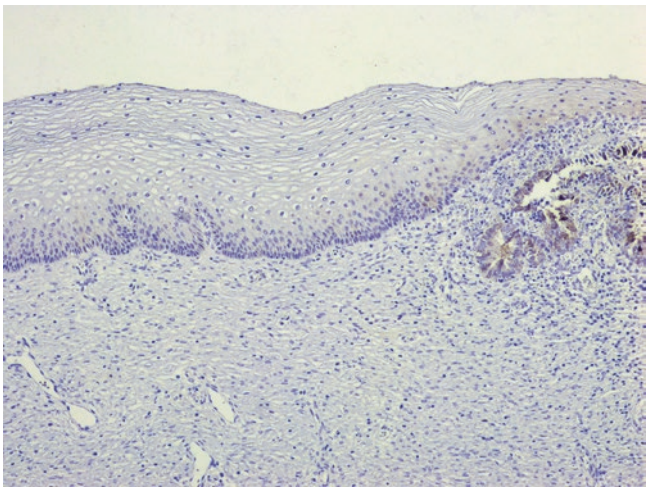

Fig. 1.13 p16 is negative (patchy pattern) in normal squamous epithelium

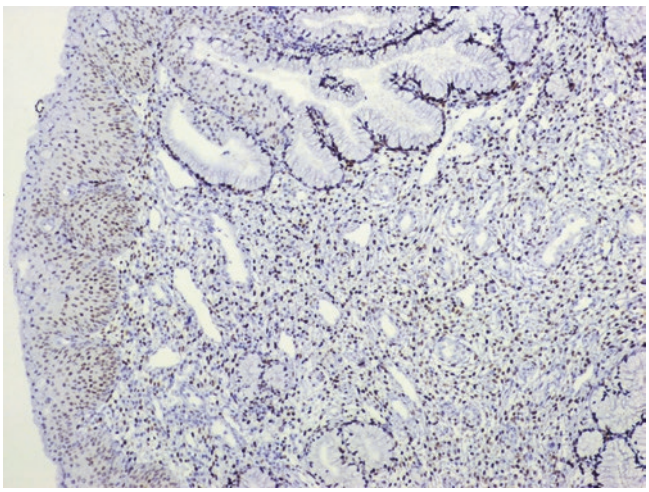

Fig. 1.14 The nuclei of the basal and parabasal cells are positive for estrogen receptors (ER); stromal cells are also positive for ER

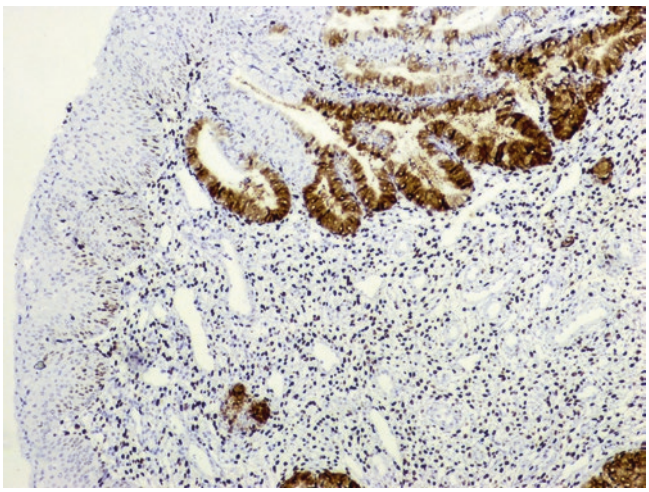

Fig. 1.15 Progesterone receptors (PRs) are positive in the parabasal layer during luteal phase and pregnancy; the picture also shows stromal cells positive for PR

1.1.1.1 Cytological Correlation in Squamous Epithelium

During microscopic examination of a Pap, three types of normal squamous cells can be identified: parabasal squamous cells, intermediate squamous cells, and superficial squamous cells. Parabasal cells are the smallest with dense, green/blue cytoplasm and a dark, large nucleus (occupying most of the cell size) with evenly distributed chromatin (Fig. 1.16). Some of these cells show only the free nuclei present in the Pap. The intermediate cells are larger, with pale blue cytoplasm and nuclei that are round and smaller than those in the parabasal cells, with finely granular chromatin (Fig. 1.17). The superficial cells are the largest with a polyhedral shape, a pyknotic, small nucleus and pink cytoplasm (Fig. 1.18). Keratinization does not normally occur in the cervix.

The proportion of superficial versus intermediate squamous cells in a Pap may vary throughout the menstrual cycle. Before ovulation, during estrogenic influence, the superficial cells predominate, whereas at midcycle, there are few intermediate squamous cells, with a background of neutrophils. This is the best time to take a cervical Pap if the patient is enrolled in a national cervical screening program. Under the influence of progesterone, intermediate cells are most numerous, with an increased background of neutrophils occurring during the late secretory phase. In postmenopausal women, the Pap shows a predominance of parabasal cells with occasional intermediate cells, while endocervical cells are usually not identified (Fig. 1.19). Similar changes occur postpartum, but squamous metaplasia, regenerative changes, and endocervical cells can be identified. Menstrual specimens contain many neutrophils, red blood cells, and endometrial cells. The endometrial cells are small with dark, coarse chromatin, round nuclei, and scant basophilic cytoplasm. These cells are usually clustered in small, darkly stained groups, but they also can be observed as individual cells.

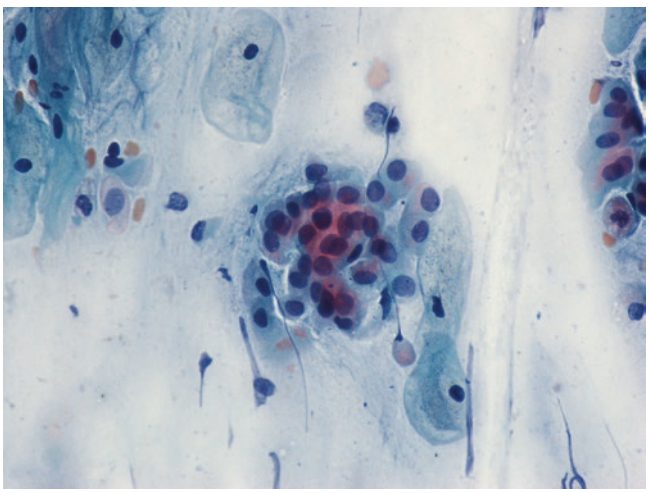

Fig. 1.16 Parabasal cells are the smallest in size, have a dense, green/blue cytoplasm, a dark, large nucleus with evenly distributed chromatin (Papanicolaou stain)

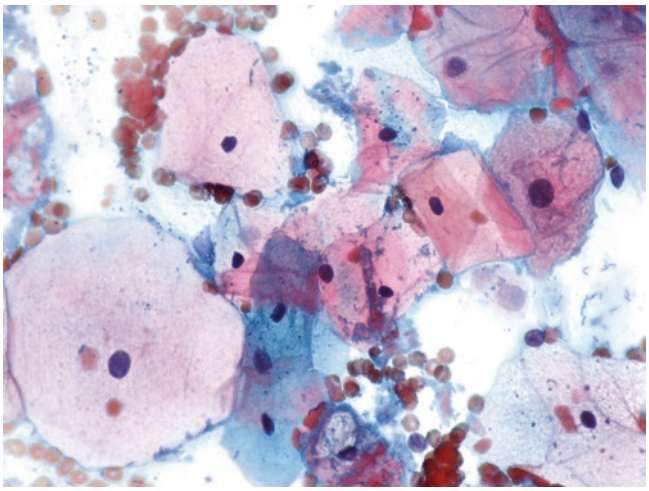

Fig. 1.18 Superficial cells are the largest and present a polyhedral shape, with a pyknotic, small nucleus and pink-staining cytoplasm (Papanicolaou stain)

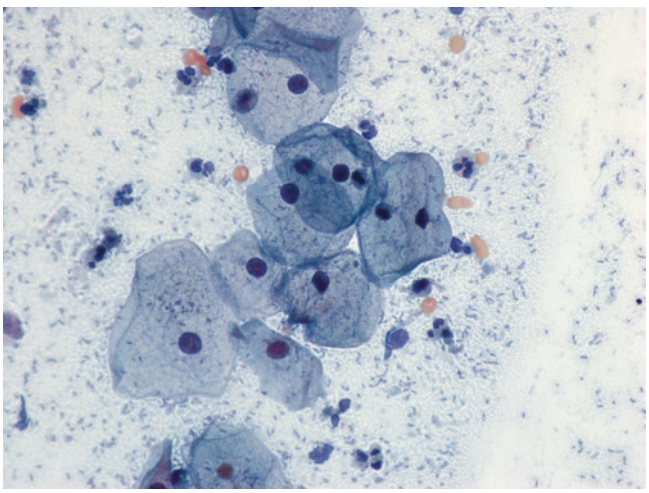

Fig. 1.17 Intermediate cells are larger, have pale blue cytoplasm, and round nuclei that are smaller than the ones in the parabasal cells, with finely granular chromatin (Papanicolaou stain)

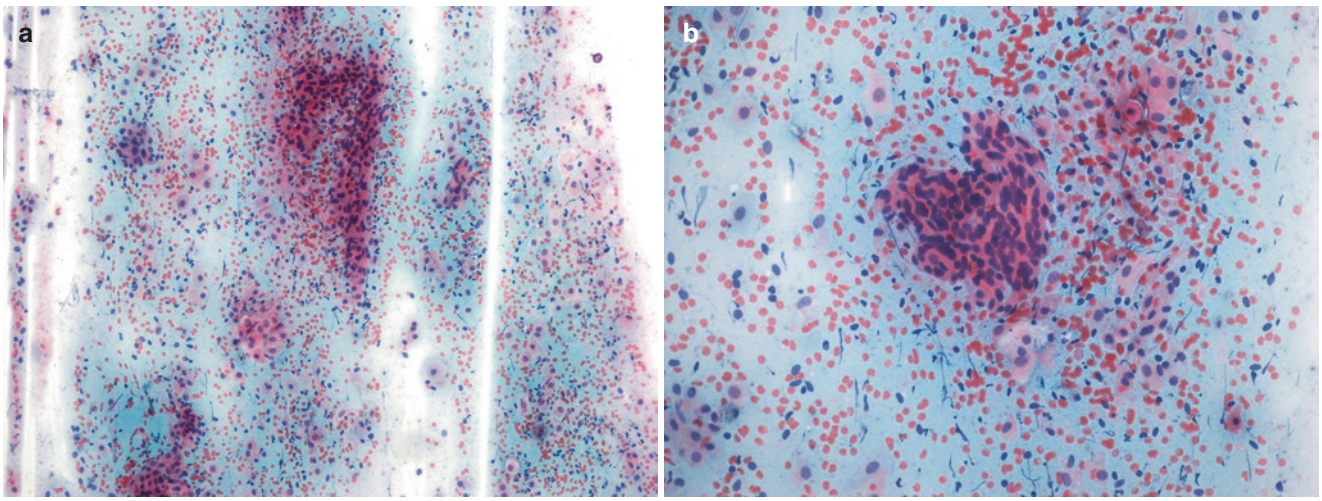

Fig. 1.19 Atrophy: The smear from a postmenopausal woman shows a predominance of parabasal cells with occasional intermediate cells (**a**); endocervical cells are usually not identified (**b**) (Papanicolaou stain)

