# Non-Neoplastic Findings

# 2

Daniel F.I. Kurtycz, Paul N. Staats, Nancy A. Young, Marluce Bibbo, Terrence J. Colgan, Marianne U. Prey, and Ritu Nayar

## 2.1 Negative for Intraepithelial Lesion or Malignancy

When there is no cellular evidence of neoplasia, this is stated in the General Categorization and/or in the Interpretation/Result section of the report. Organisms or other nonneoplastic findings are optional to report, in addition to this statement.

D.F.I. Kurtycz, MD (✉)
Department of Pathology and Laboratory Medicine, University of Wisconsin, Wisconsin State Laboratory of Hygiene, 465 Henry Mall, Madison, WI 53706, USA
e-mail: dkurtycz@wisc.edu

P.N. Staats, MD
Department of Pathology, University of Maryland School of Medicine, 22 South Greene Street, Baltimore, MD 21201, USA
e-mail: pstaats@umm.edu

N.A. Young
Department of Pathology and Laboratory Medicine, Einstein Healthcare Network, 5501 Old York Road, Philadelphia , PA 19141, USA
e-mail: youngnan@einstein.edu

M. Bibbo, MD
Department of Pathology, Thomas Jefferson University Hospital, 132 s 10th street, Philadelphia, PA 19107, USA
e-mail: marluce.bibbo@jefferson.edu

T.J. Colgan, MD, FRCP(C), FCAP, MIAC
Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, ON, Canada
e-mail: tcolgan@mtsinai.on.ca

M.U. Prey
Department of Pathology, 8829 Ladue Road, Ladue, Missouri 63124, USA
e-mail: marianne@extravirginoo.com



R. Nayar, D.C. Wilbur (eds.), *The Bethesda System for Reporting Cervical Cytology: Definitions, Criteria, and Explanatory Notes*,
DOI 10.1007/978-3-319-11074-5_2

R. Nayar, MD
Department of Pathology, Northwestern University,
Feinberg School of Medicine, Northwestern Memorial Hospital,
251 East Huron Street, Galter, 7-132B, Chicago, IL 60611, USA
e-mail: r-nayar@northwestern.edu

**Normal Cellular Elements**
- Squamous cells
- Endocervical cells
- Endometrial cells
- Lower uterine segment cells

**Nonneoplastic Findings (Optional to Report)**
- Nonneoplastic cellular variations
  - Squamous metaplasia
  - Keratotic changes
  - Tubal metaplasia
  - Atrophy
  - Pregnancy-associated changes
- Reactive cellular changes associated with:
  - Inflammation (includes typical repair)
  - Lymphocytic (follicular) cervicitis
  - Radiation
  - Intrauterine contraceptive device (IUD)
- Glandular cells status post hysterectomy

**Organisms**
- *Trichomonas vaginalis*
- Fungal organisms morphologically consistent with *Candida* spp.
- Shift in flora suggestive of bacterial vaginosis
- Bacteria morphologically consistent with *Actinomyces* spp.
- Cellular changes consistent with herpes simplex virus
- Cellular changes consistent with cytomegalovirus

## 2.2 Background

The category "negative for intraepithelial lesion or malignancy" is used for specimens that show a spectrum of nonneoplastic changes, including those associated with protective and reactive responses to inflammation, hormonal alterations, and colonizing or infectious organisms.

Cervical cytology is a screening test primarily for the detection of squamous cell carcinoma of the cervix and its precursors. Due to the wide spectrum of reactive cytomorphologic changes, criteria are not well-defined and may lack reproducibility [1–5]. The reporting of specific nonneoplastic findings is optional and at the discretion of the laboratory. Reasons for continuing to report certain nonneoplastic findings in a cervical cytology report include the following:

1. Utility as a triage tool and as documentation for laboratory regulations regarding referral for hierarchical review.
2. Fostering a discipline in applying cytomorphologic criteria during screening and sign-out.
3. Documentation of morphologic findings to explain differences in interpretation on review [6].
4. Facilitation of clinical-cytologic correlation. For example, the cytologic finding of hyperkeratosis and parakeratosis may correlate with the colposcopist's assessment of the uterine cervix.
5. Documentation of reactive cellular changes in the report to spot trends in a series of cervical cytology specimens from one woman. Studies have reported a slight increase in the incidence of squamous intraepithelial lesion (SIL) in cases interpreted as reactive compared to those interpreted as within normal limits [7, 8]. This may relate to the concept that tissues which are more frequently subjected to infection, inflammation, and other traumatic stimuli may either be more subject to high-risk HPV infection or that mutational changes occur more frequently in traumatized tissues undergoing repair [9].
6. Documentation of findings that allow education of both laboratorians and clinical practitioners as to biologic processes relevant to the patient and to cytomorphology in general.
7. Marked reactive and/or reparative changes may cause concern for or be overinterpreted as squamous or glandular neoplastic entities. These more worrisome examples of reactive changes and repair should be subject to additional hierarchical review.

Note that the list of nonneoplastic findings in TBS is not comprehensive. Additionally, these interpretive categories do not necessarily correspond to regulatory requirements for hierarchical supervisory review; within the parameters of government regulation, it is up to the laboratory to specify findings that trigger additional review.

**Negative for Intraepithelial Lesion or Malignancy (NILM)**
Specimens for which no epithelial abnormality is identified are reported as "negative for intraepithelial lesion or malignancy" (NILM). If optional nonneoplastic findings are reported, NILM should still be included as the primary interpretation or as the General Categorization to avoid ambiguity.

## 2.3 Normal Cellular Elements

It is important that persons analyzing cervical samples have an understanding of both the nuclear morphology and sizes of the cellular constituents. Pioneers of cervical cytology derived basic understanding of benign and neoplastic processes from careful measurements in conventional cytologic samples [10]. While there is no contemporary literature on such measurements in liquid-based preparations, size relationships remain important in defining diagnostic entities and functional states.

### 2.3.1 Squamous Cells

#### 2.3.1.1 Superficial Cell

Derived from the outermost layer of the cervical epithelium and usually seen in the proliferative phase of the menstrual cycle and in the presence of irritation. The nucleus is highly condensed (pyknotic) and 10–15 μm$^2$ in cross-sectional area. The cytoplasm is abundant, usually eosinophilic. Keratohyaline granules may be found in the cytoplasm, reflecting elaboration of high molecular weight keratin protein (Fig. 2.1).

#### 2.3.1.2 Intermediate Cell

Generally present in the middle or intermediate layer of the squamous epithelium. In the secretory phase, this cell type may compose both the middle and superficial layers of the normal cervical epithelium. It is particularly prominent in pregnancy and with the use of progestational agents. The nucleus is larger than that of the superficial cell, with a cross-sectional nuclear area of 35 μm$^2$ and a finely granular chromatin pattern. The nucleus is often elongate with a longitudinal nuclear groove (Fig. 2.2). *The intermediate cell nucleus serves as the basic size reference for other cells in cervical cytology specimens*. Naked intermediate cell nuclei are seen in the second half of the cycle secondary to bacterial cytolysis (Fig. 2.59).

