

## Female Reproductive System

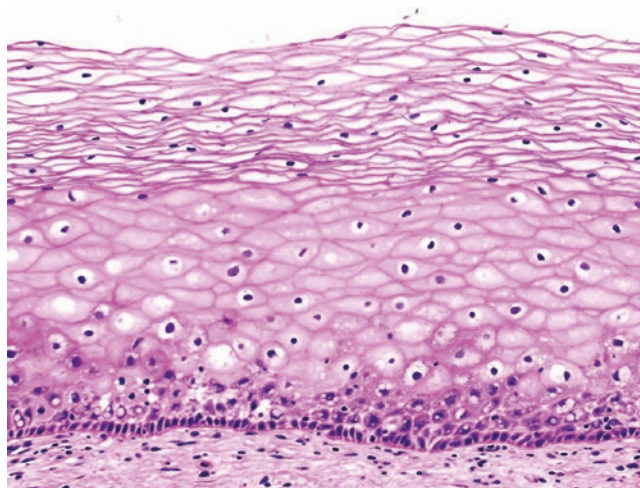
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### Ectocervix and Vagina

The Papanicolaou (Pap) test is directed primarily at obtaining exfoliated cells from the ectocervix and endocervical canal, including the squamocolumnar junction. Squamous cells from the ectocervix and adjacent vaginal canal account for the majority of cellular elements in most Pap test samples. Benign squamous cells are typically large, polygonal cells with abundant cytoplasm and centrally located nuclei (Fig. 9.1). On Papanicolaou (Pap)-stained liquid-based cytology (LBC) preparations, squamous cells may be present as single cells or in clusters. The cytoplasm of squamous cells ranges from green-blue to pink-orange [1, 2].

The appearance of squamous cells varies depending on which layer of the ectocervical lining they are sampled from and the degree of maturation [1]. The three cell types typically seen on a Pap test, in ascending order of maturation, are parabasal, intermediate (midzone), and superficial squamous cells. The maturation index (MI), although no longer used to assess the hormonal status of women, refers to the proportion (%) of parabasal:intermediate:superficial cells. Most reproductive-aged women have a predominance of superficial and intermediate cells on Pap test sampling, with superficial cells predominating during the first half (follicular phase, estrogen dominant) of the menstrual cycle and intermediate cells predominating during the second half (luteal phase, progesterone dominant) (Fig. 9.2a). Postmenopausal women and those with squamous cell atrophy have a predominance of parabasal and intermediate cells (Fig. 9.2b).

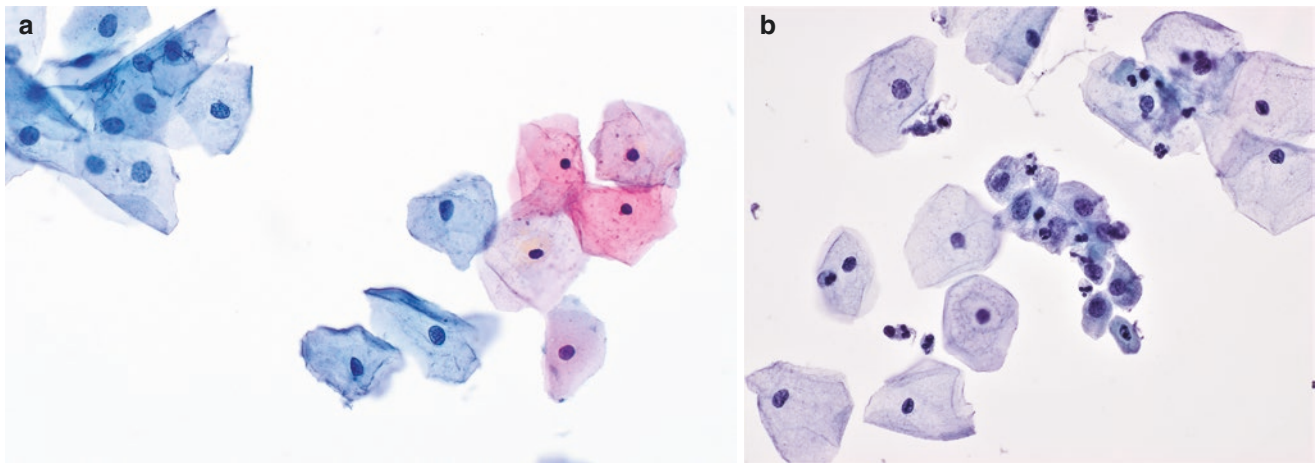


**Fig. 9.1** Normal squamous epithelium of the ectocervix. The outer surface superficial squamous cells have abundant glycogenated cytoplasm and small pyknotic nuclei, while the middle intermediate cells have larger, more pale nuclei and less intracytoplasmic glycogen. A uniform, single layer of basal-type squamous cells is present at the base. Cervical biopsy, hematoxylin & eosin, 40× magnification

Superficial cells are the largest type of squamous cell (50  $\mu\text{m}$  in diameter), located at the mucosal surface of the squamous epithelium. Their nuclei are very small (5  $\mu\text{m}$ ) and pyknotic. The cytoplasm is usually delicate and tends to be pink-orange due to intracytoplasmic keratin accumulation. Intermediate cells, just deep to the superficial cells in the squamous lining, are slightly smaller (45  $\mu\text{m}$  in diameter) with slightly larger nuclei (7–8  $\mu\text{m}$ ). The chromatin of intermediate cells is more pale than superficial cells. Like superficial cells, intermediate cells tend to be polygonal in shape but have less cytoplasm, which more commonly appears green on Pap stain. Parabasal cells are the smallest (20–30  $\mu\text{m}$  in diameter) and most deeply located of the commonly encountered squamous cells seen on a Pap test. They are typically round in shape and have small centrally located nuclei that are slightly larger than intermediate cell nuclei. Their cytoplasm is dense, often appearing similar to meta-

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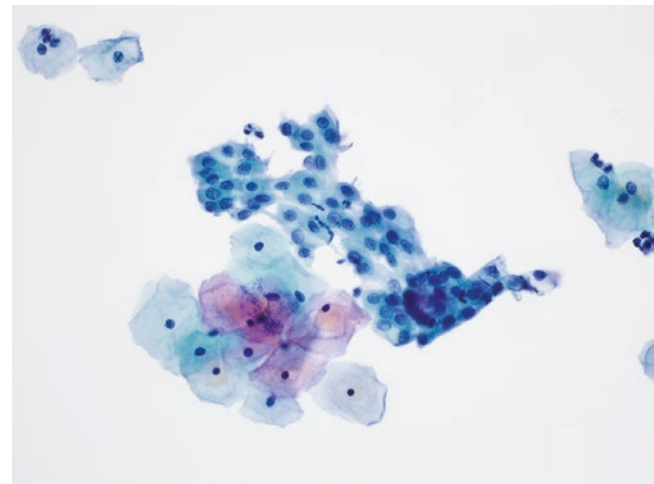
**Fig. 9.2** (a) Superficial and intermediate cells predominate in this Pap test from a woman of reproductive age. The intermediate cells have slightly larger, paler nuclei (left) than the pyknotic nuclei of the superficial cells (right). (b) Parabasal cells, shown in the center of this image,

are less than half the diameter of adjacent intermediate cells, and also have slightly larger nuclei and denser cytoplasm. Cervical Pap, ThinPrep preparation, Papanicolaou stain, 40× magnification (a, b)

plastic squamous cells. Basal (reserve) cells are uncommonly encountered in the Pap test. They are approximately 10  $\mu\text{M}$  in diameter, with very scant but dense cytoplasm, and contain central nuclei similar to those seen in parabasal cells. Reserve cells are more likely to be encountered with reserve cell hyperplasia related to microglandular hyperplasia.