1.1.2 The Epithelium of the Endocervix

The endocervical epithelium is composed of a single layer of columnar cells, with small elongated nuclei containing dense chromatin, arranged at the base of the cell, with abundant cytoplasm due to mucin secretion (Fig. 1.20). This mucin is pale blue on hematoxylin and eosin (H&E)-stained slides. Mucicarmine and Alcian blue are positive, revealing acidic mucopolysaccharides in the mucin. Neutral mucopolysaccharides may also occur in smaller quantities. Columnar cells are 20–30 μm high and 5–9 μm wide. Nuclear overlapping can be seen as a normal phenomenon at the base of these cells and should not be mistaken for endocervical glandular neoplasia (Fig. 1.21). In normal endocervical epithelium, mitotic figures are rarely observed, and nucleoli are usually indistinct. The nuclei can become larger, with prominent nucleoli, and mitotic figures are numerous in reactive and regenerative processes, as well as in various neoplastic lesions (Fig. 1.22).

In addition to these cells, other types of cells are among those found in the endocervical epithelium:

- *Goblet (caliciform) cells*. When they are numerous, the condition is diagnosed as *intestinal metaplasia* (Fig. 1.23), which is highly correlated with glandular neoplasia and must be reported even in association with discrete nuclear atypia.
- *Ciliated cells*. When they are numerous and associated with tubal type secretory cells and reserve or intercalary cells, they are diagnosed as *tubal metaplasia* (Fig. 1.24). This condition, found in up to 30% of patients, is not related to hormonal changes or inflammation; it should not be mistaken for endocervical glandular neoplasia (adenocarcinoma in situ). Tubal metaplasia does not show atypia or brisk mitotic activity; it is located close to the superficial inner third of the cervical wall. p16 is negative (patchy) and the Ki-67 index is low. Of interest, *tubo-endometrioid metaplasia* following conization can also occur. Tubo-endometrioid metaplasia is part of a spectrum of changes that include tubal metaplasia. In contrast to tubal metaplasia, tubo-endometrioid may lack cilia, imparting an appearance similar to endometrium; typical endometrial stroma is lacking. Instead, there is a vague cuff of surrounding bland and spindled stroma.
- *Reserve subcolumnar cells*. These should not be confused with lymphocytes, which may migrate into the endocervical epithelium in inflammatory processes.
- *Argyrophilic and argentaffin-positive endocrine cells*. These cells are identified in about 20% of patients and potentially represent the origin of various tumors in the cervix with neuroendocrine differentiation [19].

The endocervical epithelium covers the surface of the endocervical canal, with architecture ranging from flat to villous. The villous appearance is due to invaginations of epithelium into underlying stroma, forming elongated clefts (also called crypts) (Fig. 1.25). As a result, the underlying fibroconnective tissue assumes a papillary shape, with a central capillary. On longitudinal and transverse sections through the clefts, they resemble glands, which is a false impression. There are no true endocervical glands. About 60 years ago, Fluhmann demonstrated through serial sections and three-dimensional reconstruction that the endocervical clefts actually represent protrusions of the endocervical epithelium in the underlying stroma [20, 21]. The literature currently uses all terms, "endocervical glands" and "crypts" or "clefts." Sometimes a lobular pattern is seen, with a cystically dilated cleft associated with what appear to be acini, organized in a lobular configuration (Fig. 1.26). When the endocervical epi-

thelium lining the crypts proliferates, secondary channels appear in the adjacent stroma, giving rise to a lesion that Fluhmann termed *tunnel clusters* (also known in the literature as *Fluhmann's lumens* or *cervical adenomatous hyperplasia*). These secondary channels multiply further during pregnancy. They may have empty lumina or intraluminal secretions that appears on H&E as eosinophilic material. If the secretions are abundant, the epithelium of the channels may appear flattened.

The depth to which endocervical crypts can extend into the cervical stroma varies from person to person, but it usually does not exceed 5 mm (though it can reach up to 1 cm) [22, 23]. This information is important to keep in mind to avoid confusion with lesions such as minimal deviation adenocarcinoma of mucinous type (a well-differentiated variant of gastric type endocervical adenocarcinoma), an adenocarcinoma that features epithelium that can be difficult to distinguish from normal endocervix and in which deep neoplastic glands are often present. The details concerning this lesion are covered in Chap. 8.

The endocervical epithelium undergoes minimal morphological changes during the menstrual cycle, which are represented only by a change in the position of the nuclei; in the proliferative phase of the cycle, they are arranged in the middle of the cell, due to the presence of subnuclear vacuoles. Biochemical changes are more important, consisting of decreased viscosity and alkalization of the mucus in the proliferative phase, facilitating the penetra-

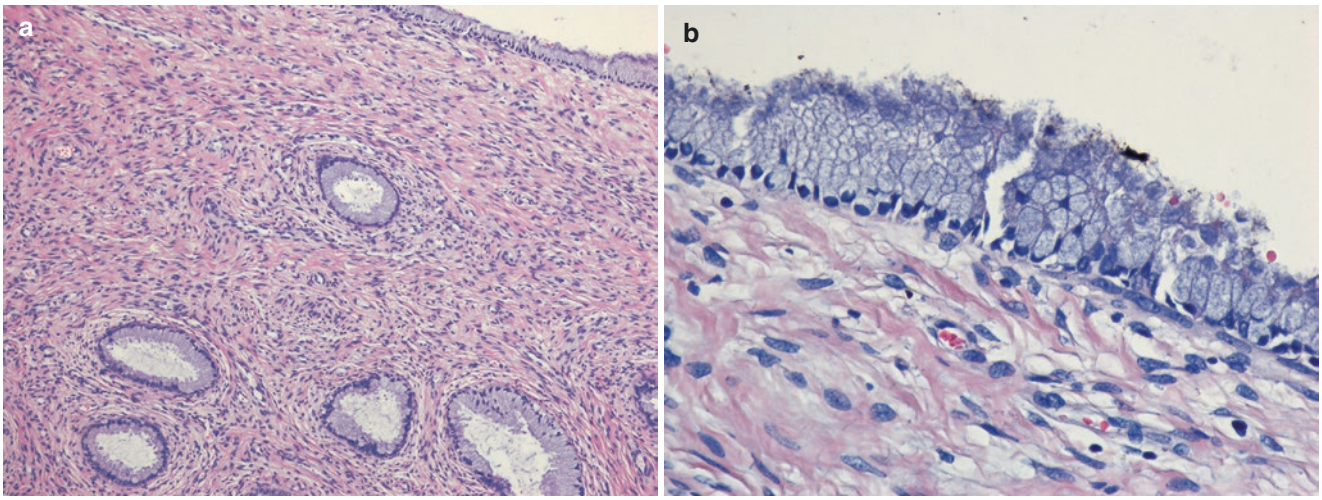

Fig. 1.20 Both superficial and glandular columnar epithelium (**a**) is composed of a single layer of columnar cells, with small, elongated nuclei, dense chromatin arranged at the base of the cell, and abundant cytoplasm due to mucin secretion (**b**)

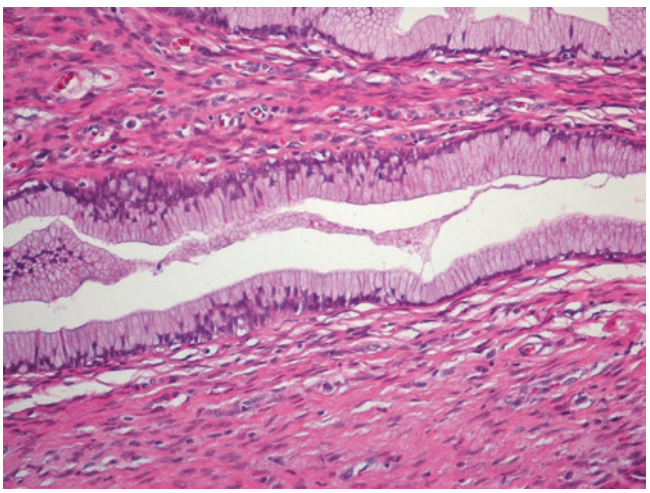

Fig. 1.21 Nuclear overlapping can be seen as a normal phenomenon at the base of these columnar cells

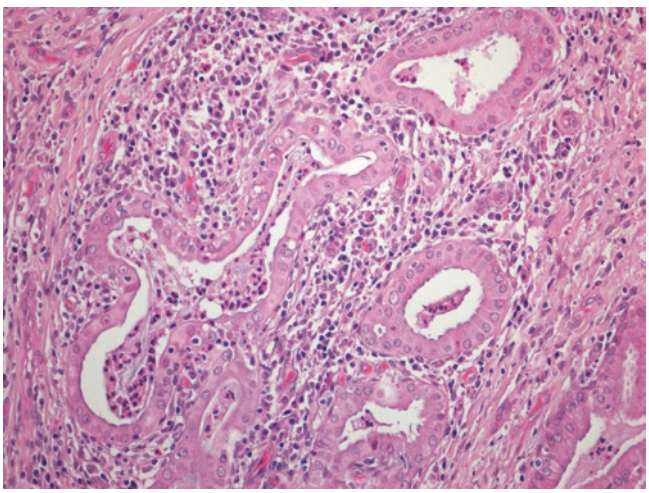

Fig. 1.22 The nuclei of the columnar endocervical epithelium can become larger, rounded, with prominent nucleoli in a reactive process in association with numerous neutrophils in various inflammatory infiltrates