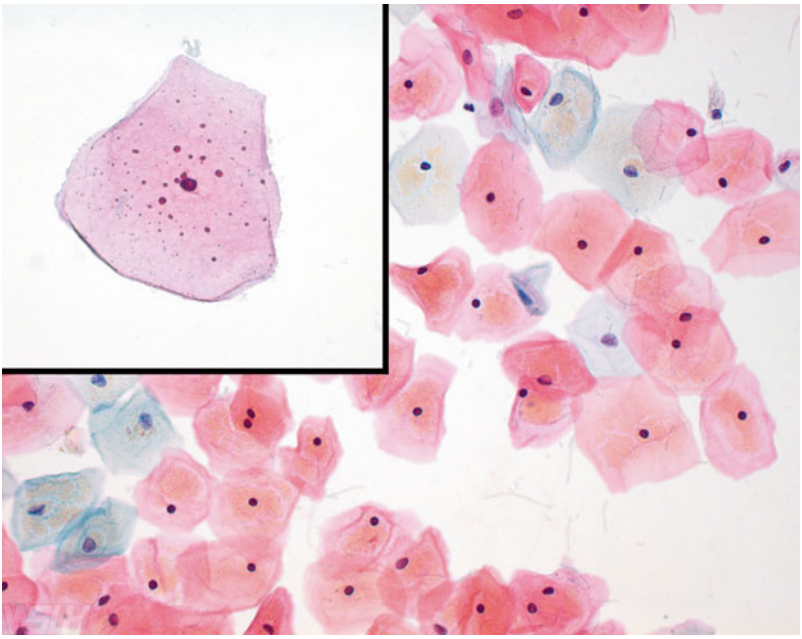

**Fig. 2.1** Superficial squamous cells (*LBP*, *ThinPrep*). Admixture of superficial and intermediate squamous cells. The superficial cells have smaller condensed (pyknotic) nuclei. Light brown glycogen is present in the cytoplasm of both cell types. The inset reveals a characteristic superficial cell at high magnification. Note the polygonal cytoplasmic profile, cytoplasmic keratohyaline granules, and pyknotic nucleus with a cross-sectional area of approximately 10 μm$^2$. The dense nucleus is opaque to light

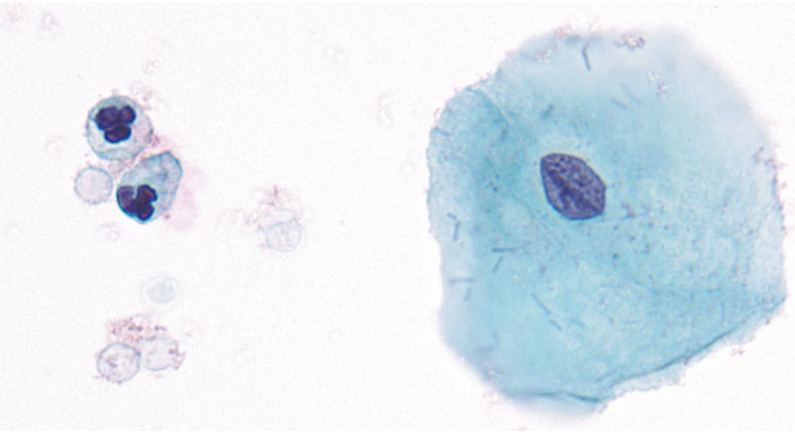

**Fig. 2.2** Intermediate squamous cell (*LBP*, *ThinPrep*). A typical intermediate cell with a polygonal cytoplasmic profile. The nucleus possesses finely granular chromatin with a longitudinal groove. The cross-sectional area of the intermediate nucleus is approximately 35 μm$^2$ and is generally used as the internal reference for size comparison. Light can pass through the intermediate nucleus due to the chromatin being more open than that of a superficial cell

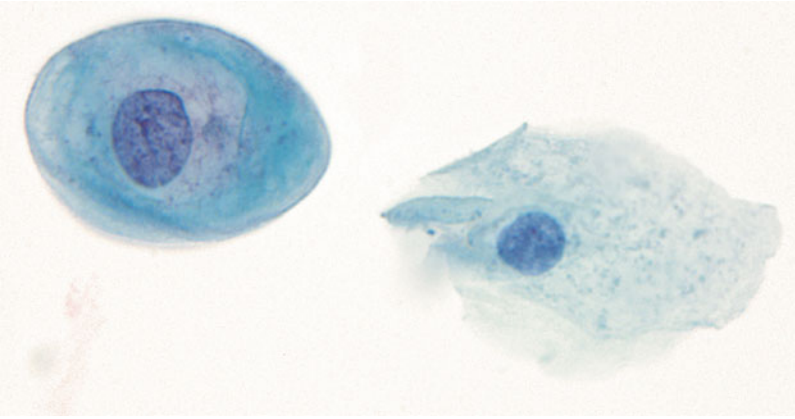

**Fig. 2.3** Parabasal cell (*LBP*, *ThinPrep*). A parabasal cell is contrasted with an intermediate cell. The parabasal cell exhibits typical features with an oval nucleus, fine chromatin, and a cross-sectional area of approximately 50 μm$^2$. The cytoplasm is dense relative to the intermediate cell, because the intermediate cell cytoplasm flattens out next to the nucleus, whereas in the parabasal cell, the cytoplasm is heaped up. If the cells were viewed from the side, an intermediate cell would be a flattened saucer with a central nuclear heap. The parabasal cell would resemble a hill with sloping sides

#### 2.3.1.3 Parabasal Cell

Along with immature squamous metaplastic cells, parabasal cells are the least mature cells in a cervical cytology sample. They are generally not present in specimens from a hormonally mature epithelium as they are derived from deep cell layers not typically sampled in cervical cytology specimens from premenopausal women. In the absence of hormonal stimulation, this cell type comprises layers of a relatively thin and atrophic epithelium. Parabasal cells may predominate in postmenopausal and postpartum states. The nuclei are larger than in intermediate cells with an area of 50 $\mu m^2$. The cytoplasmic area is smaller and the nuclear to cytoplasmic ratio is higher than in intermediate or superficial cells; and the cytoplasmic texture is more granular and dense (Fig. 2.3).

### 2.3.2 Glandular Cells

#### 2.3.2.1 Endocervical Cell

Endocervical glandular cells have nuclear sizes that are highly variable with a mean of 50 $\mu m^2$ which is slightly larger than that of an intermediate squamous cell. The nucleus shows a finely granular and evenly distributed chromatin pattern with small nucleoli. The cytoplasm is diffusely vacuolated or granular. Cells exhibit polarity with nuclei at one end of the cytoplasm and mucus present at the opposite end. The cytomorphology will differ depending on the orientation of the cells on the slide: when viewed from the side, there will be a "picket-fence" formation, whereas when viewed en face, they will have a classic "honeycomb" configuration (Fig. 2.4).

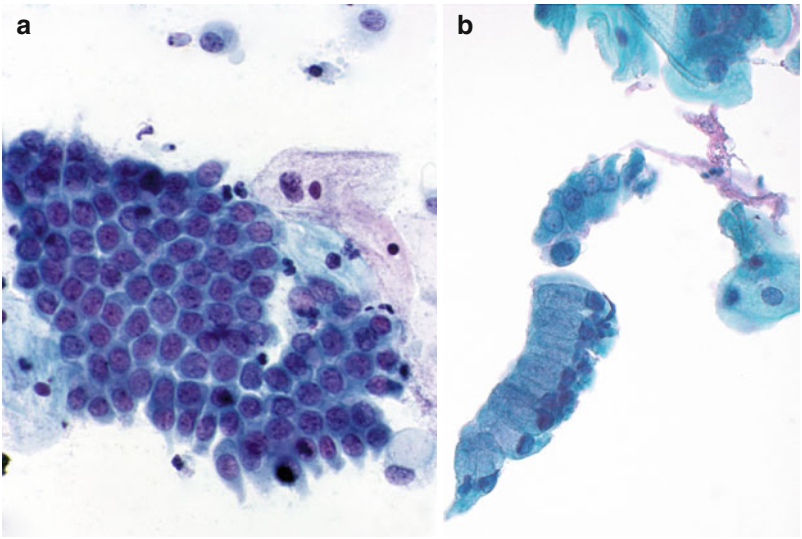


**Fig. 2.4** Endocervical cells (*LBP*, *ThinPrep*). Endocervical cells may be seen en face in a typical "honeycomb" arrangement of benign glandular epithelium (**a**). Alternatively, endocervical cells when viewed from the side present in a "picket-fence" configuration (**b**). There is normal nuclear polarity and ample evidence of apical mucin in these columnar cells

### 2.3.2.2 Endometrial Cell

Spontaneously exfoliated endometrial cells may be of epithelial or stromal origin and can occur as isolated cells or as aggregates. Endometrial glandular cells are typically smaller than endocervical cells, with a nuclear area equal to or slightly smaller than an intermediate cell nucleus (35 μm$^2$) and have a higher nuclear to cytoplasmic ratio. The nuclear chromatin tends to be dense, heterogeneous and may contain apoptotic debris due to degenerative changes. Nucleoli are generally not prominent, but may be observed in liquid-based preparations due to improved fixation. The cytoplasm is scant and may be dense or vacuolated. Exfoliated endometrial stromal cells are typically arranged in dense aggregates which often have a surrounding layer of glandular epithelium – a characteristic formation often referred to as an "exodus" ball because of its presence at the end of menstrual flow. Exfoliated endometrial stromal cells may also be isolated and have spindled tails of wispy cytoplasm. Exfoliated endometrial cells (Figs. 2.5 and 2.6, see Figs. 3.1, 3.2, and 3.4) present differently than do directly sampled lower uterine segment and endometrial cells, which are described below (Figs. 2.7, 2.8, and 2.9, see Fig. 3.5).