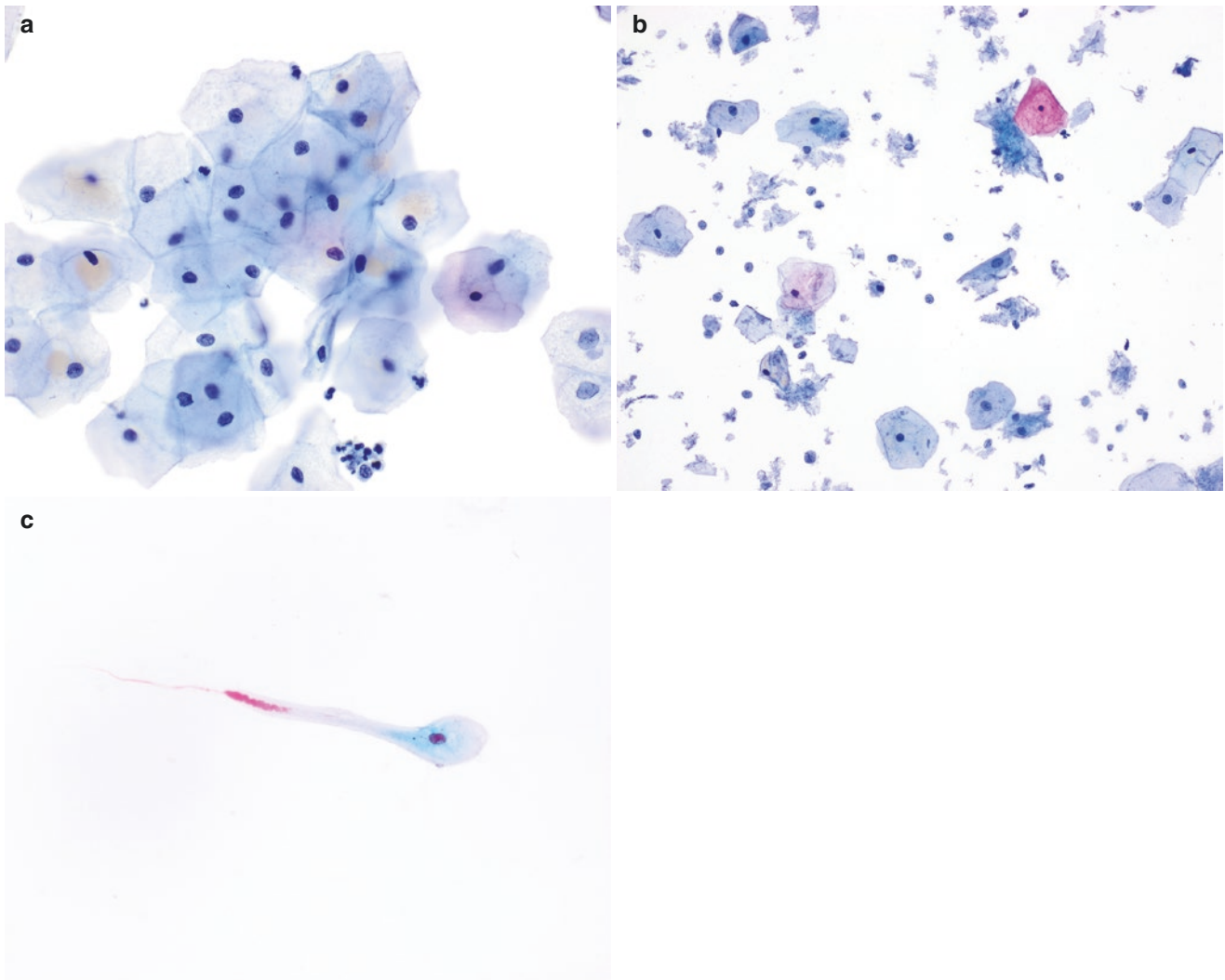
Squamous metaplastic cells are immature epithelial cells present at the squamocolumnar junction (transformation zone). They are typically present in cohesive sheets with interlocking cells that have distinctive cell borders [1, 3]. Approximately the size of parabasal cells and containing similarly dense cytoplasm, squamous metaplastic cells are difficult to distinguish from parabasal cells in practice when present singly or in small groups (Fig. 9.3). Like other benign squamous cell types, the nuclei of these metaplastic cells are smooth bordered with evenly distributed chromatin. Immature squamous metaplasia is marked by groups of small, nonkeratinizing metaplastic squamous cells with relatively scant cytoplasm which can impart a hyperchromatic appearance that resembles a high-grade squamous intraepithelial lesion (HSIL) at low to mid scanning magnification. However, nuclear overlap is minimal and the nuclei are not enlarged or irregular.

Squamous cells may show a variety of benign, physiological changes that are important to recognize, as these morphologic changes can overlap with those seen in cervical dysplasia. Navicular cells are intermediate cells seen normally during increased endogenous or exogenous progesterone exposure, such as during the luteal phase of the menstrual cycle, pregnancy, and with progesterone-based birth control [4]. They are so named because of their “boat-shaped” appearance, with a thick outer rim of dark cytoplasm and an inner portion of yellowish-brown cytoplasm



**Fig. 9.3** Squamous metaplastic cells are shown present in a cohesive streaming sheet. Nuclei are approximately the size of intermediate cell nuclei, with euchromatin and smooth nuclear borders. Cervical Pap, ThinPrep preparation, 40× magnification

that is rich in glycogen. As a result, navicular cells can take on a pseudo-koilocyte appearance. However, the nuclei remain typical of benign intermediate cells, and the yellowish appearance of the central cytoplasm is distinct from the achromic clearing seen in koilocytes. The background in navicular cell-rich Pap tests will typically show naked intermediate cell nuclei, due to cytolysis, and increased lactobacilli (Fig. 9.4a, b). The naked nuclei may resemble inflammatory cells and sometimes can mimic *Trichomonas vaginalis* parasitic organisms. Occasionally, keratinizing squamous cells can contain Herxheimer spirals, which are twisted or coiled keratin fibrils (tonofilaments) seen within



**Fig. 9.4** (a) Navicular cells are glycogenated intermediate squamous cells. Glycogen imparts a clear to yellow appearance to the cytoplasm around nuclei, and should not be mistaken for koilocytes. (b) Cytolysis occurs when lactobacilli lyse the cytoplasm of navicular cells. Naked

nuclei are abundant, and lactobacilli are seen on the surface of adjacent intact squamous cells. (c) Squamous cell with Herxheimer spiral, a coil-shaped intracytoplasmic keratin fibril. Cervical Pap, ThinPrep preparation, 20× (b) and 40× magnification (a, c)

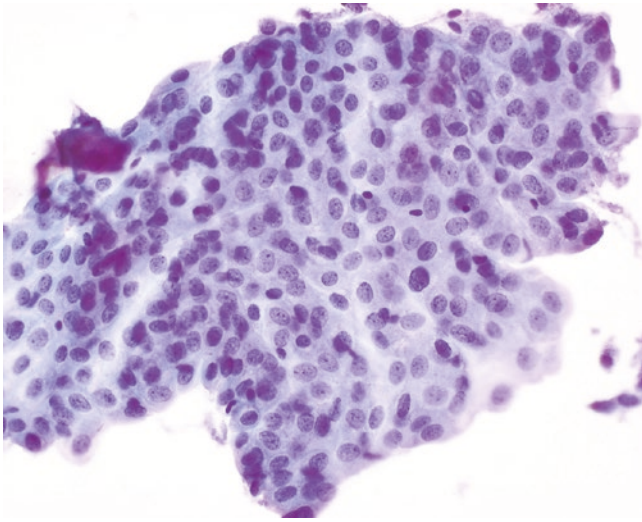
the cytoplasm of spindle-shaped squamous cells (Fig. 9.4c). These spirals may be associated with benign metaplastic cells or associated with radiation-induced change, but can also be encountered in tadpole-shaped squamous cells in keratinizing dysplasia or squamous cell carcinoma [5]. The presence of these spirals can help differentiate spindle-shaped squamous cells from smooth muscle cells or fibroblasts.

Squamous cell atrophy can be a physiological or pathological process, ultimately related to a decreased estrogenic state. Atrophic smears are typical in postmenopausal patients and in the postpartum period. In Pap tests demonstrating atrophy, parabasal cells predominate, but basal cells may also be present and should not be mistaken for HSIL [6, 7]. Though parabasal and basal cells have high nuclear to cytoplasmic ratios, their nuclei are small (10  $\mu\text{M}$ ) with evenly

distributed chromatin (Fig. 9.5). Pseudo-keratosis, marked by polygonal squamous cells with dense cytoplasm and small degenerated central nuclei, is commonly identified in both atrophic smears and other relatively high progesterone states (e.g., post-pregnancy).

Parakeratosis and hyperkeratosis are reactive, protective conditions that may be seen in both benign and malignant processes [1, 2]. Thickening (plaques) of the squamous epithelium occurs as a result of chronic irritation (e.g., uterine prolapse), and consequentially keratotic squamous cells from the mucosal surface can be sampled during a Pap test. On Pap tests, these keratinized cells are typically intensely orangeophilic. Two forms of keratosis exist: hyperkeratosis and parakeratosis. Hyperkeratosis is marked by plaques of anucleate superficial squamous cells (Fig. 9.6a). Parakeratosis is marked by keratinizing squamous cells that have dense

cytoplasm and small, pyknotic nuclei (Fig. 9.6b). Often, parakeratotic cells take on a spindled, elongated shape in comparison to the typical polygonal shape of squamous cells. In most cases, parakeratosis and hyperkeratosis are associated with benign and reactive processes, but careful examination is necessary when keratoses are present, as such protective processes may also result from dysplastic and malignant conditions. Contaminating squamous cells from the vulva or skin can mimic keratosis. Pseudo-keratosis can be seen with high progesterone states (e.g., postpartum) and



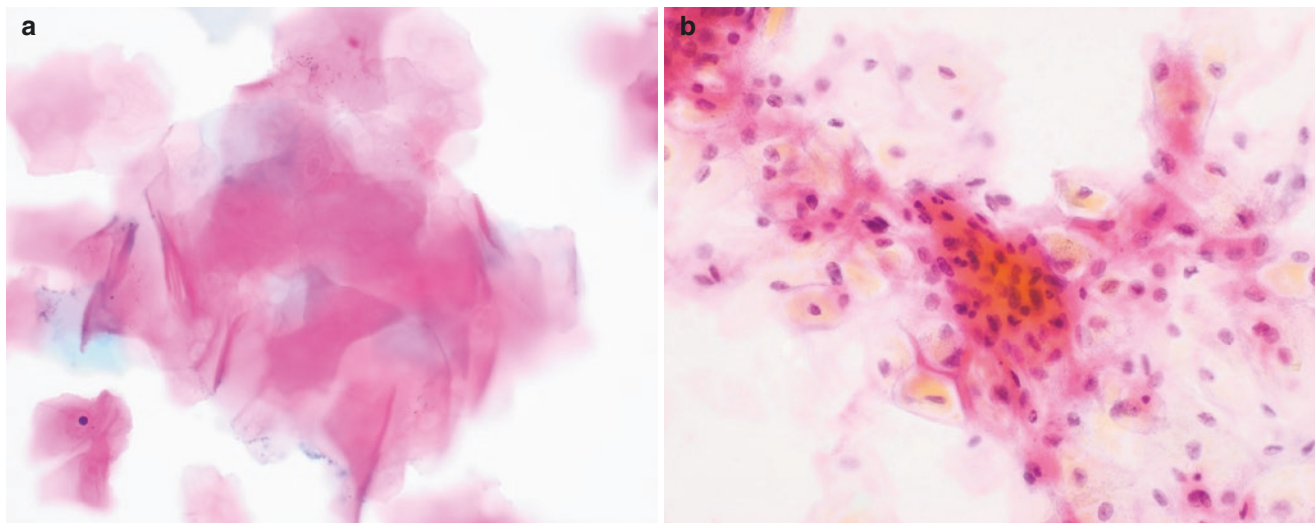
**Fig. 9.5** Tightly cohesive group of parabasal-type squamous cells in a patient with atrophy. Cervical Pap, ThinPrep preparation, 40× magnification

atrophy and is characterized by orangeophilic degenerated glandular cells with pyknotic nuclei.