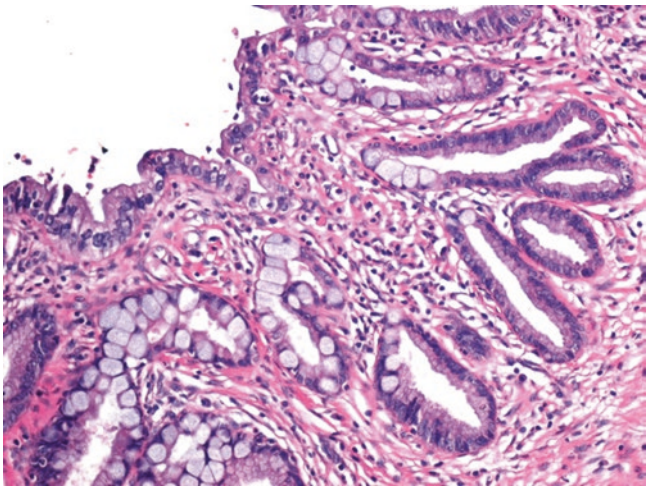

Fig. 1.23 Intestinal metaplasia: numerous goblet cells replacing the normal glandular epithelium of the cervix

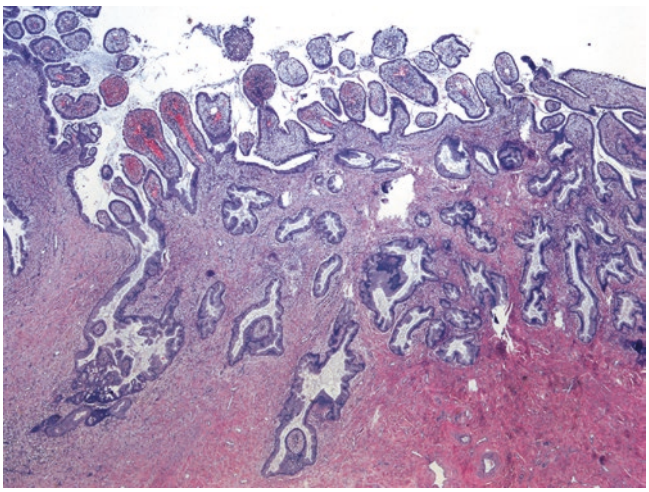

Fig. 1.25 The endocervical epithelium covers the surface of the endocervical canal with a villous architecture but also penetrates the underlying stroma, forming elongated clefts (crypts)

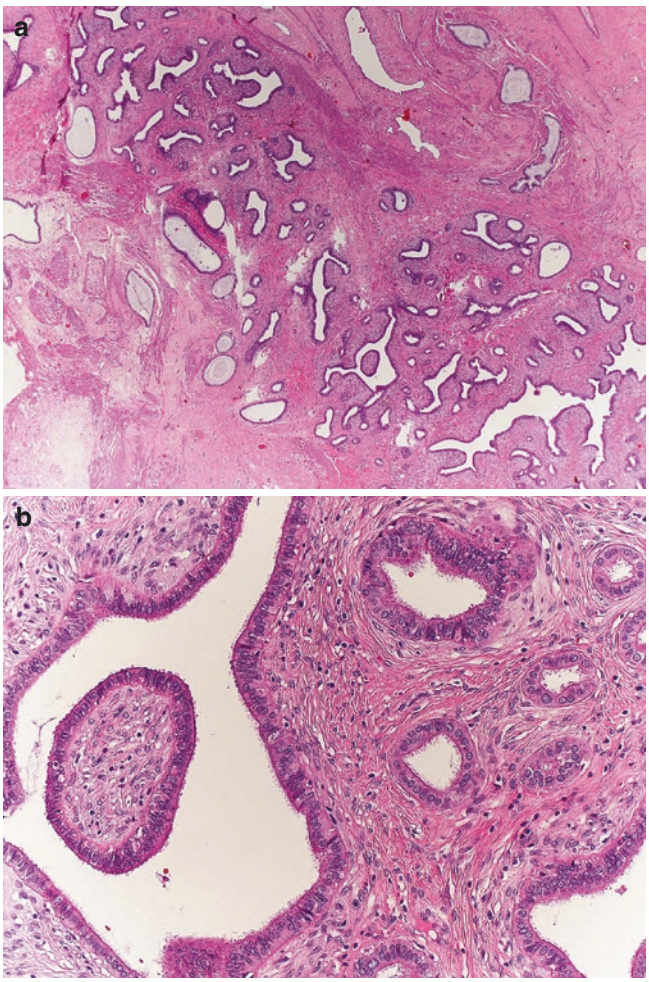

Fig. 1.24 Tubal metaplasia: Dark blue endocervical glands (**a**) presenting secretory, reserve, intercalary, and ciliated cells (**b**)

tion of sperm, whereas in the luteal phase, the viscosity of the mucus increases, it becomes acidic, and it contains numerous leukocytes, acting as a barrier against penetration. The acidic mucin content of normal epithelium changes during the menstrual cycle, with sialomucins increasing during the ovulation period and sulphomucins increasing in the secretory phase [24].

Immunohistochemically, the endocervical epithelium is positive for various types of cytokeratins, such as cytokeratin 7, 8, 18, and 19, as well as various mucin antigens such as MUC1, MUC4, and MUC5, whereas MUC2 is typically absent [8, 25–28]. Normal endocervical epithelium is also positive for EMA (epithelial membrane antigen), CEA, and PAX8, Cyclin D1, ER, PR, and HER-2 [12, 14, 15, 18]. Of interest, gastric and intestinal markers (such as CLDN18, CDH17, TFF2, SATB2) are negative in normal columnar epithelium, which can be helpful in differentiating between cervical lesions and metastases to the cervix from the gastrointestinal tract [29].

1.1.2.1 Cytological Correlation in Glandular Epithelium

Columnar endocervical cells are easily identified in a Pap, occurring in sheets or small groups, or even as isolated cells. These glandular cells are much larger than endometrial cells. They have round or elongated nuclei with finely granular chromatin, sometimes with small nucleoli and pale blue/gray, abundant cytoplasm, usually vacuolated, with indistinct cell borders and a characteristic "honeycomb" appearance when present in sheets and clusters due to the central location of the nuclei in the cytoplasm (Fig. 1.27).

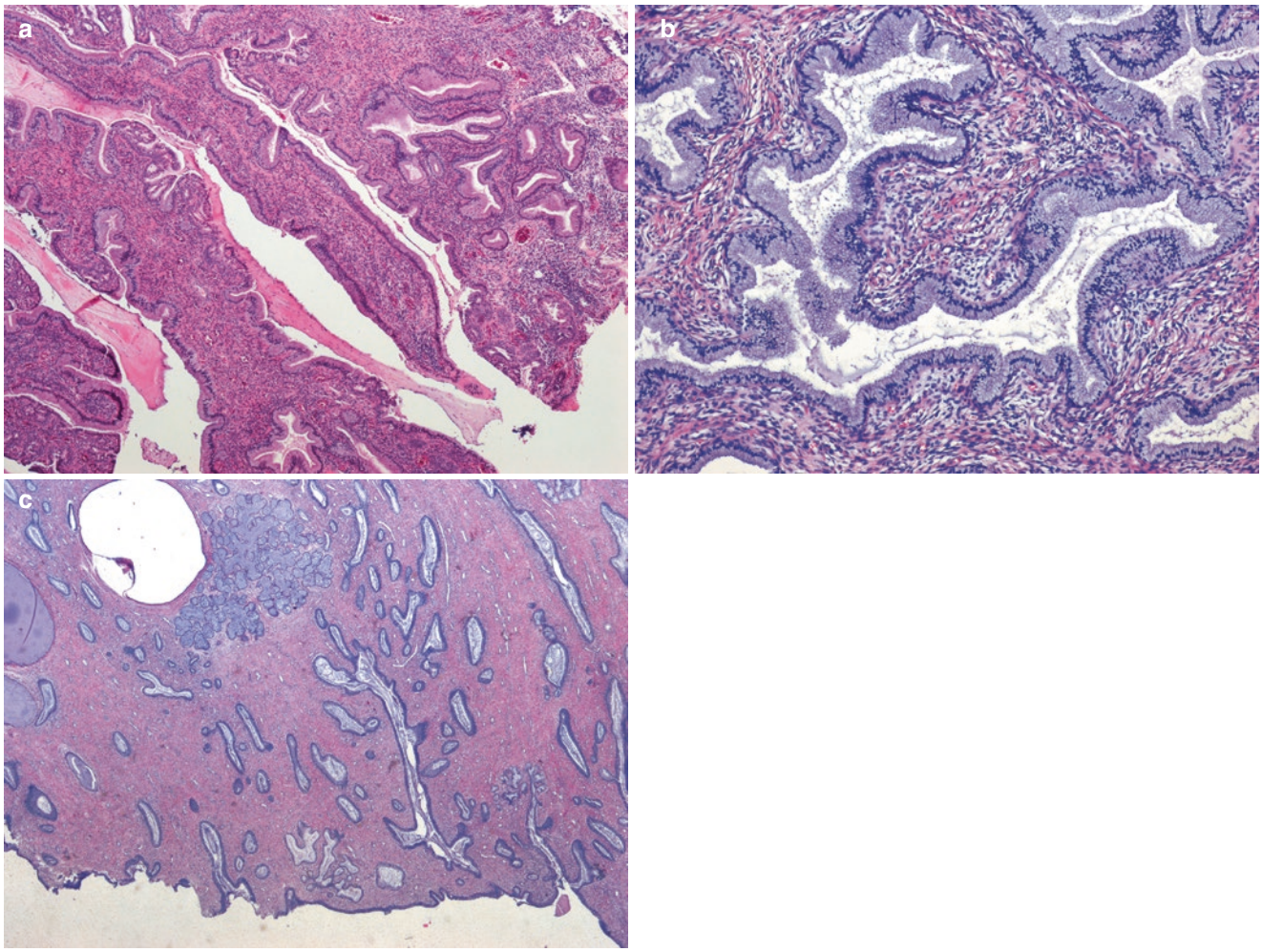

Fig. 1.26 Crypts (**a**, **b**) sometimes presenting a lobular pattern with a cystically dilated cleft and peripheral grooves organized in a uniform lobular distribution (**c**)