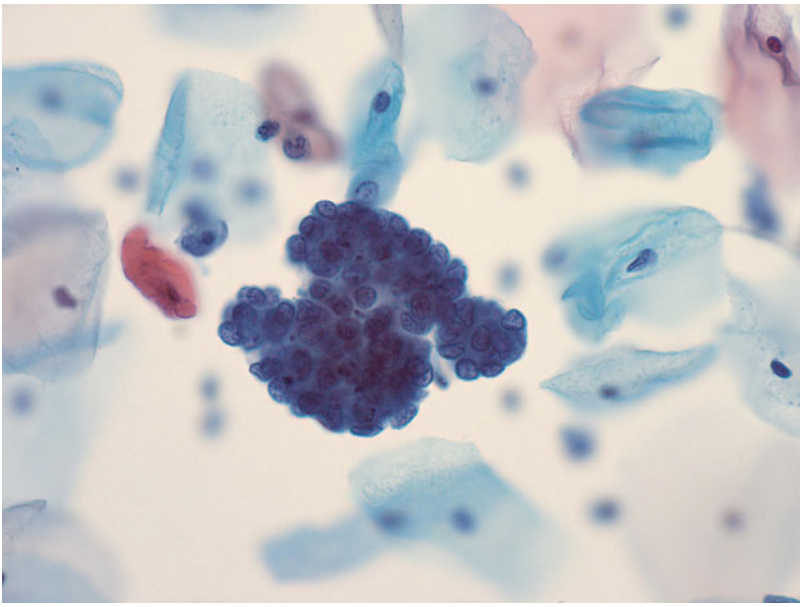

**Fig. 2.5** Endometrial cells (*LBP*, *SurePath*). A tight cluster of endometrial glandular cells with nuclei having cross-sectional areas slightly smaller than the 35 μm$^2$ of intermediate cells. Endometrial cell nuclear to cytoplasmic ratios are high and the cells tend to form three-dimensional groups. The small and monotonous nuclear size should prevent overinterpretation as a squamous, or glandular abnormality

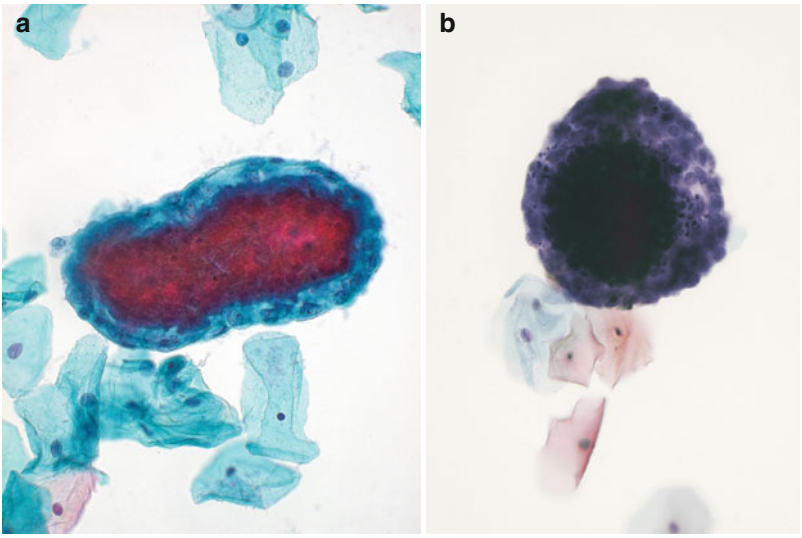


**Fig. 2.6** Endometrial cells, exodus (*LBP*, *ThinPrep*). Collections of peripheral glandular and central stromal endometrial cells (exodus ball) are typically seen between day 6 and 10 of the menstrual cycle. These clusters are among the last remnants of endometrial shedding and the cells may show degenerative changes. Both images show exodus balls from two different cases. On the left (**a**) is an intermediate magnification from a conventional preparation. More nuclear structure is observed in cells on the periphery of the exodus ball. In (**b**), from a liquid based preparation, physical forces have accentuated the rounding up of cells during fixation. The resultant three dimensional cell ball obstructs more light, is darker and may be over interpreted as a glandular abnormality

## Preparation-Specific Criteria for Normal Cellular Elements

*Liquid-Based Preparations*:
Fixation is generally improved and these preparations remove much of the background bacteria, debris, and inflammatory material that can obscure the cells of interest. Glandular cells may form three-dimensional structures, as cellular fixation occurs during suspension in liquid as opposed to preparations in which fixation occurs after smearing on a slide. Rounded benign groups can be more densely cellular and hyperchromatic. Observation of cells near the borders of the group becomes more important to determine the true origin and nature of such cell groupings. Nucleoli may be better preserved and more prominent.

*Conventional Preparations*:
Bacteria, inflammatory cells, and debris are more prominent in the background. Degenerative changes, "air-drying artifact", mechanical artifact, and other limiting factors associated with sample collection and preparation are more common. Cells may be larger as they are flattened out on the slide.

### 2.3.3 Lower Uterine Segment and Directly Sampled Endometrial Cells (Figs. 2.7–2.9)

#### 2.3.3.1 Criteria

Cells directly sampled from the lower uterine segment or endometrial cavity may present as large, cellular, hyperchromatic groups composed of both endometrial glandular and stromal cells (Fig. 2.7, see Fig. 3.5). Branching glands can be seen in some groups, with surface gland openings and palisading of nuclei in the interior of the fragments (Fig. 2.8). The glands are surrounded by stroma, which may contain small vessels that can appear to protrude from the surface of the groups in a spindled or "feathered" pattern. Smaller fragments may contain only glandular or stromal cells. Nuclear crowding and overlap are present in both epithelial and stromal components.

Directly sampled endometrial and lower uterine segment glandular cells are columnar and have round to oval, variably hyperchromatic nuclei, with moderately coarse but evenly distributed chromatin and smooth nuclear borders (Fig. 2.8).

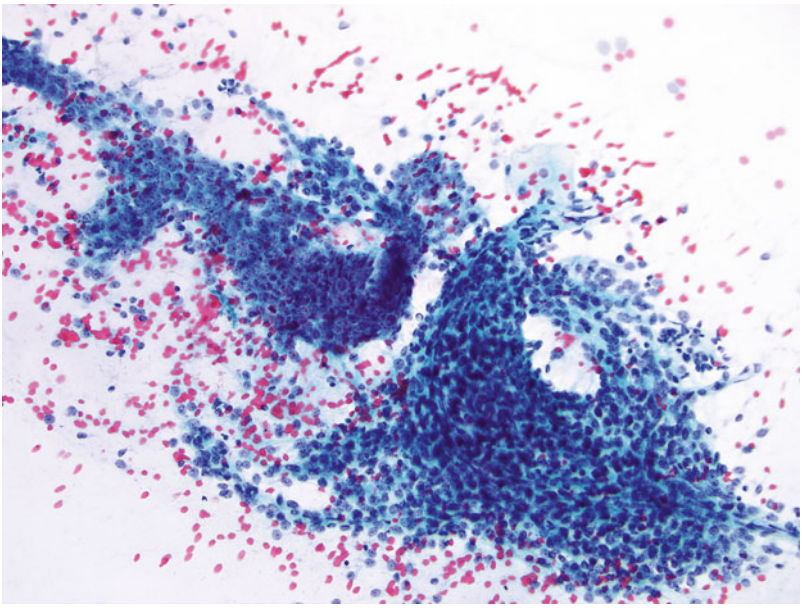

**Fig. 2.7** Lower uterine segment sampling (*CP*). Lower uterine segment sampling with ill-defined glandular cells near the upper left aspect and stromal cells loosely adherent to the glandular cells. Several blood vessels can be seen protruding from the group. Stromal and glandular components are not always easy to distinguish on cervical cytology