Transitional cell metaplasia is less common than squamous metaplasia, but it is not infrequently encountered on Pap tests. Like atrophy, it may be encountered in postmenopausal patients. It is also commonly seen in patients receiving exogenous testosterone therapy (e.g. female-to-male transgender patients) and in patients who have received partial agonist estrogen therapy (e.g., Tamoxifen) [8]. Transitional cell metaplasia exhibits cohesive sheets of squamous cells resembling squamous metaplastic cells (Fig. 9.7a). Nuclei from transitional cell metaplastic cells have smooth borders, fine chromatin, and a distinctive longitudinal nuclear groove that helps distinguish these cells from squamous metaplasia. Small parabasal-type cells (“small cell change”) are often present in conjunction with transitional cell metaplasia (Fig. 9.7b).

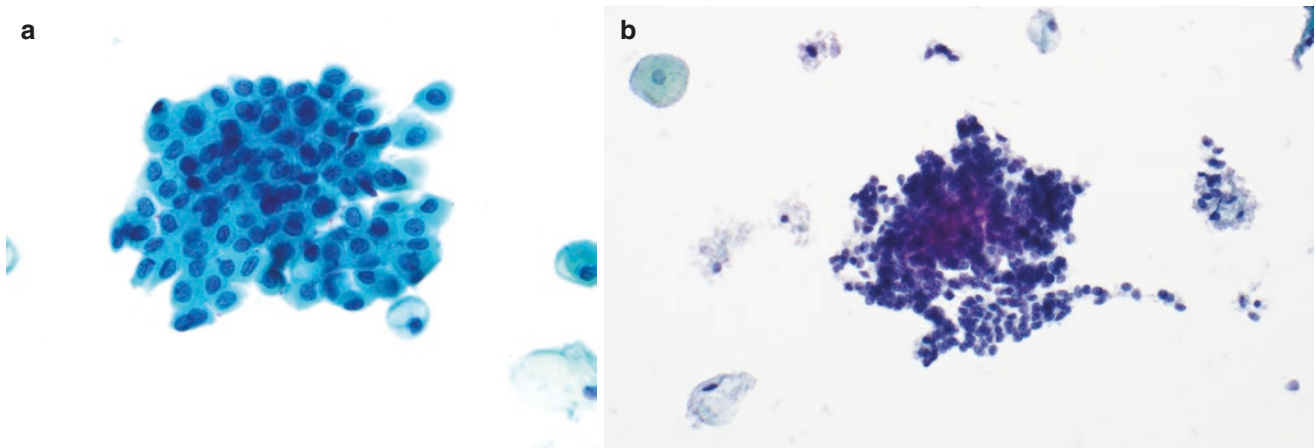
Reactive/reparative change in squamous cells is important to recognize, as they represent benign changes that morphologically overlap with invasive carcinoma. In fact, under regulations from the Clinical Laboratory Improvement Amendments (CLIA) of 1988 in the United States, all Pap tests screened as negative with reactive or reparative changes must be reviewed by a pathologist due to this morphologic overlap. However, reparative changes are not premalignant. They occur in association with typical causes of inflammation and irritation, such as infections, physical or chemical irritation, and intrauterine devices, although in many cases, an etiology is not clear.

Reactive and reparative changes are characterized by cohesive sheets of nonoverlapping squamous cells that may have a streaming appearance [6, 7] (Fig. 9.8). Nuclear enlargement is



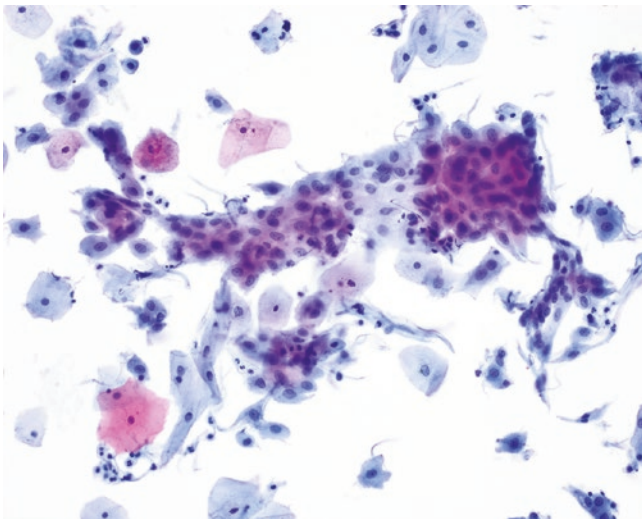
**Fig. 9.6** (a) Hyperkeratosis in this Pap test appears as anucleated superficial squamous cells, often in association with occasional nucleated forms (bottom left). (b) Parakeratosis is marked by squamous cells with

dense, keratin-rich cytoplasm and dark, elongated nuclei. Cervical Pap, ThinPrep preparation, 50× magnification (a). Cervical Pap, alcohol-fixed smear preparation, Papanicolaou stain, 50× magnification (b)



**Fig. 9.7** (a) Transitional cell metaplasia, present as cohesive group resembling squamous metaplastic epithelium. Nuclei are round to elongated with longitudinal grooves. (b) Small cell change is shown in a patient receiving Tamoxifen. These basal-like epithelial cells have

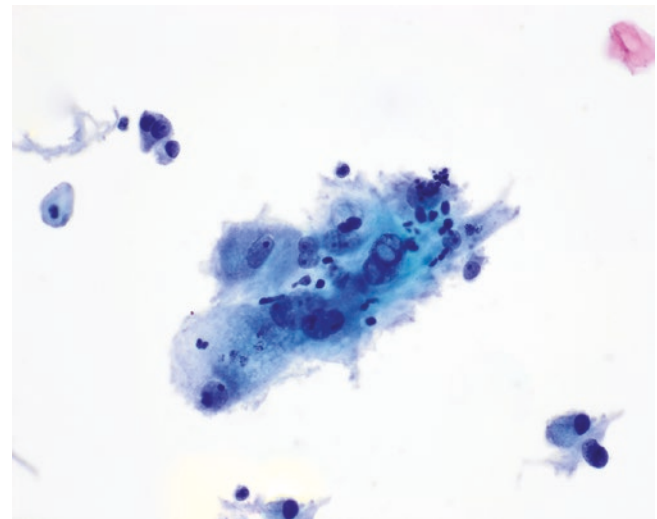
nuclei that occupy nearly the entire cell diameter. However, nuclear size has not increased and chromatin is bland. Cervical Pap, SurePath preparation, 50× magnification (a). ThinPrep preparation, 50× magnification (b)



**Fig. 9.8** Reactive changes in squamous cells include mild nuclear enlargement and central nucleoli. Note that the nuclear membranes are smooth, and chromatin is pale and open. Cervical Pap, ThinPrep preparation, 40× magnification

often present, but nuclear membranes are typically smooth and chromatin is euchromatic to mildly hyperchromatic. Multinucleation may be seen, but no koilocytic changes are present. The cytoplasm of reactive squamous cells can range from dense and metaplastic to larger more mature forms, the latter of which may display polychromatic cytoplasm that imparts a “moth-eaten” appearance. Perinuclear halos, a thin rim of cytoplasmic clearing around the nucleus, can be seen. In contrast to koilocytosis, the cleared area is smaller and nuclear changes of dysplasia are absent.