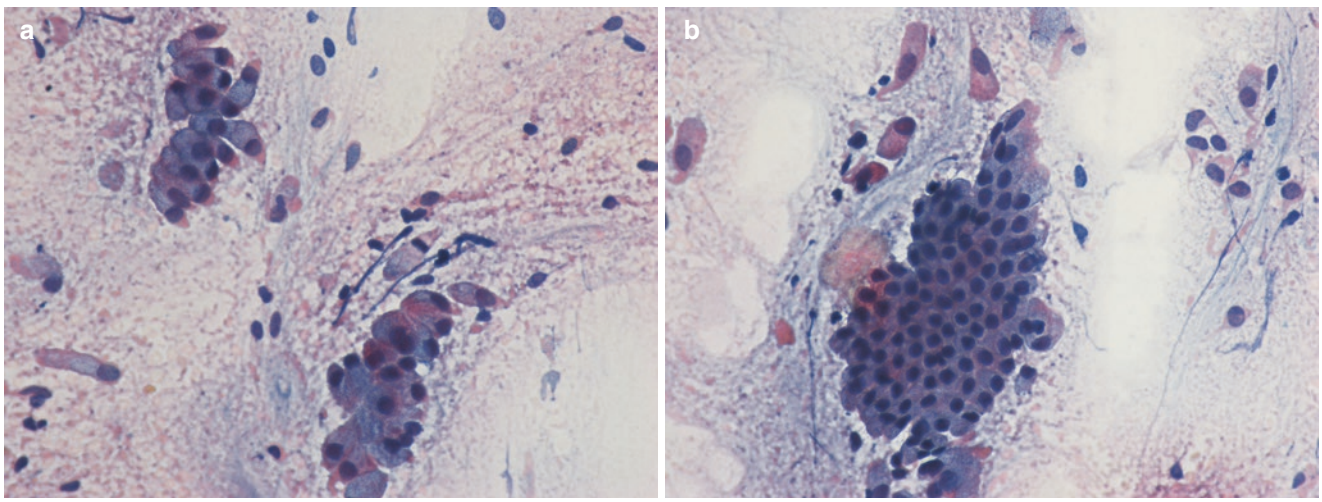

Fig. 1.27 The columnar endocervical cells are much larger than the endometrial cells, have round/elongated nuclei with finely granular chromatin, sometimes with small nucleoli and pale blue/gray abundant cytoplasm with indistinct cell borders. They may occur in sheets or small groups (**a**) or with a characteristic "honeycomb" appearance (**b**) due to the central location of the nuclei in the cytoplasm (Papanicolaou stain)

1.1.3 The Epithelium of the Transformation Zone

The endocervical epithelium has a different location throughout life as a response to hormonal stimulation. At birth, the endocervical epithelium appears in the ectocervix, but it moves to the endocervical canal during the first year of life, where it remains until the first menstrual cycle. At puberty, it moves again to the ectocervix, more prominently on the anterior lip than the posterior lip. As the cervix grows larger, the endocervical epithelium runs further outward onto the ectocervix (more markedly anteriorly and posteriorly than at the sides), giving rise to ectropion (cervical ectopia). Ectropion is even more pronounced during pregnancy or after progesterone therapy. Some authors have termed this lesion an "erosion" because upon inspection the ectropion epithelium appears red (as the columnar epithelium is one cell layer thick and transparent to the underlying blood vessels) and rough (related to the villous pattern of the endocervical tissue).

This phenomenon is important when pathologists refer to the type of tissue they examine under the microscope. Most pathologists do not refer to the position of the tissue from which it was sampled, but rather to the type of the tissue. Thus, a biopsy specimen consisting of stroma and glandular epithelium in the form of surface epithelium or crypts may be described as "endocervical," although the tissue may come from the ectocervix, which can be clinically misleading.

Throughout the reproductive years, the endocervical epithelium in the ectropion is gradually replaced by squamous epithelium (squamous metaplasia) resulting in the areas referred to as the *transformation zone* or *T zone*. The term "squamocolumnar junction" is used in two settings: at birth, it is the area where the ectocervical squamous epithelium is contiguous with the endocervical glandular epithelium (*original squamocolumnar junction*); during reproductive life, the junction includes squamous metaplasia (*functional squamocolumnar junction*). The area between the original and functional junctions, the transformation zone, is considered to be the area where most HPV-associated neoplastic processes occur, and because it is located in the ectocervix during the reproductive life, it can be seen on colposcopic examination.

During premenopause, the functional squamocolumnar junction migrates to the outer opening of the cervical canal. Later, because of the decrease in cervical size, it is located in the endocervical canal, a phenomenon called *inversion*, which explains why the transformation zone cannot be visualized with the naked eye or during colposcopic examination in postmenopausal patients.

1.1.3.1 Transformation Mechanisms

Two mechanisms are responsible for the transformation of endocervical epithelium into squamous epithelium: squamous epithelialization and squamous metaplasia [30]:

During *squamous epithelialization*, direct development of mature squamous cells occurs in the ectocervix. These cells initially appear between the basal membrane of the epithelium and the endocervical glandular cells. As they grow, they push the glandular cells outward. The degenerated columnar cells will subsequently detach from the epithelium. Initially, squamous epithelialization occurs in the openings of endocervical clefts (Fig. 1.28), and subsequently, the clefts are also involved. If epithelialization of the cleft openings causes their obstruction by continuous accumulation of mucus, the clefts dilate cystically and Nabothian cysts appear (Fig. 1.29). Crypts in which the columnar epithelium has been replaced by squamous epithelium, especially in cross sections, should not be confused with invasive squamous cell carcinoma. Even though the cells in squamous epithelialization have enlarged nuclei and nucleoli, they do not present anaplasia, pleomorphism, chromatin abnormalities, or mitotic figures. They do not infiltrate the adjacent stroma, and there is no stromal desmoplasia. Squamous epithelialization is not accompanied by tissue granulation, but only by a chronic inflammatory infiltrate.

Squamous metaplasia is part of a process that involves an initial proliferation of endocervical reserve cells and their subsequent differentiation into squamous cells [20, 31]. This phenomenon is also called *epidermalization*, and the stimulus for this phenomenon is thought to be the increased acidity of the vaginal environment compared with that of the cervical canal. The reserve cells are cuboidal, with round nuclei and scant cytoplasm, and are located beneath the columnar epithelial cells (Fig. 1.30). They resemble the basal or parabasal cells of the ectocervical squamous epithelium. Their origin has been widely discussed; some authors consider that they derive from columnar mucin-producing cells, basal cells of the squamous epithelium, embryonic remnants of urogenital origin, and yet others suggest that they derive from stromal cells [32, 33]. Immature squamous metaplasia is covered by a layer of endocervical cells. As these reserve cells proliferate and stratify, their cytoplasm becomes more abundant as they differentiate towards squamous cells (*immature squamous metaplasia*). Later, these cells acquire cytoplasmic glycogen, identical to the superficial cells of the exocervical epithelium (*mature squamous metaplasia*) (Fig. 1.31). Neither condition (mature and immature squamous metaplasia) is associated with a risk for developing subsequent malignancy. Immature squamous metaplasia should not be confused with squamous intraepithelial lesion (SIL). Although cells constituting immature squamous metaplasia have reduced cytoplasm with an increased nuclear/cytoplasmic ratio and elongated, sometimes hyperchromatic nuclei, the nuclei are nonetheless uniform, without chromatin changes, there are few mitoses, and the nucleoli are distinct. Unlike most SILs, the Ki-67 index is less than 15% in squamous metaplasia and p16 is negative or displays patchy staining, but not block-like staining, which is a surrogate for the presence of integrated

high-risk HPV, seen in nearly all high-grade SILs and a significant proportion of LSILs [34, 35]. Immunohistochemically, the cells in immature squamous metaplasia are cytokeratin-positive, and some may be positive for mucicarmine, MUC2, and MUC5 [27].

Immature squamous metaplasia may sometimes have nuclear atypia, which is described as atypical immature metaplasia [36]. Distinguishing between atypical immature metaplasia and HSIL can therefore be difficult. In atypical immature metaplasia, the nuclei of the cells are only slightly enlarged and round, with fine chromatin and prominent nucleoli, whereas in high-grade SIL, the nuclei are more pleomorphic and hyperchromatic, and mitotic figures appear, some of which may be atypical. High-grade SIL displays greater cellularity and cellular disorganization with lack of polarity. Transitional metaplasia is an incidental finding occurring in postmenopausal patients that shares some features with atrophy. It involves the ectocervix but sometimes also the endocervix; it is composed of basal and parabasal cells with high nuclear/cytoplasmic ratio, oval or fusiform nuclei, and longitudinal grooves that are oriented vertically in the deep layers and horizontally in the superficial ones, extending throughout the thickness of the epithelium, resembling transitional epithelium [37, 38] (Fig. 1.32). Some authors call this cervical Walthard islands. The nucleoli are small or indistinct, and mitoses are absent or rare. Frequently, cells have a clear perinuclear halo, but the nuclear/cytoplasmic ratio is usually low. Immunohistochemically, transitional cell metaplasia (like urothelium epithelium) expresses cytokeratin 13, 17, and 18, but in contrast to the urothelium, it is negative for cytokeratin 20.