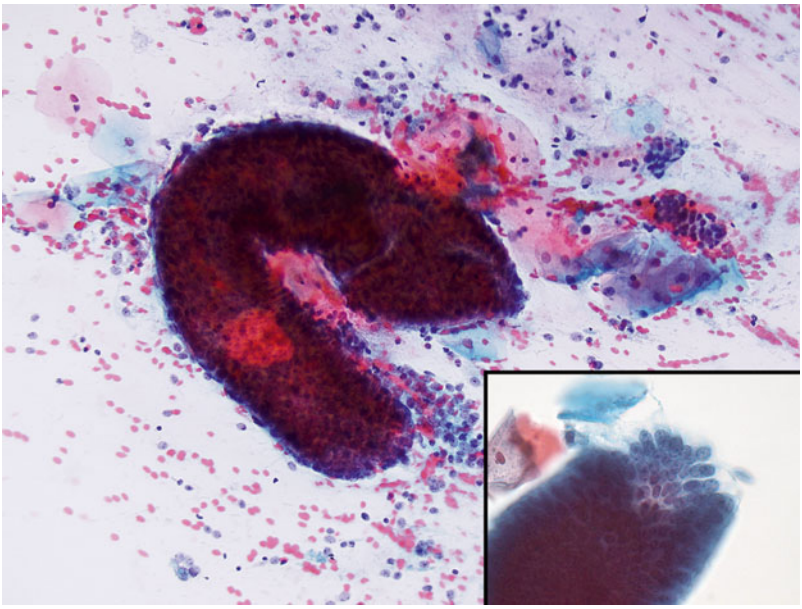

**Fig. 2.8** Lower uterine segment sampling (*CP*). A well-preserved endometrial gland presenting as a tubular structure. A stromal component is also visible at the lower right side of the epithelial tube. The inset shows columnar endometrial glandular cells that have round to oval, variably hyperchromatic nuclei, with moderately coarse but evenly distributed chromatin and smooth nuclear borders (*CP*)

Nucleoli are inconspicuous and mitotic figures may be seen, particularly during the proliferative phase. Ciliated cells may be present in the case of coexistent tubal metaplasia.

Stromal cell groupings are arranged in a disorganized pattern (Fig. 2.9). The cells have oval to elongate nuclei and scant, spindled cytoplasm. Nuclei have smooth contours and an evenly distributed, finely granular chromatin pattern. Nucleoli are inconspicuous and mitotic figures are rare.

### Preparation-Specific Criteria

In liquid-based preparations, lower uterine segment and directly sampled endometrium tends to exhibit small dense cellular groups containing only epithelium or stroma (Fig. 2.8). In conventional preparations, large cellular groups may have a "stretched" configuration and glands and blood vessels are more commonly noted (Fig. 2.7, see Fig. 3.5).

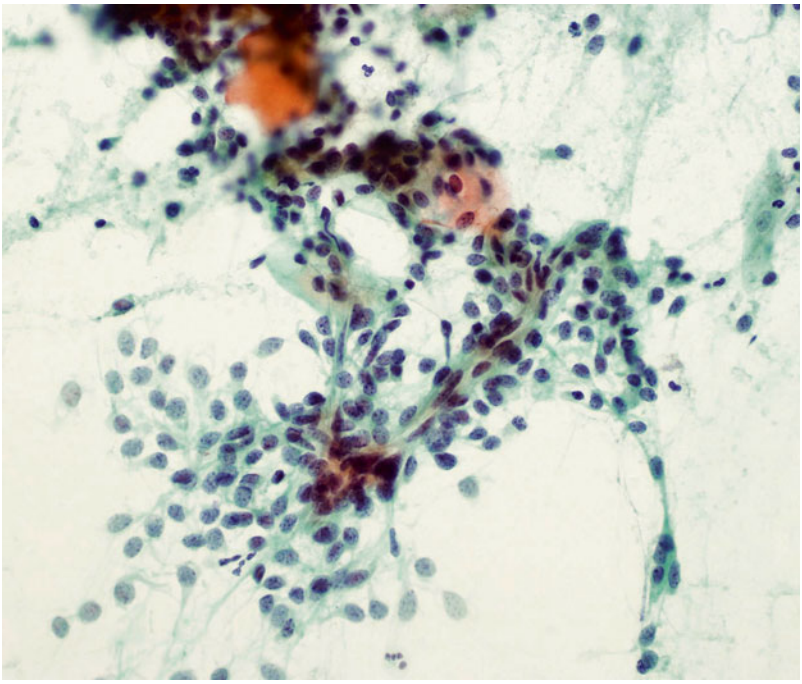

**Fig. 2.9** Lower uterine segment sampling (*CP*). Endometrial stromal cells adherent to blood vessels and flattened against the slide in a fanlike pattern

### 2.3.3.2 Explanatory Notes

Sampling of the lower uterine segment and endometrium may occur because of closer proximity to the cervical os following an excisional procedure (loop electrosurgical excision or conization) that shortens the endocervix [11] or a trachelectomy (a fertility-sparing resection of the cervix, upper vagina, and adjacent tissue, for minimally invasive squamous cell carcinoma) [12, 13]. Direct endometrial sampling can occasionally be present in women with an intact cervix secondary to the vigorous use of an endocervical brush or broom sampling device.

Directly sampled endometrial tissue may mimic glandular neoplastic abnormalities or rarely high-grade squamous lesions due to the presence of hyperchromatic crowded groups with nuclear crowding, nuclear overlap, and high nucleus to cytoplasmic ratios. In contrast to spontaneously exfoliated endometrial cells, direct brushing of endometrial tissue may yield large cellular fragments that can recapitulate their native in situ architecture (so-called organoid differentiation). This appearance may include branching tubular glands amid stroma composed of round to spindle-shaped cells. Peripheral palisading may be evident. The low-power recognition of branching glands and glandular-stromal complexes can avoid confusion with

atypical glandular cells (AGC) or glandular neoplasia. In liquid-based preparations, smaller rounded groups may have only one visible component. The most helpful clues in this situation are small nuclear size (approximating that of an intermediate nucleus); smooth, regular nuclear contours; and evenly distributed chromatin. In addition, groups of endometrial stromal cells may contain small vessels that protrude from the surface of the organoid groups, a feature not seen in neoplastic epithelial abnormalities.

## 2.4 Nonneoplastic Cellular Variations

### 2.4.1 Squamous Metaplasia (Figs. 2.10–2.13)

#### 2.4.1.1 Criteria

Squamous metaplastic cells which show a range of cytoplasmic differentiation from immature parabasal-like cells to those that approximate the appearance of differentiated intermediate/superficial cells (Fig. 2.10). The mean nuclear area is larger than that of the intermediate cell and similar to the parabasal cell at 50 $\mu m^2$.

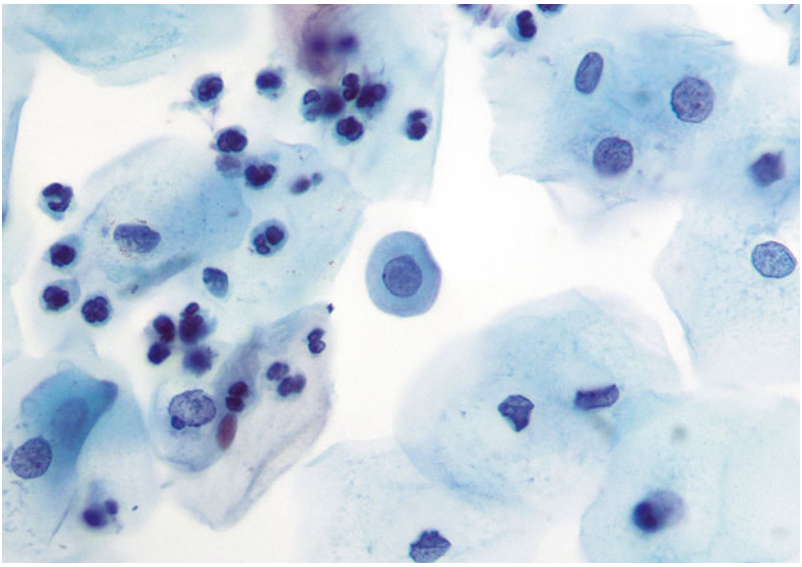

**Fig. 2.10** Squamous metaplasia (*LBP*, *SurePath*). A characteristic metaplastic cell is found in the center of the field. The nucleus is round to oval with fine, evenly distributed chromatin. The nuclear to cytoplasmic ratio is variable, and in this instance, it approaches one to one. These cells should not be overinterpreted as ASC-H or HSIL

### Preparation-Specific Criteria

Cells having spindled cytoplasmic projections ("spider cells") are often seen in conventional preparations due to disruption of the cohesion of cellular attachments by the force of the smearing procedure (Fig. 2.11).