Reactive and reparative changes can show different backgrounds and cytologic appearances depending on the under-

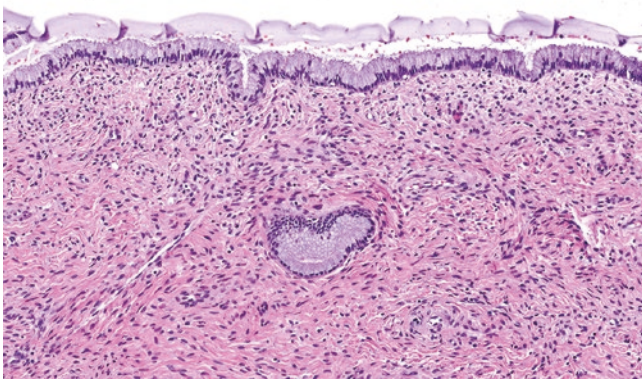


**Fig. 9.9** Radiation change in squamous cells, exhibiting increased nuclear size but low nuclear to cytoplasmic ratios. Characteristic smudgy chromatin is notable. Cervical Pap, ThinPrep preparation, 60× magnification

lying cause [1, 6, 7]. Bacterial vaginosis, trichomonas infection, and herpes virus infection are associated with polymorphous leukocytic (acute) inflammation. A lymphocytic background, with polymorphous lymphocytes and tingible body macrophages (follicular cervicitis), can be seen in chlamydial infections and other reactive inflammatory conditions. Radiation change includes markedly increased cell size with preserved nuclear to cytoplasmic ratios, anisonucleosis, multinucleation, and degenerated “smudgy” chromatin (Fig. 9.9). Nucleoli can be prominent and multiple. Cytoplasm is abundant, frequently vacuolated, and polychromatic.

## Endocervix

The surface epithelium of the endocervix is composed of glandular cells with columnar morphology, which are approximately twice as tall as wide (Fig. 9.10) [1, 7]. When present in groups, depending on the direction of their long axis, they display either honeycomb (en face) or picket-fence arrangements (long axis visible). En face endocervical cells show evenly spaced nuclei (Fig. 9.11a). The nuclei, which are slightly larger than intermediate squamous cell nuclei, are basally located, which becomes apparent in picket-fence arrangements (Fig. 9.11b). Like benign squamous cells, endocervical cells have even chromatin distribution and smooth, round nuclear borders. Multinucleated endocervical



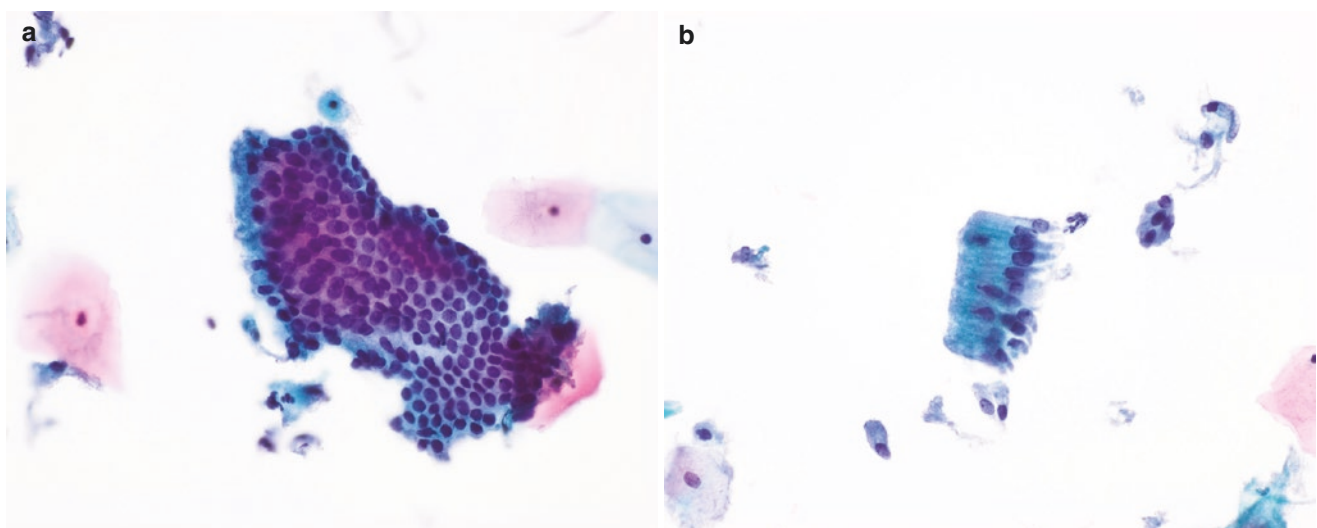
**Fig. 9.10** Normal endocervical mucosa composed of a single layer of surface columnar epithelium. Nuclei are basally located and uniform. An endocervical gland is also present in the superficial cervical stroma. Cervical biopsy, hematoxylin & eosin, 20× magnification

cells are sometimes seen. Nucleoli are more commonly visible than in squamous cells. The cytoplasm is better appreciated in picket-fence arranged cells, where moderately abundant granular or vacuolated apical cytoplasm is appreciated. The cytoplasm appears less abundant in honeycomb cell arrangements.

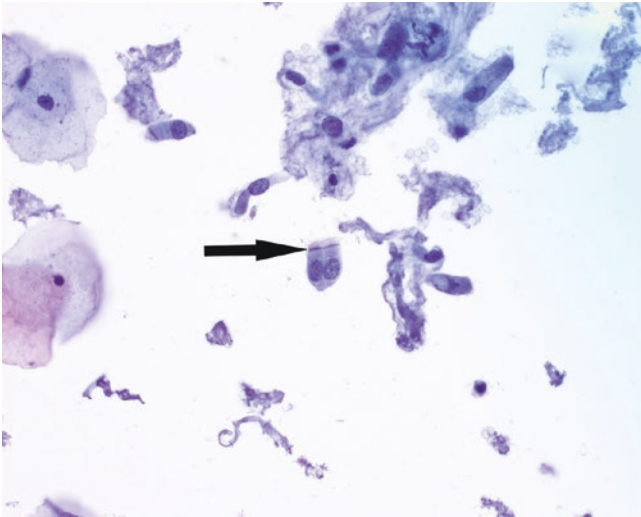
Tubal metaplasia is a common finding in the Pap test, with endocervical cells undergoing metaplastic change to ciliated epithelium [9]. The apical surface of the cells contains terminal bars with attached cilia (Fig. 9.12). The basally located nuclei may be slightly enlarged with prominent nucleoli, which can be mistaken for atypical endocervical cells on a Pap test when cilia and terminal bars are obscured from view.

Reactive change is also commonly encountered in endocervical glandular cells. As with squamous cells, they may be associated with a variety of inflammatory or directly irritating factors. Streaming of reactive endocervical cells can give a whorled appearance to the endocervical cell groups. Significant nuclear enlargement of up to twice the size of nonreactive endocervical epithelium is common, and nucleoli can become more prominent. However, nuclear membranes remain smooth and chromatin is evenly distributed (Fig. 9.13). Of note, mitoses can be encountered in benign reparative change. As with squamous cells, the background may include various inflammatory elements [6, 7].

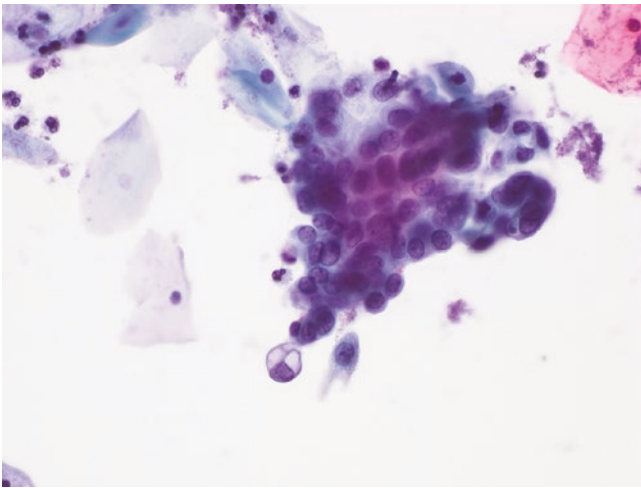
Cytoplasmic vacuolization can be encountered in reactive endocervical cells, particularly when associated with an intrauterine device. Such vacuoles may be quite large, occupying most of the visible cytoplasm, in some cases displacing the nucleus and imparting a signet ring appearance. Endocervical polyps can show reactive and degenerative changes in endocervical cells, including reactive nuclear



**Fig. 9.11** (a) Endocervical cells shown in honeycomb arrangement and (b) “picket-fence” arrangement, with even nuclear size and shape throughout. Cervical Pap, ThinPrep preparation, 40× magnification (a, b)



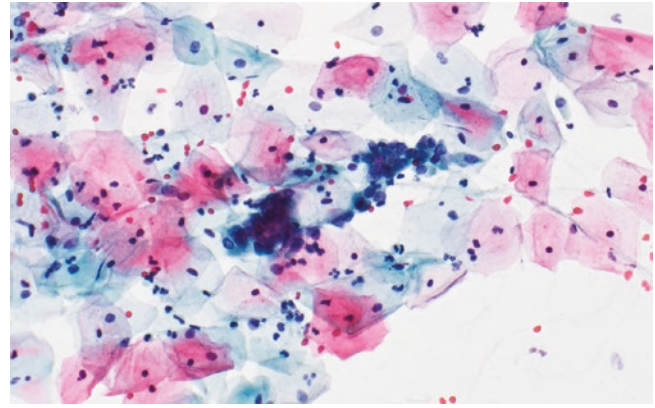
**Fig. 9.12** Tubal metaplasia in endocervical cells is shown in the center of the image, with a visible apical terminal bar and attached cilia (arrow). Cervical Pap, ThinPrep preparation, 40× magnification



**Fig. 9.13** Endocervical cells with reactive change in a patient with an intrauterine device. Mild nuclear enlargement and nucleoli are visible. Note the single vacuolated cell. Cervical Pap, ThinPrep preparation, 50× magnification

change and cytoplasmic vacuolization which can resemble signet ring cells.