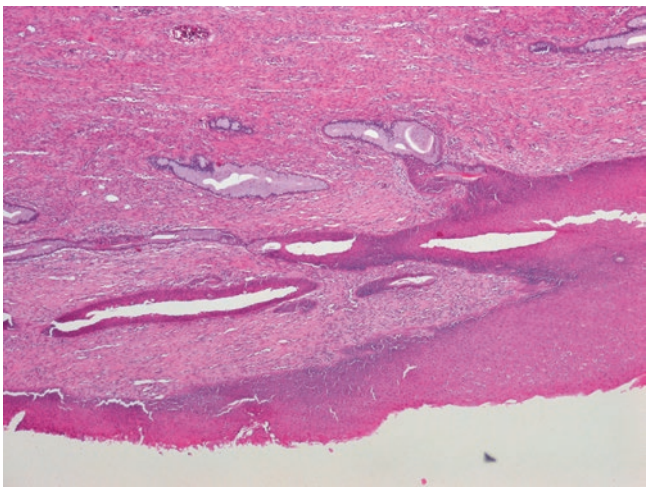

Fig. 1.28 Squamous epithelialization occurred in the openings of endocervical clefts, causing obstruction

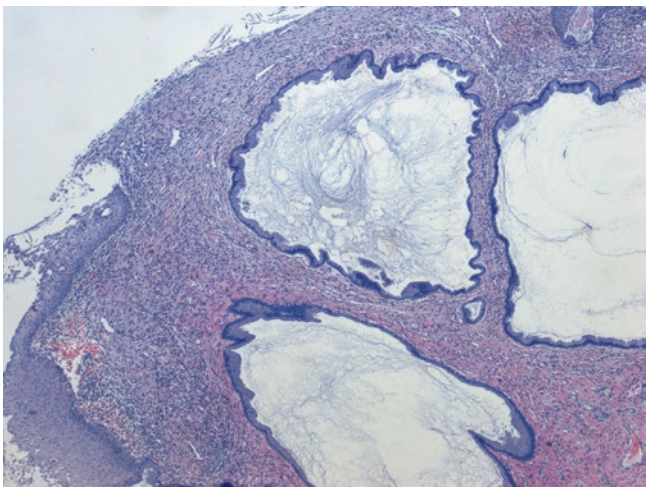

Fig. 1.29 From continuous accumulation of mucus, the clefts dilate cystically, forming Nabothian cysts

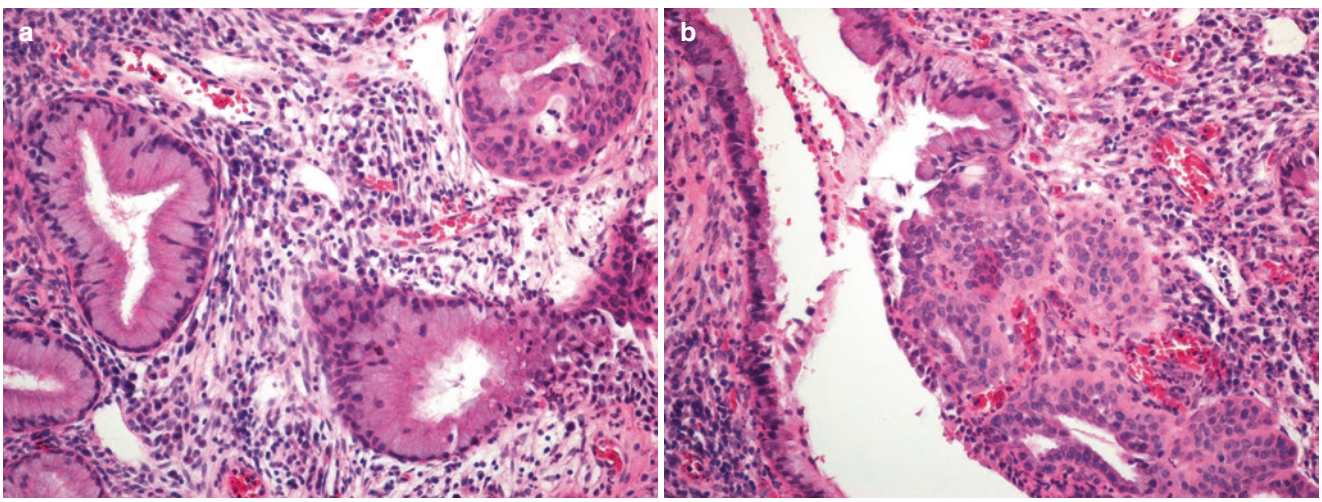

Fig. 1.30 Immature squamous metaplasia: proliferation of endocervical reserve cells (upper right) and their subsequent differentiation into squamous cells (**a**) with cuboidal shape, round nuclei, and scanty cytoplasm, located beneath the columnar epithelial cells (**b**)

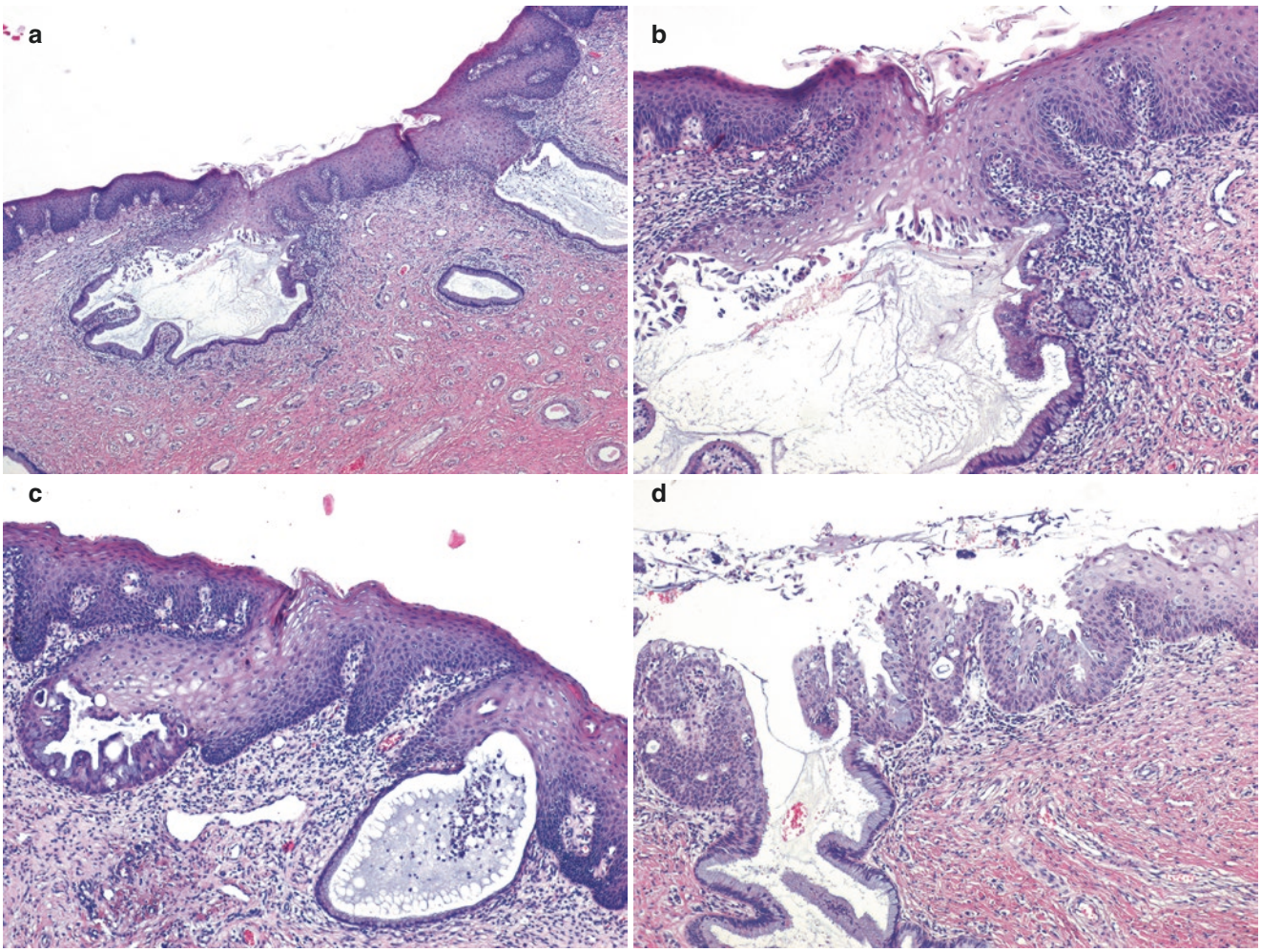

Fig. 1.31 Mature squamous metaplasia replacing the original glandular epithelium (**a**); the mature squamous cells are larger, and the cytoplasm is filled with glycogen (**b**). These cells can extend into the clefts (**c**), sometimes coexisting with immature squamous metaplasia (left) (**d**)

The cervical squamocolumnar junction and the "T zone" are the sites of the recently described embryonic cell population that was proposed as the cell of origin for cervical carcinoma and its precursors. This discrete population of cuboidal to low columnar cells has a unique genetic and immunohistochemical profile (squamo-columnar markers such as cytokeratin 7, AGR2, GDA, and MMP7) shared with high-grade SIL, a subset of low-grade SIL infected with high-risk HPV, adenocarcinoma in situ, and invasive cervical cancer [39, 40]. Crum et al. [36] proposed that direct infection of squamo-columnar embryonic cells by high-risk HPV results in transdifferentiation of these cells with an outgrowth of subjacent squamous cells (so-called top-down differentiation) leading to SIL, most often of high grade and with the propensity to progress [41]. In contrast, infection of the keratinocytes derived from ectocervical or squamous metaplastic epithelium usually results in low-grade SIL that is likely to regress [39, 40]. The presence of embryonic cells would also explain the markedly different risks for vaginal and cervical HSIL and cancer [42].

In routine practice, the term *squamous metaplasia* is used by the pathologist to designate both epithelialization and squamous metaplasia. Also, squamous metaplasia is an irreversible permanent process; the cells will never change into glandular epithelium. When the transformation zone moves back to the cervical canal, the squamous epithelium moves too, lining the cervical canal. Squamous metaplasia involves not only the surface epithelium, but also the clefts (sometimes only focally). Caution must be taken when examining the cervix, as these areas should not be interpreted as invasive carcinoma.