### 2.4.1.2 Explanatory Notes

The process of metaplasia represents the replacement of one type of epithelium (in this case endocervical) with another (squamous) as a protective response. Squamous metaplastic cells can exhibit a spectrum of morphology from relatively undifferentiated small round cells to highly differentiated intermediate/superficial squamous cells. In metaplasia, stimuli such as infection, inflammation, or other type of trauma cause an alteration in the pathway of development of new cells replacing those lost by wear and tear. The newly generated cells become progressively more

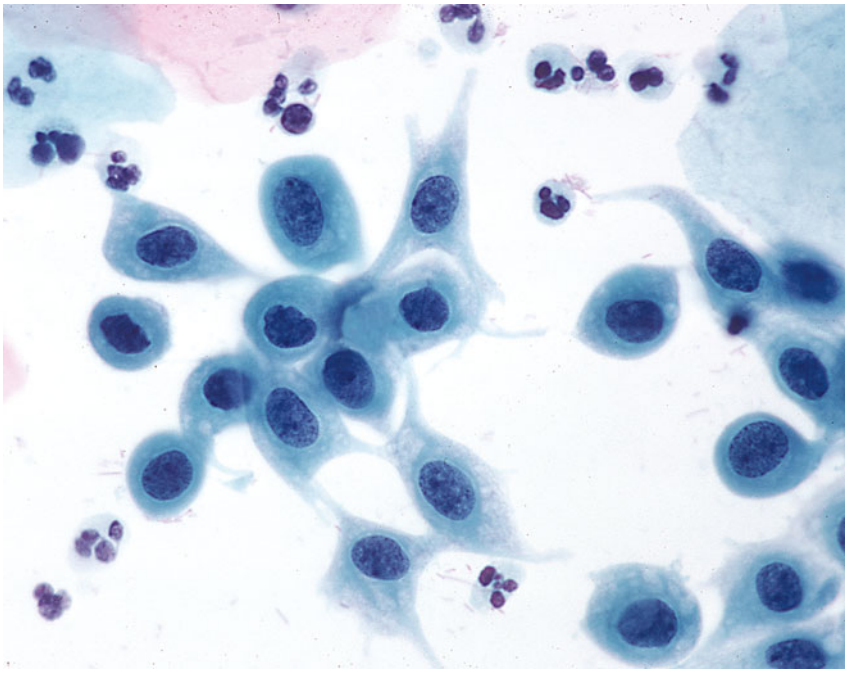

**Fig. 2.11** Squamous metaplasia (*CP*). Routine screening from a 27-year-old woman, day 8 of menstrual cycle shows reactive metaplastic cells with "spidery" cytoplasmic processes, a feature that is seen more often in conventional smears. Follow-up cytology was NILM

differentiated along the squamous pathway in response to the noxious stimulus. The metaplastic surface epithelium may eventually become indistinguishable from other squamous mucosa; however, the histologic finding of glandular spaces filled by endocervical or metaplastic squamous cells beneath the surface is a marker of the cervical transformation zone and an indication that the overlying epithelium was once glandular (Fig. 2.12).

One of the most difficult tasks in day-to-day cytologic practice is the evaluation of metaplastic cells, especially those with high nuclear to cytoplasmic ratios. Nuclear enlargement without other nuclear abnormalities in squamous metaplastic cells should lead to cautious evaluation, so as not to overinterpret the sample. One should evaluate single nuclei in intact cells. A nuclear to cytoplasmic ratio of less than 50 %, smooth nuclear contours, and even distribution of chromatin all favor benign squamous metaplasia (Fig. 2.13). A higher nuclear to cytoplasmic ratio in conjunction with hyperchromasia and/or nuclear contour irregularities, such as notching or grooving, should prompt consideration of a HSIL or ASC-H designation.

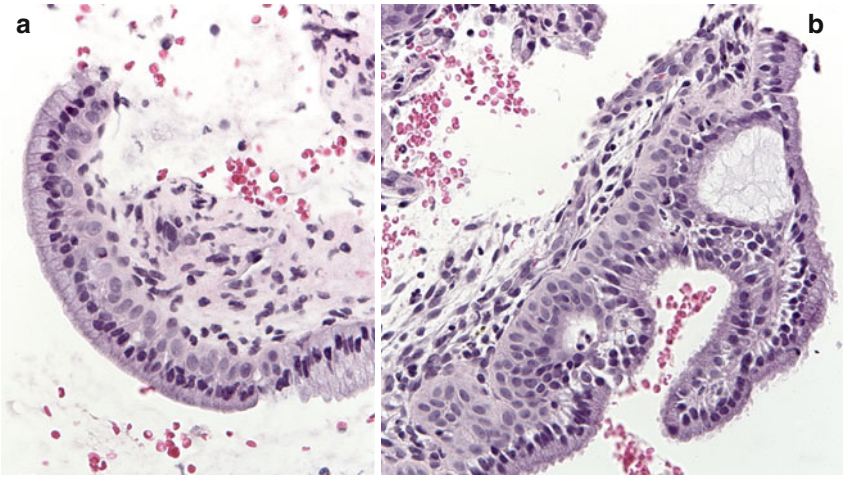


**Fig. 2.12** Squamous metaplasia (histology, H&E). (**a**, *left*) Early squamous metaplasia in an endocervical sample. A variety of stimuli can trigger an altered pathway of differentiation in the stem cell population that was committed to generating endocervical cells. The cells underneath the mucus secreting epithelial cells have rounded up, lost their ability to secrete mucin, and assumed a protective role, increasing the thickness of barrier between the stimulus and the underlying tissue. (**b**, *right*) A later stage in squamous metaplasia where multiple layers of metaplastic cells are seen under the surface epithelium

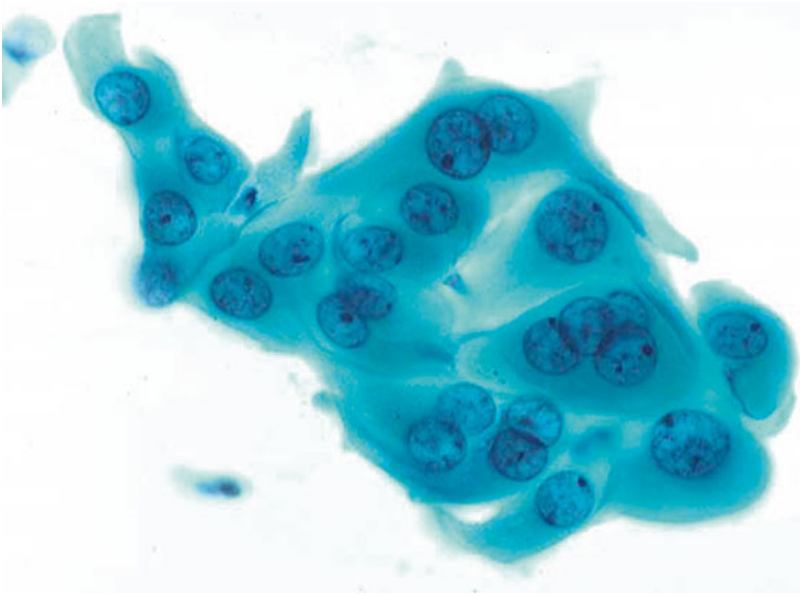

**Fig. 2.13** Squamous metaplasia (*CP*). Squamous metaplastic cells show nuclear size similar to parabasal cells. This cohesive group of cells also shows some modest nucleolar prominence that is consistent with reactive/reparative changes

### 2.4.2 Keratotic Cellular Changes (Figs. 2.14–2.17)

Normally, the cervix is a nonkeratinizing, stratified squamous epithelium. Keratotic changes usually occur as a protective reactive phenomenon or in association with human papillomavirus (HPV)-induced cell changes. Both of these processes lead to hypermaturation of the native squamous epithelium, more closely approximating the normal appearance of skin. Keratotic changes can be considered a second-order protective reaction for subepithelial tissues with metaplasia being the first-order reaction.