Although glandular cells seen on a Pap test are primarily of endocervical origin, benign glandular cells resembling endocervical epithelium can occasionally be seen in post-hysterectomy patients (Fig. 9.14). These may represent vaginal adenosis or glandular rests in vaginal mucosa [1].



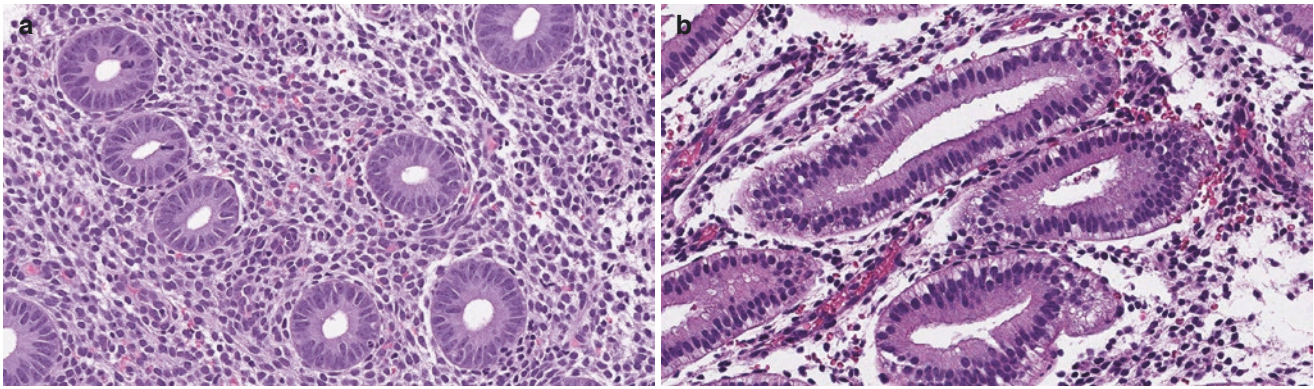
**Fig. 9.14** Vaginal adenosis in a patient status-post-hysterectomy. Benign glandular cells in the center of this image, resembling endocervical glands, can be encountered in vaginal Pap tests. Vaginal Pap, ThinPrep preparation, 40× magnification

## Endometrium

Normal endometrial glands are present in the proliferative phase during the first half of the menstrual cycle and the secretory phase in the second half (Fig. 9.15a, b). Proliferative-phase endometrium is more commonly encountered on Pap tests. Endometrial glandular cells are identified in 2–3% of Pap test cytology specimens, and they may be acquired either as a result of normal shedding during the menstrual cycle or direct sampling of the lower uterine segment [10]. Diagnostic endometrial cytology sampling is also occasionally performed [11].

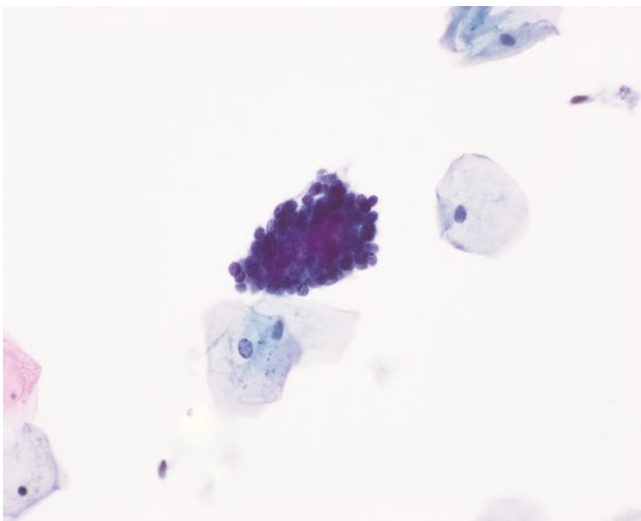
Endometrial cells are most commonly encountered in reproductive-aged women during the first half of the menstrual cycle. Endometrial sampling during days 1–5 of the menstrual cycle typically reveals small clusters of endometrial glandular cells in a background of blood and cellular debris. Later in the cycle, on days 6–10, the Pap test commonly shows an “exodus” pattern, with cellular clusters composed of a central portion of endometrial stromal cells surrounded by benign endometrial glandular cells. Cytoplasmic vacuolization may be seen in endometrial glandular cells during exodus, as well as histiocytic inflammation.

Exfoliated endometrial cells are usually present in packed, three-dimensional clusters with scant cytoplasm (Fig. 9.16). Normal endometrial glandular cells are very small and are about a third the size of endocervical cells and comparable to basal squamous cells [10]. Their nucleus occupies nearly the entire cytoplasmic diameter. However, these nuclei are small, approximately the size of an intermediate squamous cell



**Fig. 9.15** Normal endometrial glands in (a) proliferative phase have a tubular appearance with frequent mitotic figures while (b) secretory glands have more abundant cytoplasm with secretory vacuoles, and

mitoses are absent. Endometrial biopsy, hematoxylin & eosin, 40× magnification (a, b)



**Fig. 9.16** Exfoliated endometrial cells. Small clusters of endometrial glandular cells are shown. These cells are tightly cohesive, with very scant cytoplasm and nuclei that are of the size of adjacent intermediate squamous cells. Cervical Pap, ThinPrep preparation, 40× magnification

nucleus (7–8  $\mu$ M). Single cell necrosis (karyorrhexis) is often evident, but mitoses are absent. Endometrial stromal cells are less commonly encountered on a Pap test, but have a similar appearance to glandular cells and are present in tightly cohesive clusters. The nuclear density of benign endometrial stroma is quite high, and the nuclei are often compressed and somewhat elongated or spindled. When present, endometrial stroma is often surrounded by endometrial glandular tissue. Endometrial stromal cells can resemble histiocytes and may also mimic the appearance of HSIL.

Although endometrial cells are considered an unremarkable Pap test finding in younger women during the first half

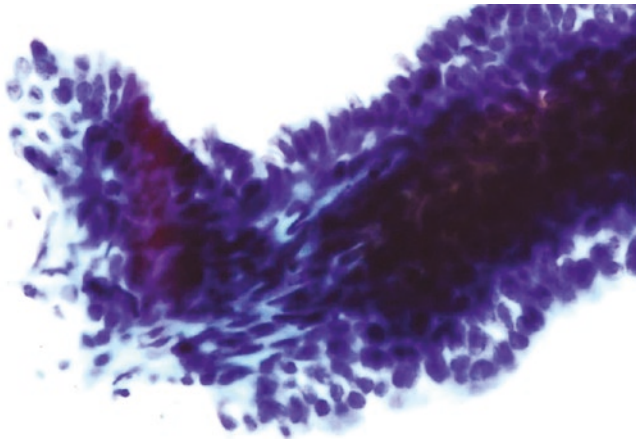
of the menstrual cycle, the presence of endometrial cells in women 45 years of age or older should be reported according to the Bethesda System (third edition) guidelines. This is due to the presumed increased risk of endometrial hyperplasia and endometrial carcinoma when endometrial cells are identified in perimenopausal and postmenopausal patients, even in the absence of cellular atypia.

Direct sampling of the endometrium occurs when high sampling of the endocervical canal results in abrasion of the endometrium in the lower uterine segment (LUS). It occurs most frequently in Pap tests obtained from patients who have had prior excisional cervical procedures. In contrast to exfoliated endometrial cells, directly sampled endometrium has no increased association with endometrial hyperplasia or neoplasia on a Pap test, and is not considered a reportable finding [12].

Whereas shed endometrial cells are often present in small, three-dimensional groups, directly sampled endometrial fragments are larger and two dimensional (Fig. 9.17). The fragments are tubular shaped and may be branched [12]. In the proliferative phase, directly sampled endometrium shows endometrial glandular cells that are tightly packed with scant cytoplasm and indistinct cell borders. Nucleoli are inconspicuous, and mitoses may be encountered. Endometrial stromal cells can sometimes be seen at the edges of cell groups. Secretory endometrium appears similar to proliferative endometrium, but the cytoplasm of endometrial cells is often more abundant and vacuolated, and mitoses are not identified. Atrophic endometrium appears similar to proliferative endometrium, although decreased nuclear density may be encountered, and mitoses are absent.