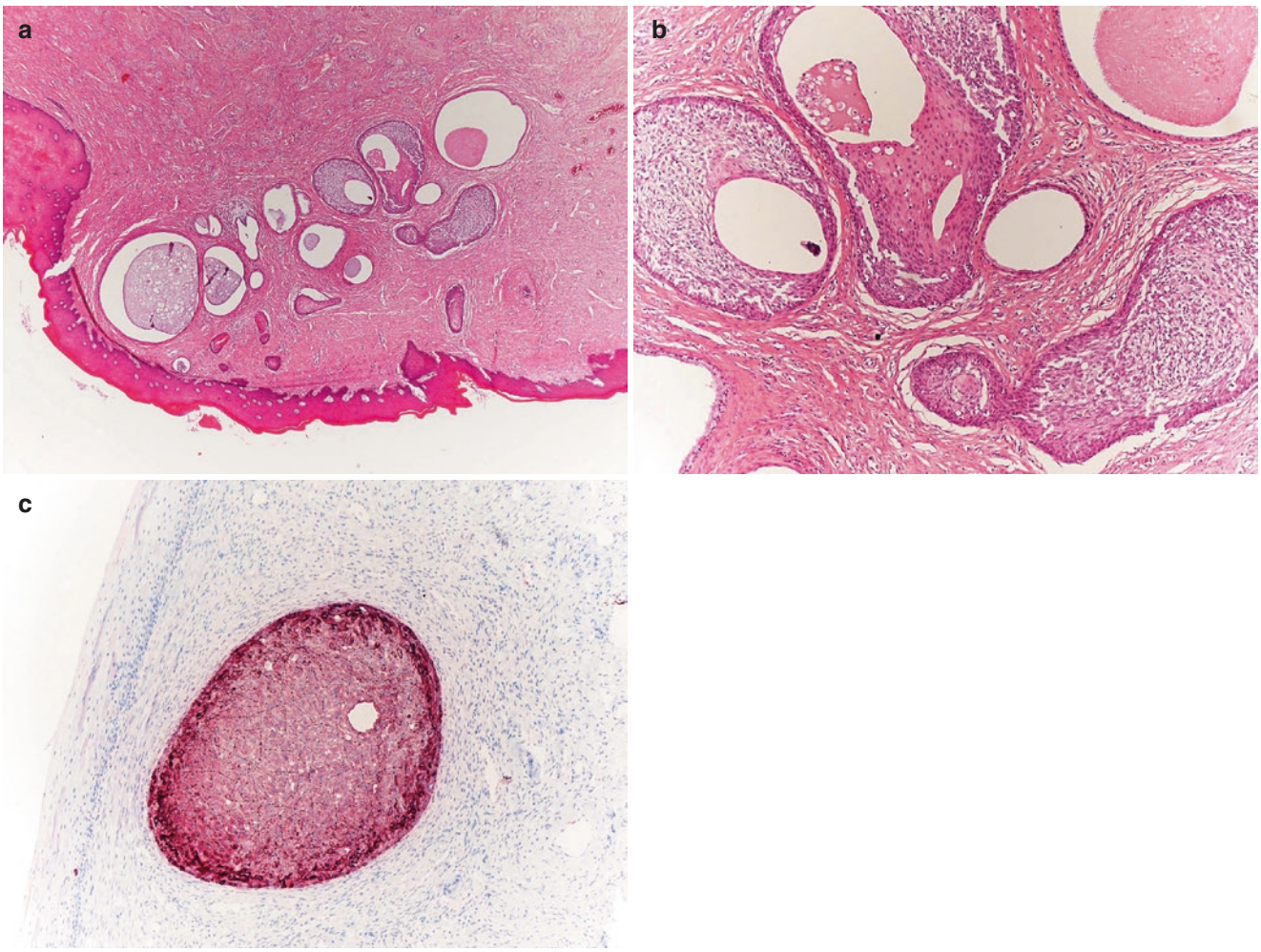

Fig. 1.32 Transitional metaplasia: endocervical glands in which the columnar epithelium has been replaced by basal and parabasal cells, which extend throughout the thickness of the epithelium (**a**), with high nuclear/cytoplasmic ratio, oval or fusiform nuclei, small nucleoli, no mitotic figures, and longitudinal grooves oriented vertically in the deep layers and horizontally in the superficial ones (**b**). Immunohistochemically, they express cytokeratin 7 (**c**)

1.1.3.2 Cytological Correlation in the Epithelium of the Transformation Zone

Both mature and immature squamous cells can be identified on microscopic examination of Paps. Immature squamous metaplasia cells are small and round with large, dark hyperchromatic nuclei with coarse chromatin, occasional nucleoli and basophilic green, dense cytoplasm with well-defined cell borders. They usually occur in small groups. The cells with mature squamous metaplasia have more abundant blue or pink cytoplasm and a larger nucleus with fine chromatin (Fig. 1.33).

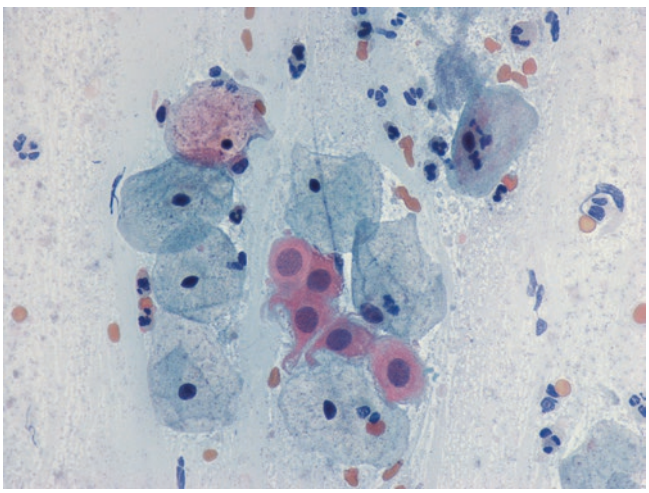

Fig. 1.33 Mature squamous metaplastic cells have a more abundant blue or pink cytoplasm and a larger nucleus with fine chromatin (Papanicolaou stain)

1.2 Cervical Stroma

The cervical stroma is composed of a mixture of fibrous, muscular, and elastic tissue, with a predominance of the fibrous component (Fig. 1.34). In the upper portion of the

cervix, the stroma merges with the endometrial stroma. The cervical stroma is predominantly fibrotic, whereas the corpus stroma is more muscular in appearance, but the demarcation between the two types of stroma may be unclear because of a hybrid endocervical-endometrial appearance. Some pathologists use the type of stroma to assign the anatomical site of origin of a tumor, but this can lead to erroneous conclusions because of the hybrid nature of the stroma at the junction between the lower uterine segment and the upper endocervix.

The muscular layer is more abundant in the endocervix than the ectocervix due to extension of the muscular fibers from the myometrium inferiorly along the periphery of the portio supravaginalis of the cervix, allowing marked dilation of the cervix at the time of birth. The cervical stroma contains many blood vessels (Fig. 1.35). At the epithelial-stromal junction, there is a rich capillary network, which sometimes may be prominent; however, this should not be confused with a hemangioma (Fig. 1.36).

The endocervical stroma normally has a lymphoid population, sometimes forming lymphoid aggregates, with or without germinal centers (Fig. 1.37). The lymphoid cells are involved in mucosal immunity and defense mechanisms against viral and bacterial pathogens. The lymphoid cells of the endocervix are part of the mucosal-associated lymphoid system (MALT) and secrete IgA. They can also migrate into the endocervical epithelium, giving the appearance of "clear cells" (these cells being formerly considered reserve cells). In the cervical stroma, there is also a population of scattered plasma cells and dendritic cells (also called Langerhans cells), some of which contain Birbeck granules. For the diagnosis of chronic cervicitis, a large number of inflammatory cells is required. There is a tendency to incorrectly diagnose *chronic cervicitis* in the presence of normally occurring, small inflammatory infiltrates in the cervical stroma [43]. This should be avoided.

Mesonephric remnants may be identified in the cervical stroma in more than 22% of cases, especially in the lateral portion of the cervix, in the deep stroma, but sometimes also below the endocervical epithelium [44]. In the embryo, the kidneys develop from the mesonephros, and in males, the mesonephros gives rise to the epididymis and its appendages, as well as the epididymis and rete testis; the mesonephric duct is responsible for the formation of the deferential vessels, the seminal vesicles, and part of the urethra and prostate [45]. In the female, however, in the absence of testosterone, there is regression of the mesonephric tubules and ducts, so that only mesonephric remnants occur in adults, and their function is not well known. In women, the incompletely developed Wolffian system can be divided into two areas: the upper part, derived from mesonephric tubules, and the lower part of the Gartner or Wolffian duct, which extends laterally along the uterine corpus and cervix and ends with an ampullary dilatation in the vagina. Mesonephric remnants come from the lower Wolffian system [45, 46] and in optimally oriented tissue, there is an elongated central duct, surrounded by smaller tubular structures. These round glands are lined by a single layer of cuboid cells with small nuclei (Fig. 1.38). The cytoplasm lacks mucin and is therefore PAS and mucicarmine negative. The lumen contains eosinophilic material, which is PAS-positive. Mesonephric remnants should not be confused with invasive adenocarcinoma. Typically, mesonephric remnants are variably positive for calretinin, CD10, and GATA3, helping to differentiate these embryological remnants from diagnostic mimics other than mesonephric adenocarcinoma [47, 48]. Mesonephric remnants can be associated with mesonephric hyperplasia or mesonephric adenocarcinomas of the cervix.

In the cervical stroma, tissues derived from the ectoderm also may appear, including epidermis and appended structures such as sebaceous glands, mature hyaline cartilage, or bone (Fig. 1.39). Multinucleated giant cells (isolated or multifocal) also may appear, sometimes with enlarged, hyperchromatic nuclei that are bizarre in appearance, with smudged chromatin and prominent nucleoli (Fig. 1.40). These cells have no mitotic activity and should not be confused with a neoplastic process. Immunohistochemistry can assist, as these cells have a low Ki-67 index, are invariably positive for vimentin, ER, PR and androgen receptor (AR), and are occasionally and focally positive for CD10 and smooth muscle markers such as actin, desmin, and h-caldesmon [49–53]. Atypical stromal cells are consistently negative for cytokeratins, EMA, myogenin, CD34, factor VIII, and macrophage marker [54].