"Keratosis," "hyperkeratosis," "parakeratosis," and "dyskeratosis" are descriptive terms for keratotic cellular changes which have been used inconsistently in the past. These terms are not specifically listed in Bethesda terminology due to lack of consensus definitions. They are included parenthetically for clarification only. Although some cytologists may choose to include such terms to describe a morphologic feature that may correlate with leukoplakia on colposcopy, they should not be used as an interpretive category in cytology reports.

After metaplastic conversion, continued trauma may lead to formation of cytoplasmic keratohyaline granules (Fig. 2.14). In rare examples, the epithelium may come to resemble skin with a granular layer.

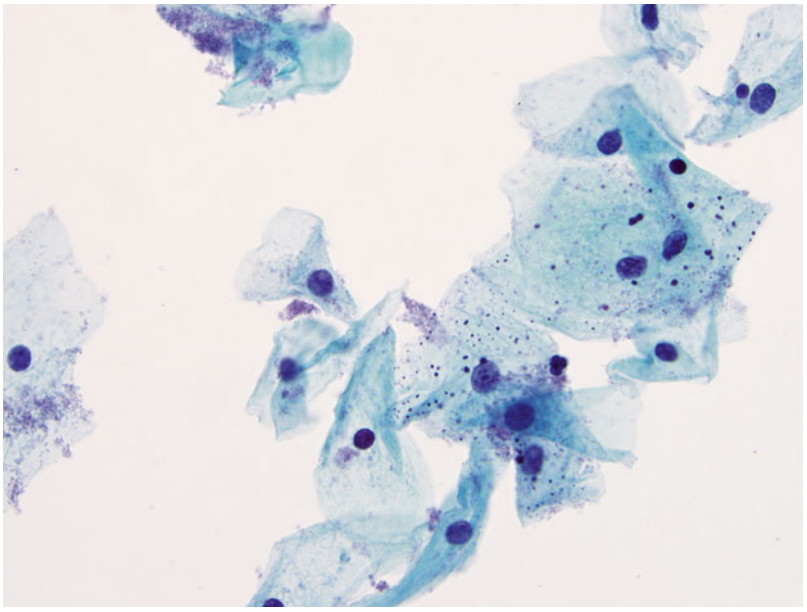

**Fig. 2.14** Keratotic cellular changes (*LBP*, *ThinPrep*). Intermediate squamous cells showing prominent cytoplasmic keratohyaline granules, a precursor to full keratinization

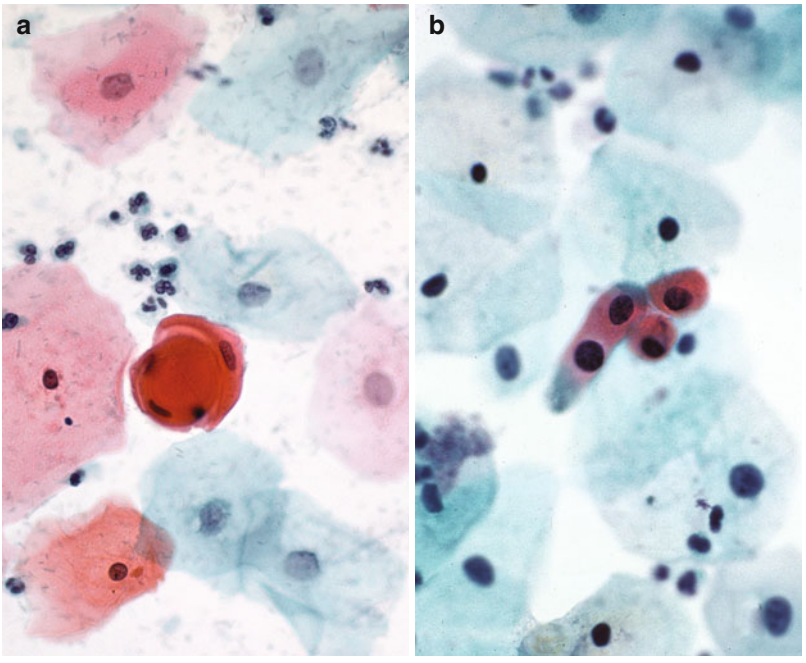


**Fig. 2.15** Keratotic cellular changes (*CP*). Keratotic cellular changes, "typical parakeratosis." On the left side (**a**), note the "squamous pearl" formation in this specimen from a 49-year-old woman being followed up after treatment for SIL. On the right side (**b**) is a small cluster of miniature squamous cells. Both are examples of "typical parakeratosis" showing miniature squamous cells with small bland, pyknotic nuclei

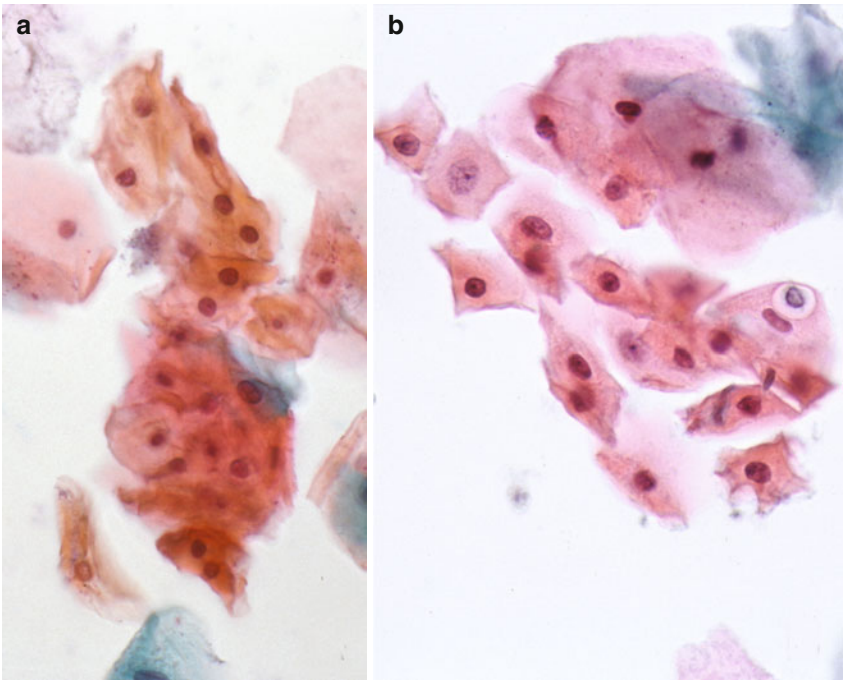


**Fig. 2.16** Keratotic cellular changes. Keratotic cellular changes, "typical parakeratosis". On the left (**a**, *CP*) is an orangeophilic cluster, and on the right (**b**, *LBP*, *ThinPrep*) are more eosinophilic squamous cells with small, opaque nuclei. Human papillomavirus (HPV) testing, performed as part of co-testing on the liquid-based specimen, was negative

### 2.4.2.1 Typical Parakeratosis (Figs. 2.15 and 2.16)

#### 2.4.2.1.1 Criteria

Miniature superficial squamous cells with dense orangeophilic or eosinophilic cytoplasm. Cells may be seen in isolation, in sheets, or in whorls; cell shape may be round, oval, polygonal, or spindle shaped.

Nuclei are small (approximately 10 μm$^2$ in cross-sectional area) and dense (pyknotic).

If atypical nuclear changes are present, an atypical squamous cell (ASC-US/ASC-H) or SIL interpretation should be considered, but if nuclei are round, regular, and resemble neighboring nuclei, a designation as abnormal is not warranted.

### 2.4.2.2 Hyperkeratosis (Fig. 2.17)

#### 2.4.2.2.1 Criteria

Anucleate but otherwise unremarkable mature polygonal squamous cells, often associated with mature squamous cells showing keratohyaline granules.

Empty spaces or "ghost nuclei" may be noted.