Pregnancy/progesterone-related changes seen in endometrial glandular and stromal cells can occasionally appear in Pap test cytology [13]. Prominent decidualization of endo-

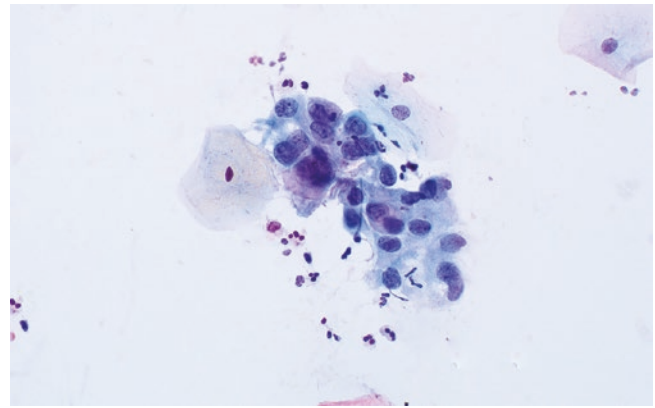




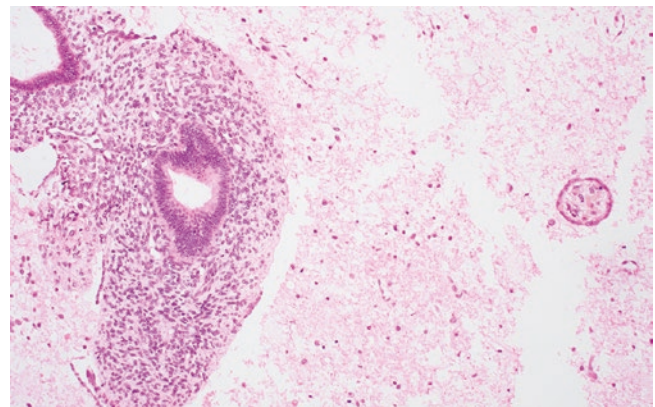
**Fig. 9.17** Branching and folding sheets of directly sampled lower uterine segment endometrium are shown. Cervical Pap, SurePath preparation, 40× magnification

metrial and cervical stroma occurs late in pregnancy and in the postpartum period. Like endometrial cells, the stromal cells have abundant granular cytoplasm, well-defined and distinct cell borders, and a central, round nucleus with a prominent nucleolus. They can appear singly or in clusters in a Pap test. Arias-Stella reaction is a prominent glandular change that may occur in endometrial and endocervical mucosa associated with pregnancy. Cytologically, this resembles clear cell carcinoma, so clinical history and recognition of this change is important. Glandular cells with Arias-Stella reaction show severe nuclear atypia, marked enlargement, hyperchromasia, and vesicular chromatin, with vacuolated clear to eosinophilic cytoplasm (Fig. 9.18). Syncytiotrophoblasts, the multinucleated form of placental trophoblastic cells, are occasionally encountered. Their cytoplasm is dense to coarsely granular, and often there is a “tail” of cytoplasm at one end. The nuclei are bland with distinct nucleoli and can be difficult to distinguish from histiocytic nuclei.

Endometriosis is defined by the presence of ectopic endometrial tissue, and it is occasionally encountered in a Pap test and in pelvic washing cytology [14]. It is classically diagnosed by the presence of at least two of the following: endometrial glands, endometrial stroma, and hemosiderin-laden macrophages. It can occur anywhere in the gynecologic tract, including the cervix. Endometriosis can also form mass lesions which may be targeted by fine needle aspiration (FNA) biopsy. Endometrial glands and hemosiderin-laden macrophages tend to predominate in FNA preparations, although intact endometrial glandular and stromal tissue is sometimes seen (Fig. 9.19).



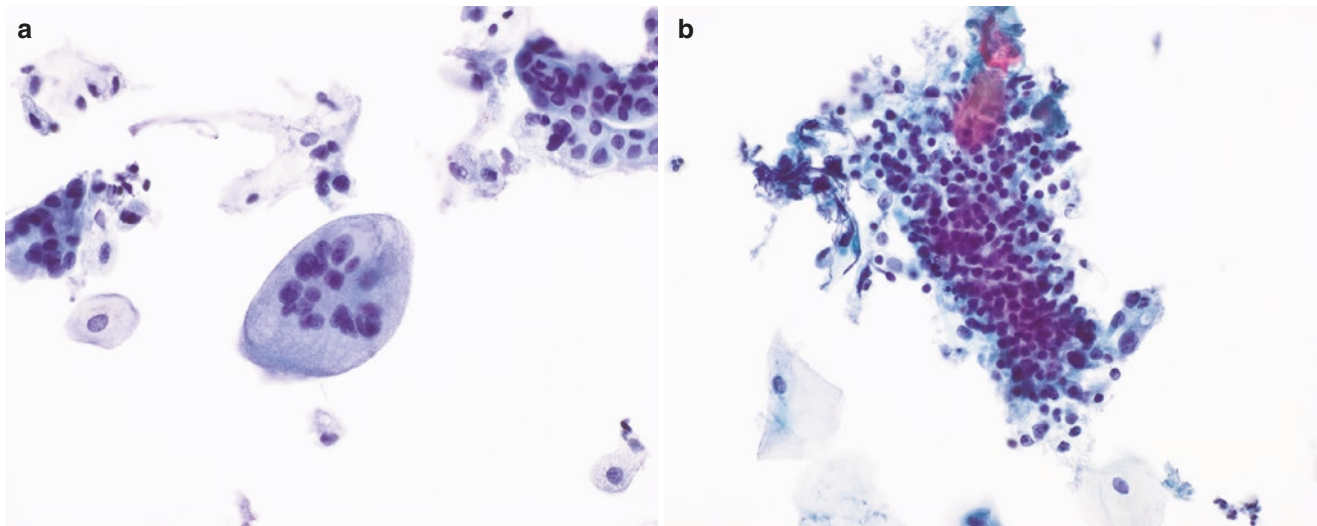
**Fig. 9.18** Glandular cells with Arias-Stella reaction in a peripartum patient. Note the marked nuclear enlargement and chromatin irregularity seen in this physiological, pregnancy-related change. Cervical Pap, ThinPrep preparation, 50× magnification



**Fig. 9.19** Intact fragments of endometrial-type glands and stroma in a patient with endometriosis, with epithelium and associated hemosiderin-laden macrophages noted on the right side. Pelvic washing, cell block, hematoxylin & eosin, 40× magnification

## Inflammatory Elements

Pap tests may contain histiocytes that appear singly and in loose aggregates of cells with moderately abundant cytoplasm, which can range from being foamy, granular to dense [1]. These macrophages may contain hemosiderin, as well as other cellular and acellular debris. The shape of their nuclei ranges from bean-shaped to round, and their chromatin is characteristically bland with a finely granular appearance. Histiocytes are commonly encountered in Pap tests during exodus (days 6–10) and are considered a normal finding during the perimenstrual period. They are also commonly encountered in patients with prior cervical procedures, and



**Fig. 9.20** (a) Multinucleated giant cell is shown in a patient who has undergone prior instrumentation. (b) Follicular cervicitis is shown marked by the presence of many polymorphous lymphocytes. Cervical Pap, ThinPrep preparation, 40× (a) and 50× (b) magnification

when a foreign body (e.g., intrauterine device) is present. In postmenopausal patients, foamy histiocytes or hemosiderin-laden histiocytes may be associated with endometrial carcinoma, and in this clinical setting should thus prompt careful examination for associated endometrial cells or other cellular glandular abnormalities.

Multinucleated giant cell forms of histiocytes can be seen in patients with prior pelvic surgery or radiation history, and may also be encountered in the postpartum period and in postmenopausal patients. Multinucleated giant cells have cytoplasm similar to that of associated mononuclear histiocytes, and their nuclei are likewise bland and uniform (Fig. 9.20a).

Polymorphonuclear leukocytes (neutrophils) and lymphocytes are the most commonly encountered inflammatory cells seen in Pap tests [1, 15]. Neutrophils are slightly larger (~10  $\mu\text{m}$  in diameter), with multilobed nuclei and scant pale cytoplasm. They are the predominant infiltrate of acute inflammation and can be associated with many benign and malignant conditions, even obscuring underlying abnormal cells. In many cases, no obvious cause for acute inflammation is present, although bacterial vaginosis and atrophic vaginitis are common etiologies. Lymphocytes are slightly smaller (around 8  $\mu\text{m}$  in diameter) and have unilobed, round nuclei that occupy the bulk of the cell diameter. A thin rim of dense cytoplasm is present. In cervical samples, small lymphocytes tend to predominate, with pyknotic nuclei. However, in follicular cervicitis, when germinal centers form within cervical epithelium, polymorphous lymphocytosis is seen (Fig. 9.20b). Small lymphoid cells are intermixed with larger, more immature cells that can display more vesicular chromatin. In such cases, tingible body macrophages can also be evident. Although classically associated with