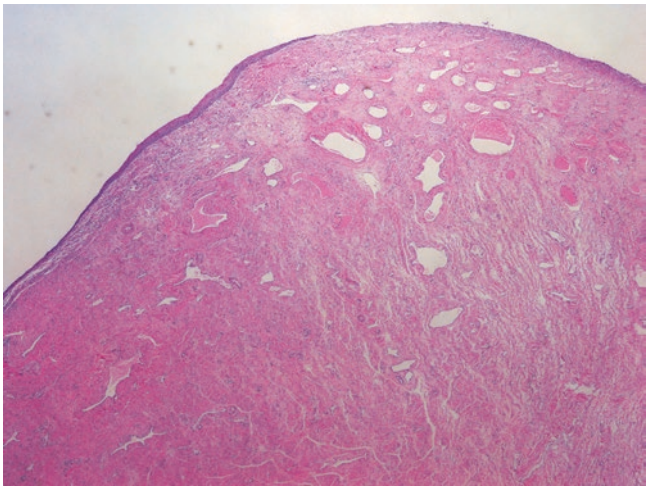

Fig. 1.34 Cervical squamous epithelium lines the cervical stroma

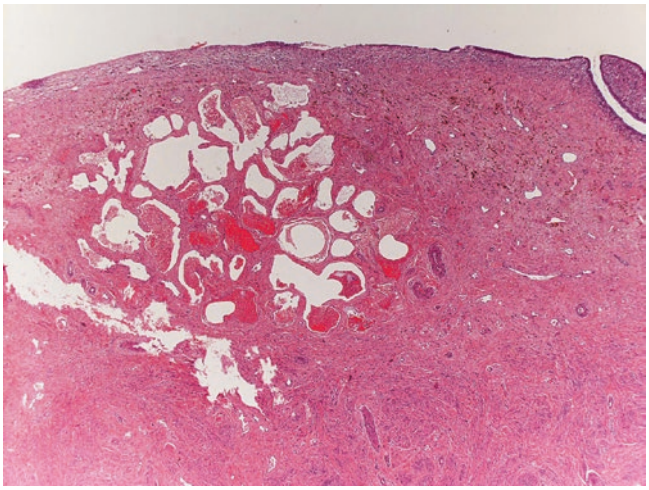

Fig. 1.36 Cervical hemangioma: proliferation of blood vessels forming a mass at the junction between epithelium and stroma

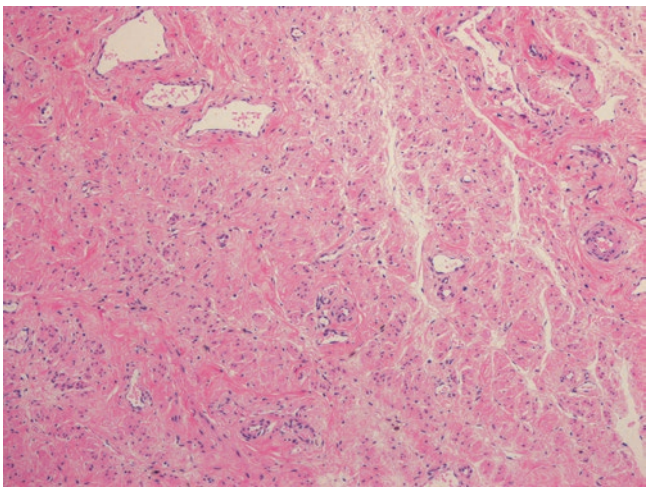

Fig. 1.35 The cervical stroma is predominantly fibrotic and contains a large number of vessels

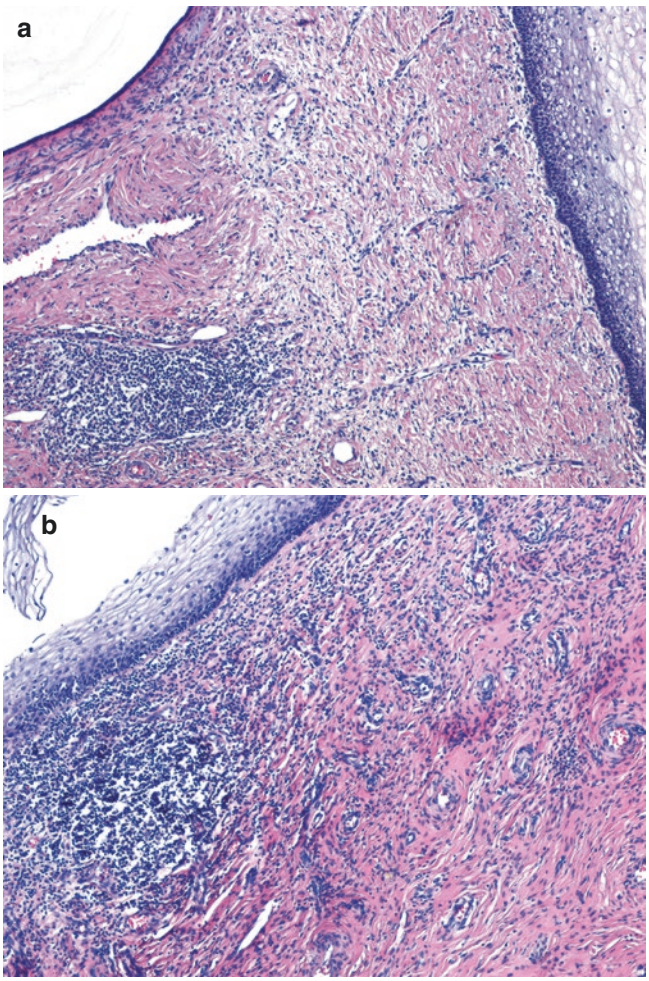

Fig. 1.37 The endocervical stroma presents a lymphocyte population (**a**), forming lymphoid aggregates, with or without germinal centers (**b**)

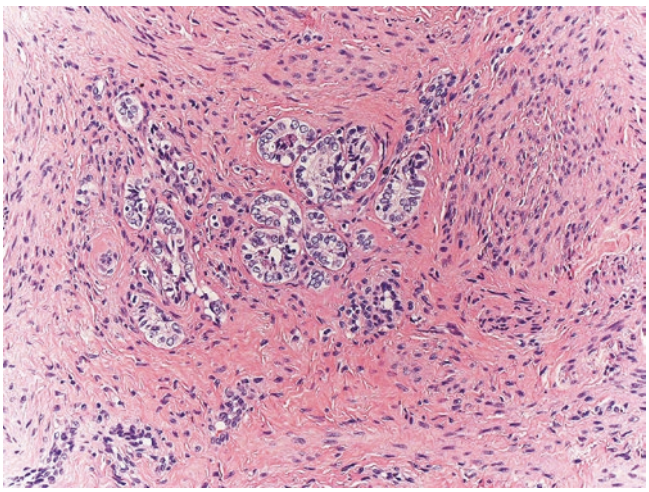

Fig. 1.38 Mesonephric remnants: tubular, round structures lined by a round, central nucleus and a single layer of cuboid cells

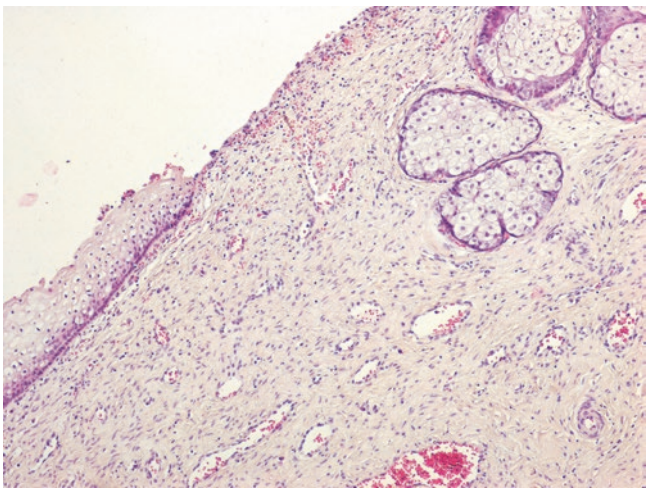

Fig. 1.39 Sebaceous glands in the cervical stroma

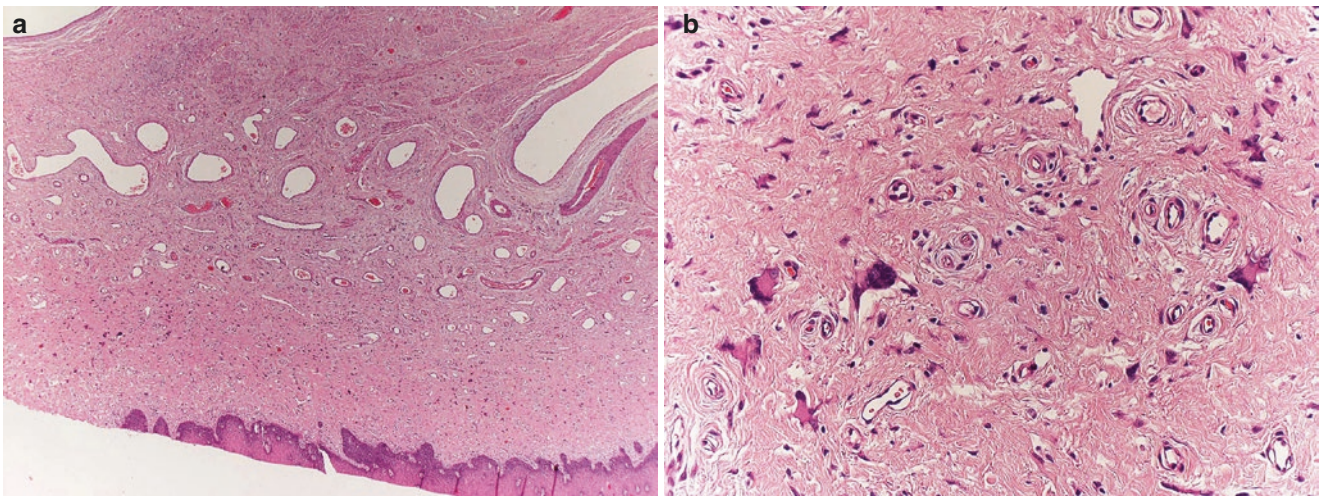

Fig. 1.40 Multinucleated giant cells, isolated or multifocal (**a**), sometimes show enlarged, hyperchromatic nuclei that are bizarre in appearance, with smudged chromatin and containing prominent nucleoli but without mitotic activity (**b**)

1.3 Cervical Adventitia

In the external part of the cervical wall, there is a layer called *adventitia*, made up of loose connective tissue with numerous vessels (Fig. 1.41). This layer extends in the lower part of the cervix to the vaginal fornix; in the upper part, it is continuous with the isthmus and the uterine body, up to the part where the peritoneum is attached, where serosa begins.