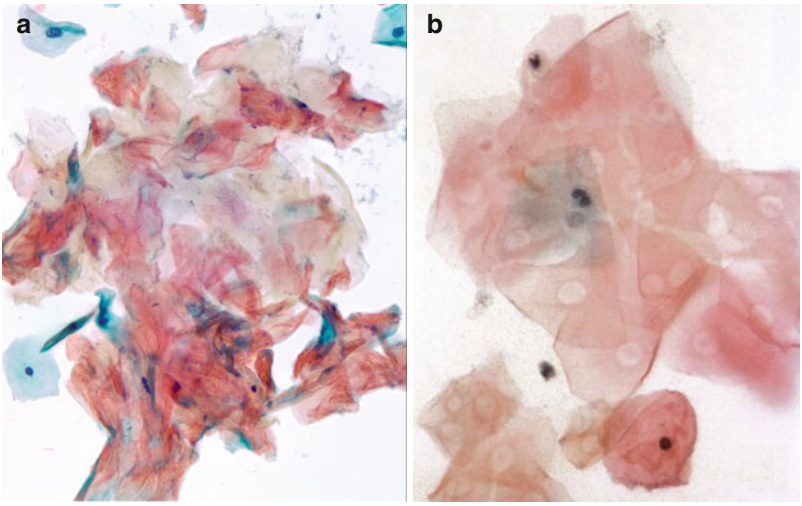


**Fig. 2.17** Keratotic cellular changes, "hyperkeratosis." On the *left* (**a**, *LBP*, *ThinPrep*) is a group of anucleate squames at low power. On the *right* (**b**, *LBP*, *ThinPrep*) are anucleate, mature polygonal squamous cells with ghostlike "nuclear holes" ("**b**" is reprinted with permission from Williamson et al. [15])

#### 2.4.2.3 Explanatory Notes

The Bethesda classification and interpretation of such keratotic changes depends on the nuclear alterations present. Miniature squamous cells with small pyknotic nuclei and orangeophilic to eosinophilic cytoplasm ("parakeratosis") are a nonneoplastic reactive cellular change. However, single cells or cell clusters that demonstrate pleomorphism of nuclear shape and/or increased nuclear size and/or chromasia ("atypical parakeratosis," "dyskeratosis," or "pleomorphic parakeratosis") are representative of an epithelial cell abnormality. Such findings should be categorized as atypical squamous cells (ASC) or as a squamous intraepithelial lesion (SIL), depending on the degree of cellular abnormality identified (see Figs. 4.15, 4.16, 5.8, 5.9, 5.26, 5.42, 5.43, 5.44, 5.56, and 5.59) [14].

Anucleate, but otherwise unremarkable mature, squamous cells ("hyperkeratosis") constitute a nonneoplastic cellular change. Inadvertent contamination of the specimen with vulvar material may also introduce anucleate squamous cells into the cervical cytology specimen. When extensive hyperkeratosis is present, an underlying neoplastic or nonneoplastic process may be associated and should be considered when evaluating such cytologic preparations [15]. Thick plaques of pleomorphic anucleate squamous cells with irregular contours may rarely be the only clue to an underlying squamous cell carcinoma [16]. Similar to parakeratosis, hyperkeratosis alone does not constitute a specific interpretive category.

### 2.4.3 Tubal Metaplasia (Figs. 2.18–2.21)

#### 2.4.3.1 Definition

Tubal metaplasia is a metaplastic phenomenon in which the normal endocervical epithelium is replaced by an epithelium that recapitulates that of the normal fallopian tube. This metaplastic epithelium includes several cell types (ciliated cells, peg cells, and goblet cells) [17] (Fig. 2.18). Tubal metapalsia is a frequent finding in the upper endocervical canal/lower uterine segment.

#### 2.4.3.2 Criteria

Columnar ciliated endocervical cells that may occur in small groups or as pseudostratified crowded groups (Figs. 2.19 and 2.20).

Nuclei are round to oval and may be enlarged, pleomorphic, and often hyperchromatic.

Chromatin is evenly distributed and nucleoli are usually not seen.

Nuclear to cytoplasmic ratio can be high.

The cytoplasm may show discrete vacuoles or goblet cell change (Fig. 2.21).

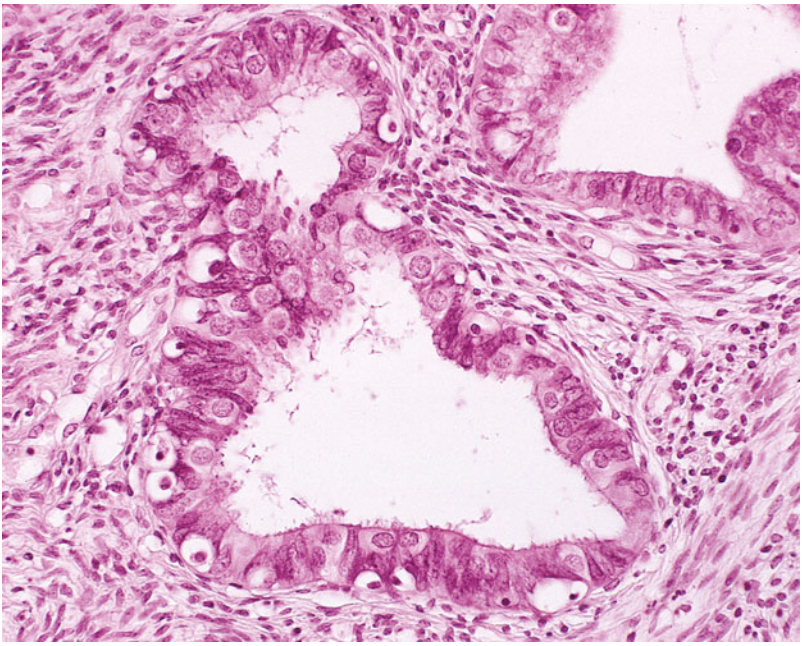

**Fig. 2.18** Tubal metaplasia (histology, H&E). Endocervical gland with tubal metaplasia amid cervical stroma. The ciliated cells of tubal metaplasia show prominent terminal bars at the base of the cilia

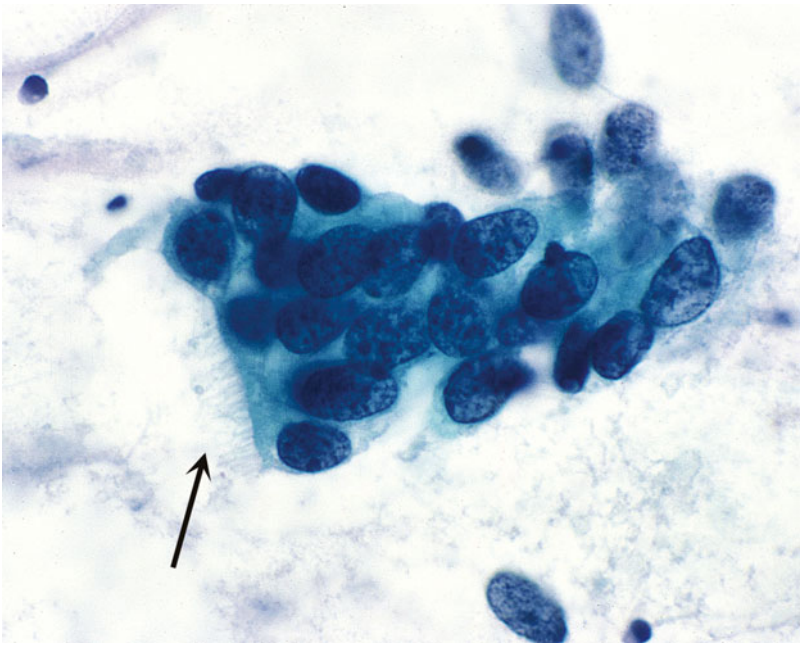

**Fig. 2.19** Tubal metaplasia (*CP*). Ciliated cells derived from tubal metaplasia. Note terminal bar and cilia at left edge (*arrow*). Tubal metaplasia shows prominent pseudostratification and can have enlarged nuclei that make it a look-alike for endocervical adenocarcinoma in situ