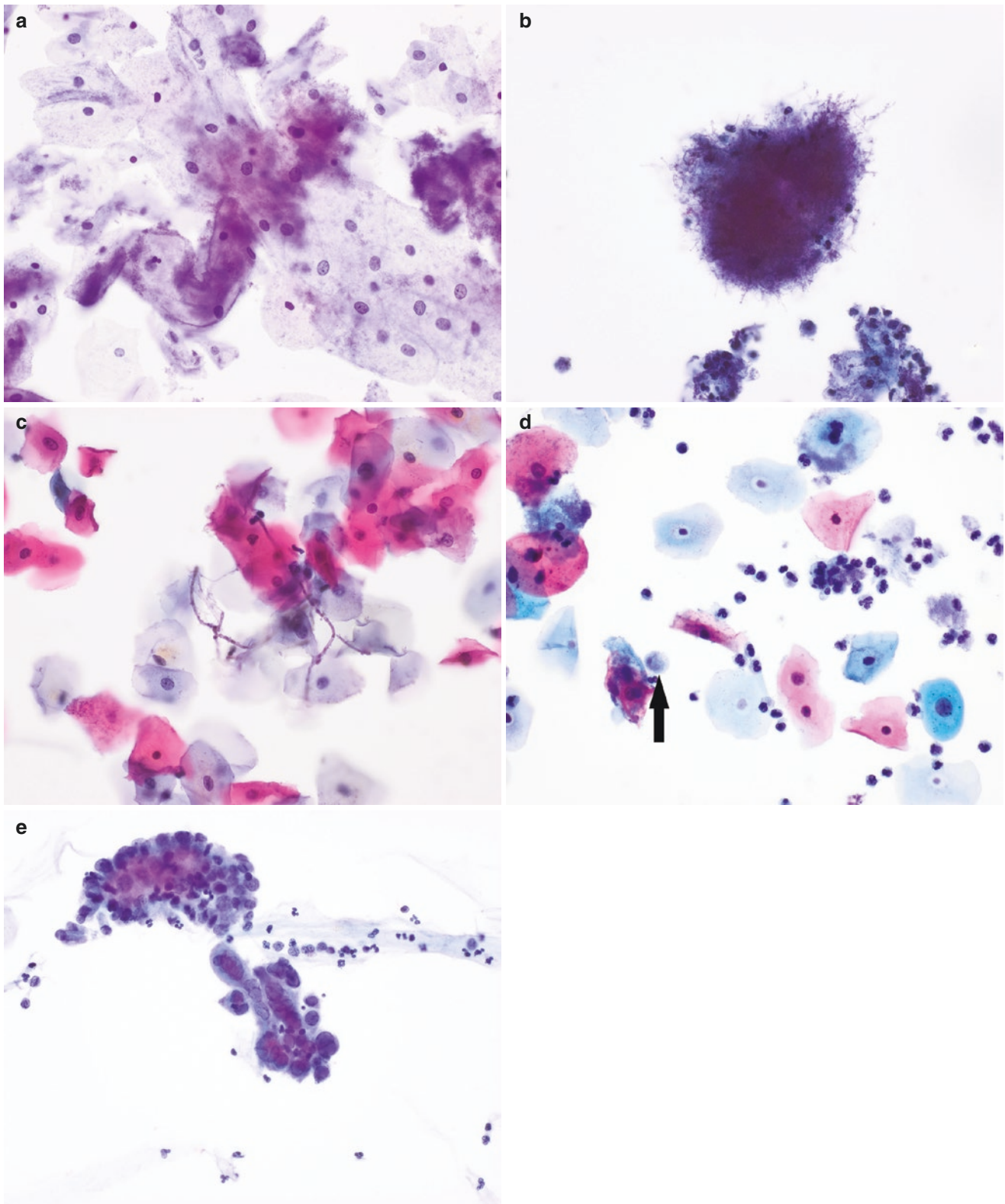
chlamydial infection, many cases of follicular cervicitis are associated with prior instrumentation or are idiopathic in nature.

## Common Microorganisms

A number of microbial organisms are commonly encountered in routine Pap tests, including normal vaginal flora, opportunistic organisms, and pathogenic infectious organisms. A focused approach to the most commonly seen microbial elements is described here.

Lactobacilli (previously called Doderlein bacilli) are facultative anaerobic, Gram-positive bacterial rods that are part of the normal vaginal flora [1]. They maintain the acidic vaginal pH through lactic acid production. They appear as thin, rod-shaped bacilli on the surface of squamous cells and in the background. They induce cytolysis of intermediate cells (Fig. 9.4b), releasing glycogen which allows for production of lactic acid and maintenance of the vaginal pH. As a result, naked nuclei and fragments of cytoplasm from intermediate squamous cells are frequently seen in combination with lactobacilli in a Pap test.

Bacterial vaginosis is a polymicrobial infectious state caused by an overgrowth of anaerobic bacteria, with *Gardnerella vaginalis* being the most common microorganism [15]. In contrast to the thin rod shape of lactobacilli, Gardnerella are coccobacilli. When present, these coccobacilli often coat the surface of squamous cells, imparting the classic “clue cell” appearance seen on Pap tests (Fig. 9.21a). Clumps of coccobacilli can also be seen in the background. Squamous cells often show associated reactive change, and associated acute inflammation is often present.



**Fig. 9.21** (a) Bacterial vaginosis. Squamous cells are coated with coccobacilli, imparting a “shaggy” appearance to the cytoplasm. (b) Actinomyces presenting as a tangle of filamentous bacteria. (c) Branching pseudohyphae of *Candida* spp. (d) *Trichomonas* organism (arrow) appears as a small ovoid cell with a tiny, pale nucleus that may

be hard to visualize. Note the reactive perinuclear clearing in adjacent squamous cells and background associated neutrophils. (e) Cytopathic effect of herpes simplex virus (HSV). The nuclei of infected epithelial cells appear glassy, with margined chromatin and frequent multinucleation. Cervical Pap, ThinPrep preparation, 40× magnification (a–e)

Actinomyces are Gram-positive filamentous bacteria commonly present in the Pap test of patients with intrauterine devices, and infections are often asymptomatic [1, 15]. Actinomyces presents as dense balls of radiating filamentous bacteria, giving a “fluffy ball” or “dust bunny” appearance (Fig. 9.21b). Macrophages and neutrophils may be seen in the background, along with reactive epithelial change.

Candida subspecies are also frequently encountered in Pap tests. In many cases, Candida infections are asymptomatic and may be associated with varying degrees of acute inflammation and reactive squamous cell change. Candida on Pap tests presents as round to oval budding yeasts (~5 uM) with pseudohyphae (Fig. 9.21c). Candida also tends to “skewer” squamous cells, creating a characteristic “shish kabob” appearance [1].

*Trichomonas vaginalis* is a common parasitic sexually transmitted infection, which may be asymptomatic or associated with vaginal discharge [1, 15]. Trichomonads are small oval to pear-shaped organisms, 15–30 uM in size, which may be present singly or in small aggregates. Their cytoplasm is pale gray, with red granules occasionally visible. Nuclei are eccentrically located, pale-staining, and small, approximately a quarter the size of an intermediate squamous cell nucleus (Fig. 9.21d). They have a single flagellum which is not typically visible on Pap tests. Acute inflammation is typically abundant, and collections of neutrophils (“poly balls”) are often present. Reactive changes in squamous cells include a small perinuclear clearing of cytoplasm (“trich halos”) that should not be mistaken for koilocytosis. Leptothrix, a long Gram-positive bacterium (several times longer than lactobacilli), is often seen in the background, and its presence almost always indicates trichomonas infection.

*Chlamydia trachomatis* is the most common sexually transmitted infection [1]. Chlamydia are obligate intracellu-

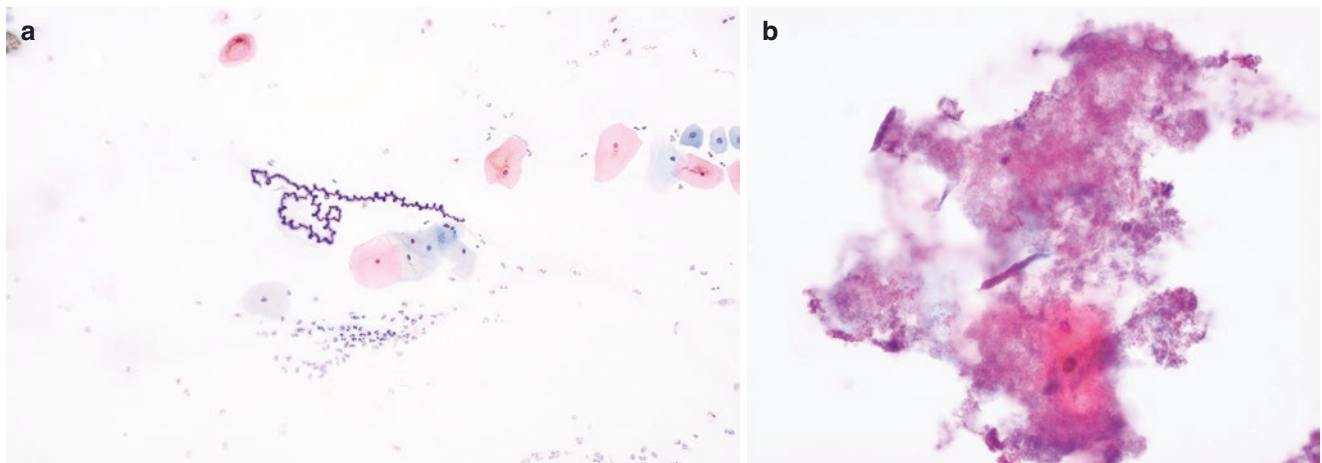
lar Gram-negative bacteria. Changes associated with chlamydia on a Pap test are often nonspecific and include follicular cervicitis. Intracellular inclusion bodies (“aggregate bodies”) in squamous or endocervical cells can be seen in some cases. These are large clear intracytoplasmic vacuoles that can resemble mucin at scanning power, within which small elementary bodies or larger reticulate bodies may be seen.

Herpes simplex virus is another common sexually transmitted infection [1, 15]. Infected epithelial cells commonly display multinucleation, nuclear molding, and chromatin margination (Fig. 9.21e). Acute inflammation is typically abundant.

### Miscellaneous Cellular and Acellular Elements

A number of miscellaneous structures and acellular or foreign substances are often identified in Pap tests and/or abdominopelvic washings at the time of gynecologic surgery. Recognition of these elements is important to avoid confusion with infection or neoplasms.

Mucin is normally produced by endocervical glandular cells and is often collected at the time of Pap test acquisition [1]. Mucin is more commonly encountered on conventional smears, as LBC removes most noncellular elements. However, abundant mucin material can be seen on LBC slides; Curschmann spirals, identical to those seen in respiratory specimens, can sometimes be encountered (Fig. 9.22a). With high estrogen states (e.g., prior to ovulation, during pregnancy) cervical mucus can form a fern-like pattern (ferning) on a slide. This finding is only noticeable on conventional Pap smear slides.