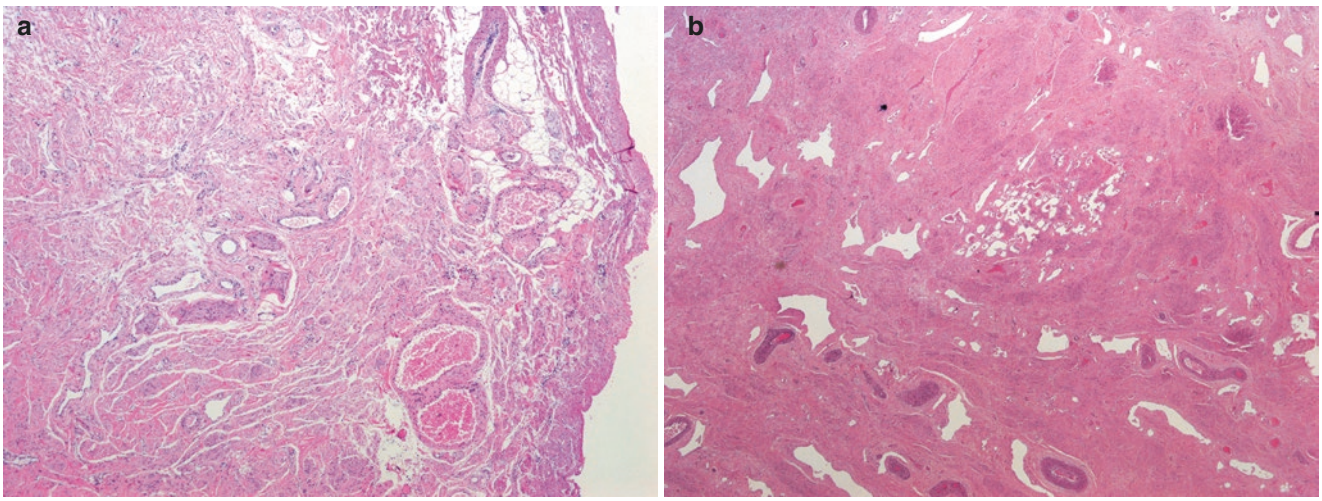

Fig. 1.41 Adventitia: In the external part of the cervical wall, there is a layer made up of loose connective tissue (**a**), with numerous vessels (**b**)

1.4 Cervical Vascularization and Innervation

The lymphatic drainage of the cervix is organized into three beds: one underlying the squamous and endocervical epithelium, one deeper into the stroma, and a third one at the outer surface of the cervix. All these lymphatic vessels connect with pelvic lymph nodes divided into external iliac, internal iliac, and common iliac nodes.

The blood supply of the cervix is provided by the descending branches of the uterine arteries entering the lateral walls along the upper margin of the paracervical ligaments. The innervation is limited to the endocervix and peripheral area of the ectocervix and is provided by nerves from the pelvic autonomic system, including the superior, middle, and inferior hypogastric plexuses.

1.5 Changes of the Cervix During Pregnancy

During pregnancy, the ectropion is obvious, with a proliferation of endocervical epithelium leading to a consequent increase in the surface area of mucus-secreting glandular epithelium. One result is a more papillary appearance of the endocervical canal epithelium, along with the appearance of *endocervical glandular hyperplasia* or *endocervical microglandular hyperplasia* (not to be confused with an identical lesion produced by oral contraceptive medication) (Fig. 1.42a). These glands secrete an increased amount of viscous mucus, which acts as a barrier between the vagina and the uterine cavity. In the endocervical stroma, collagen fibers degenerate and acidic mucopolysaccharides accumulate, causing the cervix to have a soft consistency and allow it to become flattened during pregnancy. At the same time, there is an increase in vascularization and edema accompanied by an acute inflammatory infiltrate (see Fig. 1.42b).

Another change is the *Arias-Stella reaction*, which consists of the focal transformation of the endocervical epithelium into an epithelium containing large cells with clear, vacuolated, abundant cytoplasm and enlarged and hyperchromatic nuclei with indistinct nucleoli (Fig. 1.43). Biotin vacuoles in the form of nuclear pseudoinclusions can also be present. The nuclei can project into the glandular lumen in a hobnail pattern. Typically, there is no mitotic activity, but rare mitoses are occasionally present. This change should not be confused with clear cell carcinoma, especially since the Arias-Stella reaction does not form a tumor mass.

A cervical stromal change that occurs in one third of cervices examined during pregnancy is *stromal pseudodecidualization*, a phenomenon that is mediated by high levels of progesterone during pregnancy; it disappears by 2 months after birth. In cervical pseudodecidualization, the cervical stromal cells are identical to gestational decidual cells of the endometrium: they are large, with abundant pink cytoplasm and well-defined borders. The nuclei are not atypical, which can help differentiation from invasive squamous carcinoma in a scant biopsy. Immunohistochemistry can also assist, as stromal cells with pseudodecidualization are negative for cytokeratins and p16.

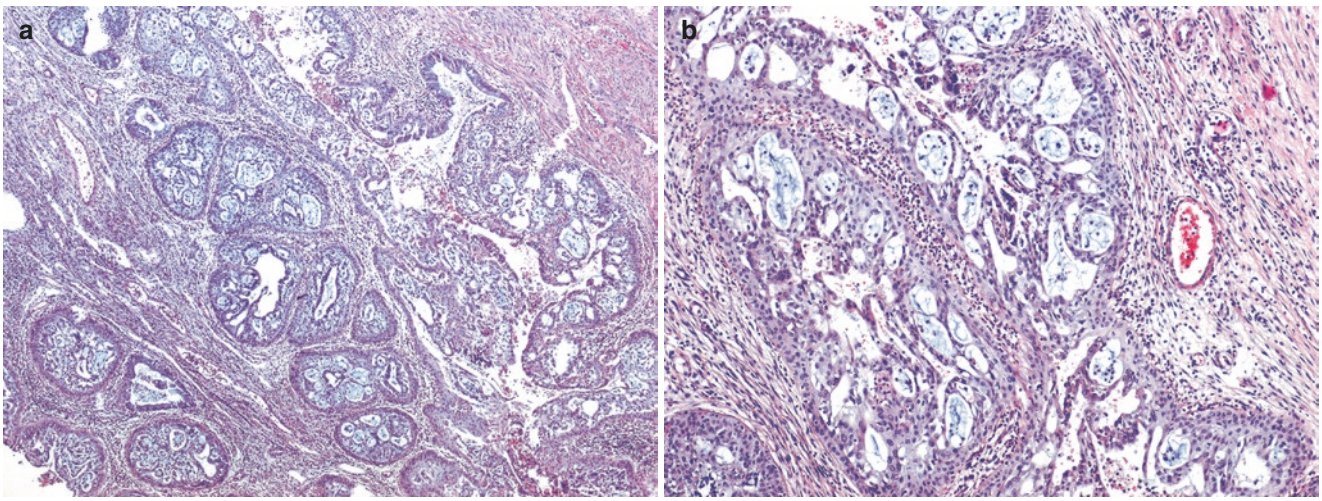

Fig. 1.42 Pregnancy-related changes: Hyperplasia of the endocervical glands, presenting squamous metaplasia (**a**) with increased vascularization and acute inflammatory infiltrate into the stroma (**b**)

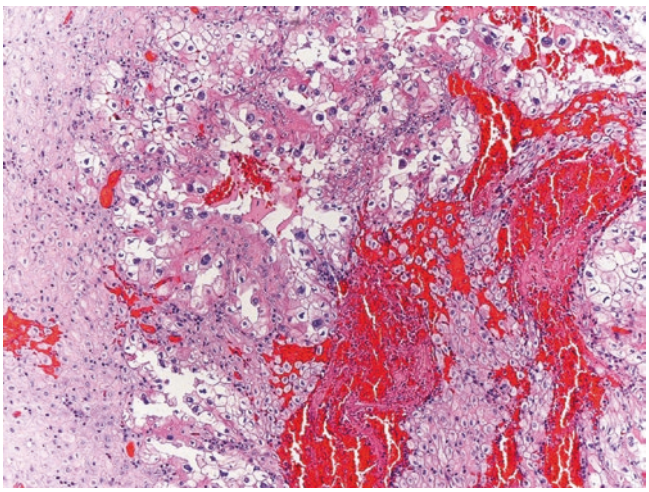

Fig. 1.43 Arias-Stella changes replacing normal epithelium of endocervical glands

1.6 Colposcopy of the Cervix

Colposcopy is the standard of care for evaluation of an abnormal Pap test. Pap screening, with cytologic evaluation and HPV testing, is used for population-based cervical cancer screening. Patients are referred for more specific, diagnostic evaluation by colposcopy for a wide range of abnormal findings on Pap, including abnormal cytologic findings, HPV positive tests, and for inadequate cell sampling. Colposcopy can also be indicated for evaluation of visible or palpable cervical abnormalities on examination. The goal of colposcopy is to identify precancerous lesions or cancer.

Colposcopy involves close examination of the cervix and vagina with various magnifying lenses (colposcope) and with targeted biopsy sampling when indicated. The cervix and upper vagina are inspected grossly and after application of 3–5% acetic acid to identify any areas of whitening (acetowhite changes) under white light and with a green or blue light filter. Lugol's solution (aqueous iodine) can be used as well to identify areas that do not stain brown with application of the solution to the cervix and upper vagina. It is important to inspect and document visibility and findings on the squamocolumnar junction. In 2015, the American Society for Colposcopy and Cervical Pathology (ASCCP) initiated an effort to standardize colposcopy technique and terminology within the United States [55]. Colposcopic evaluation needs to include systematic examination and description of findings. Per ASCCP recommendations [56], this should include:

1. Indication for colposcopy
2. Examination of the vulva, vagina, and cervix grossly
3. Description of cervical and transformation zone visibility
4. Presence and description of acetowhite lesions, vascular changes, non-staining areas with Lugol's solution, other lesions or abnormalities
5. Colposcopic impression of normal/benign versus low-grade lesions versus high-grade lesions versus cancer
6. If biopsies are indicated, biopsies should be taken at the squamocolumnar junction, with documentation of location
7. Document whether endocervical sampling, with curettage or brush, was performed.

Beyond the absence of abnormal findings, normal colposcopic findings include visualization of smooth squamous epithelium, either with a premenopausal, well-vascularized appearance or with a postmenopausal, atrophic, and pale appearance. Benign lesions such as cervical ectropion, Nabothian cysts, and gland openings can be identified and are not concerning.

Colposcopy is a cornerstone of global cervical cancer screening and prevention, and the importance of performing and documenting colposcopy in a standard, systematic manner cannot be overstated.

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