**Fig. 9.22** (a) Curschmann spirals are shown comprised of twisted strands of mucin material. (b) Grainy lubricant material shown obscuring squamous cells from view. Cervical Pap, alcohol-fixed smear prepa-

ration, Papanicolaou stain, 20× magnification (a). Cervical Pap, ThinPrep preparation, 40× magnification (b)

Like mucin, lubricant gel material often used on instruments used to perform a Pap test is largely removed in LBC preparations [16]. However, amorphous “blobs” of lubricant material can occasionally make their way through the filtration process. When overly abundant, this contaminating material may obscure the visibility of squamous cells (Fig. 9.22b).

Psammoma bodies are laminated calcifications formed from the degeneration of cellular elements. They are rarely present on Pap tests but are not infrequent in abdominopelvic washings. They are associated with a range of nonneoplastic, benign, and malignant conditions including endosalpingiosis, the presence of intrauterine devices, and a range of serous neoplasms from benign to high-grade malignancy [17].

Cockleburrs may be encountered in Pap tests, often in association with pregnancy. They are radiating arrays of club-shaped orange crystalline bodies surrounded by histiocytes. They may mimic actinomycosis.

Collagen balls are commonly identified in abdominopelvic washings, but rarely in spontaneous effusions [17]. They may also be occasionally seen in Pap test cytology. Collagen balls are three-dimensional collections of dense hyalinized matrix material surrounded by benign mesothelial cells with indistinct cell borders. The nuclei of involved cells may become crowded, but they are small and ovoid to flattened in shape without atypia.

Pollen and other plant material (e.g., *Alternaria* fungus) can occasionally be seen as a contaminant in Pap test cytology specimens, which can be identified by their thick refractile cell walls. Sperm can also sometimes be seen in postcoital Pap tests. Mature sperm have small pale nuclei, scant cytoplasm, and a single long flagellum at one end.

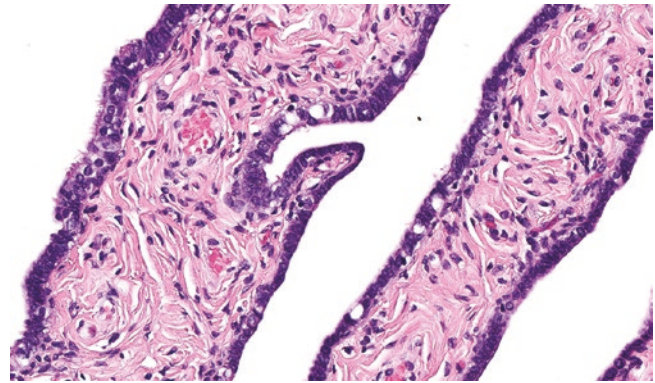
## Fallopian Tubes

The fallopian tubes are not routinely sampled by FNA or directed brushing sampling. However, fallopian tube cellular elements may be encountered in numerous cytology preparations including the Pap test, either from direct exfoliation or in patients with fallopian tube prolapse. Fallopian tube epithelium may also be encountered in pelvic washing cytology at the time of gynecologic surgery, as well as at the frozen section bench in touch and smear preparations.

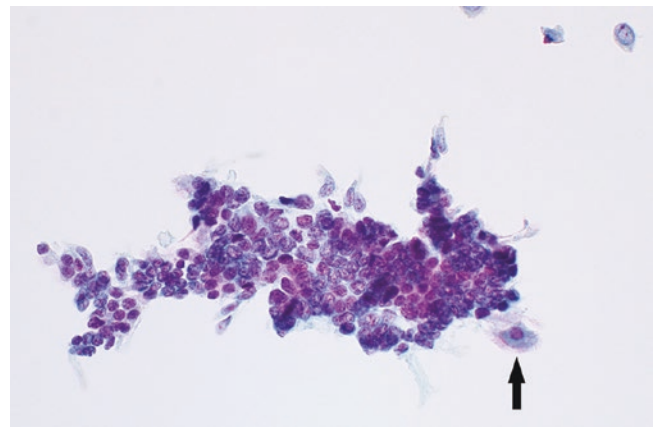
The fallopian tubes are lined by a single layer of columnar to cuboidal epithelium (Fig. 9.23). Classically, three epithelial cell types are described: ciliated cells, secretory cells, and intercalated (“peg”) cells. Ciliated cells are the most abundant, particularly in the distal end of the tube. They are typically columnar in shape, with a terminal bar at the apical end to which fine cilia are attached. Secretory cells are more numerous in the proximal fallopian tube. Secretory cells

appear similar to ciliated cells, but lack terminal bars and cilia, and may display foamy cytoplasm. The existence of smaller intercalated cells is controversial, and they are not routinely identified in cytology preparations.

Whether directly sampled or identified through passive exfoliation, fallopian tube epithelial cells may be present singly, in clusters, or in large sheets (Fig. 9.24) [17]. When in sheets, they tend to form honeycomb monolayers similar to other benign glandular epithelium. Ciliated cells are usually columnar, with a basally located, round nucleus and apical terminal bar. Ciliary “tufts” (detached fragments of apical cytoplasm from ciliated cells called ciliocytophthoria) can also be identified in Pap tests and pelvic washing specimens. Secretory cells are also columnar, and sometimes contain finely vacuolated cytoplasm. Clusters of benign fallopian tube epithelium can show moderate anisonucleosis and nuclear pleomorphism. However, they lack mitotic figures, and the presence of cilia indicates their benign nature.



**Fig. 9.23** Fimbriated end of the fallopian tube lined by single layers of ciliated columnar epithelium. Fallopian tube resection, hematoxylin & eosin, 40× magnification



**Fig. 9.24** Fallopian tube epithelium is shown in two-dimensional sheets. Nuclei are round and uniform, and occasional ciliated forms can be seen (arrow). Cervical Pap, ThinPrep preparation, 50× magnification

Endosalpingiosis is defined by the presence of ectopic, benign fallopian tube-like epithelial deposits outside the fallopian tube. This can be encountered in pelvic washings and Pap tests. Cytologically, the epithelium is indistinguishable from cells of the benign fallopian tube lining [17]. Occasionally, this process may be associated with hemosiderin-laden macrophages.

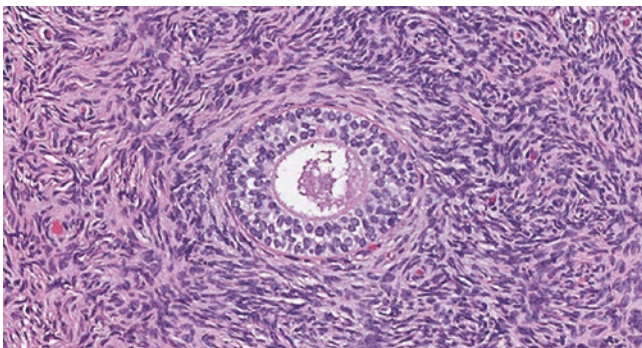
## Ovaries

Like the fallopian tube, directed cytologic sampling of the ovary is uncommonly performed. However, cells of ovarian origin can rarely be seen in pelvic washings and Pap tests. In addition, FNA is occasionally performed for ovarian cysts and masses [18].

The primary cells of the ovarian follicle are the granulosa cells, which surround the maturing Graafian follicles (Fig. 9.25). Follicle cyst fluid may sometimes be sampled in patients of reproductive age. Granulosa cells are small, round cells with central round nuclei and a small but distinct rim of cytoplasm, which appear singly and in loose sheets on cytology preparations (Fig. 9.26). Their chromatin is coarsely granular, without distinct nucleoli, and occasional nuclear grooves may be present. Mitotic figures may also occur. Theca cells, the outer lining cells of the follicle, are less commonly encountered in cytologic preparations. They have more abundant, vacuolated cytoplasm than granulosa cells, with eccentrically located nuclei.

The functional corpus luteum is composed of luteinized granulosa cells, which are distinct from the nonluteinized granulosa cells of the follicle. They have more abundant granular cytoplasm, which may be vacuolated. Nuclei are eccentric, with prominent nucleoli. Corpus luteal cysts can grow several centimeters in size, and like follicle cysts, their fluid is occasionally sampled by FNA.

The ovarian surface is lined by a layer of simple cuboidal epithelial cells. When sampled by FNA, ovarian surface epi-



**Fig. 9.25** An ovarian follicle, lined by granulosa cells, surrounded by ovarian spindle cell stroma. Ovary resection, hematoxylin & eosin, 40× magnification



**Fig. 9.26** A loose sheet of granulosa cells is shown from ovarian follicle cyst fluid, exhibiting central round nuclei and scant granular cytoplasm. Ovarian cyst fluid, cell block, hematoxylin & eosin, 20× magnification

thelium is present in tight sheets of bland, evenly spaced cells resembling mesothelium. Nuclei are round to oval and pale. Reactive change, including pleomorphism and prominent nucleoli, may occur in the setting of chronic irritation (e.g., from surface adhesions). Ovarian stroma may occasionally also be encountered on FNA. Stromal cells are spindle-shaped, with elongated nuclei, and are typically present in tightly cohesive groups. They may resemble a spindle cell neoplasm. However, their nuclei are uniform in shape and staining.

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