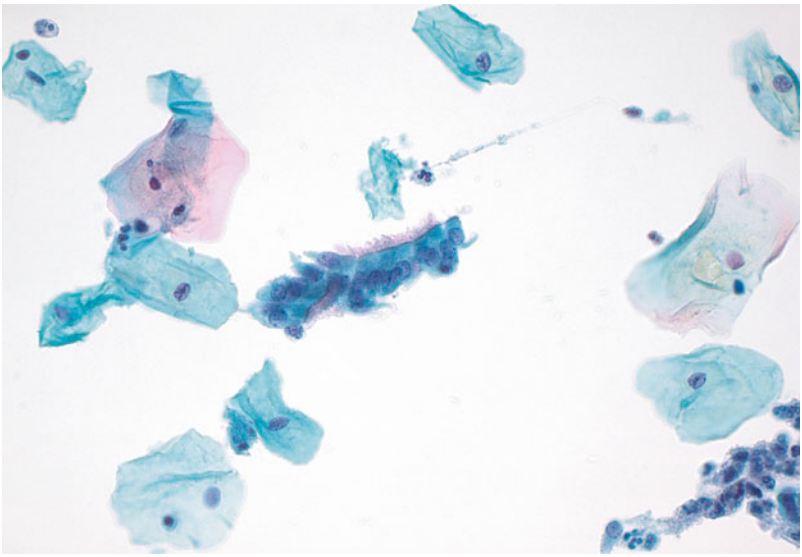

**Fig. 2.20** Tubal metaplasia (*LBP*, Thin Prep). A linear array of cells showing tubal metaplasia

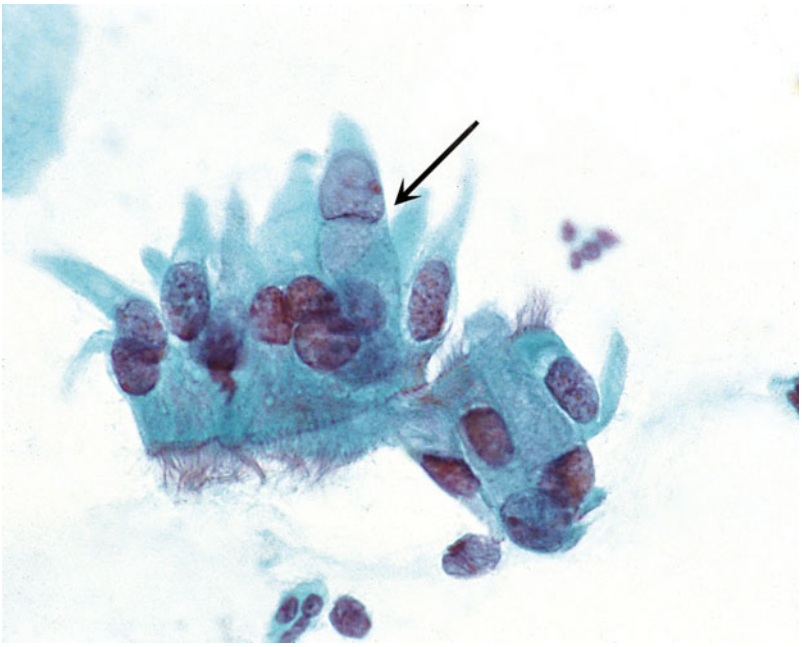

**Fig. 2.21** Tubal metaplasia (*CP*). Ciliated columnar endocervical cells. A goblet cell is seen at the center with its nucleus closer to the top of the image (*arrow*)

Presence of cilia and/or terminal bars is characteristic, but single ciliated cells in isolation are not sufficient for the designation.
Mitoses may be present.

#### 2.4.3.3 Explanatory Notes

Tubal metaplasia is among the most common benign processes to be misinterpreted as endocervical atypia or neoplasia. This is due to the tendency toward enlarged nuclei, crowded nuclei, and nuclear stratification. However, terminal bars and cilia establish a benign interpretation (see Figs. 6.12, 6.13, and 6.14).

### 2.4.4 Atrophy (Figs. 2.22–2.27)

#### 2.4.4.1 Definition

Atrophy is a normal aging phenomenon associated with lack of hormonal stimulation that leads to thinned epithelium consisting of only immature basal/parabasal cells (Fig. 2.22).

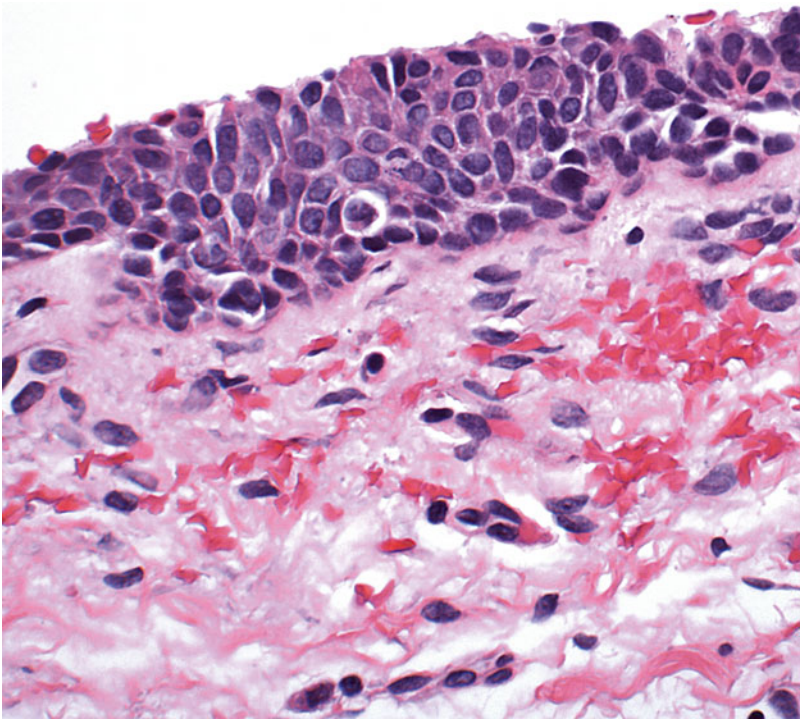

**Fig. 2.22** Atrophy (histology, H&E). The cervical squamous epithelium is remarkably thinned and made up entirely of parabasal cells. This is a consequence of waning hormonal support. In such cases, p16 immunostain would be negative

### 2.4.4.2 Criteria

Flat, monolayer sheets of parabasal-like cells with preserved nuclear polarity and little nuclear overlap in individual focal planes (Fig. 2.23).

Dispersed parabasal-type cells may predominate.

Generalized nuclear enlargement may occur with a slight increase in nuclear to cytoplasmic ratio.

Intermediate cells tend to be normochromatic, but parabasal-type cells may have mild hyperchromasia and tend to have more elongated nuclei.

Chromatin is uniformly distributed and nuclear contours are regular.

Autolysis may result in the presence of stripped nuclei.

An abundant inflammatory exudate and basophilic granular background that resembles tumor diathesis may be present in examples of extreme atrophy (atrophic vaginitis) (Figs. 2.24 and 2.25).

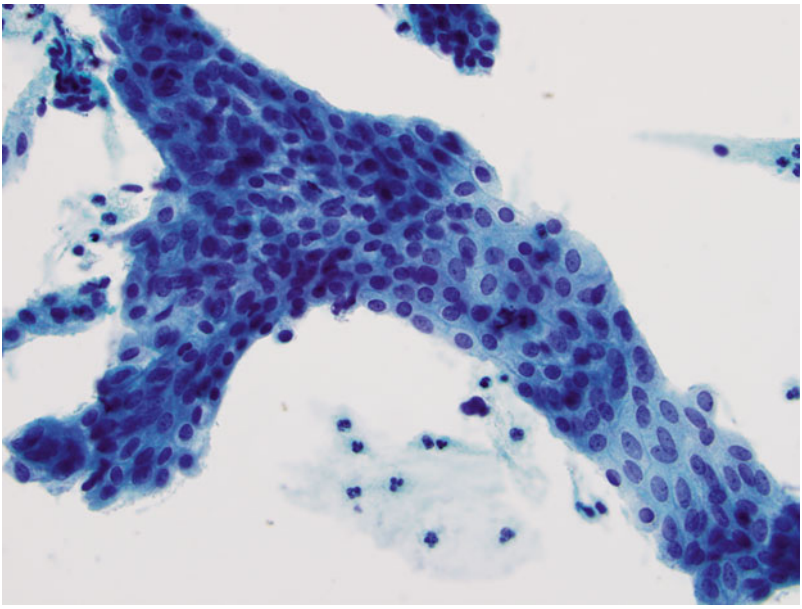

**Fig. 2.23** Atrophy (*LBP*, *ThinPrep*). Note flat, monolayer sheet of parabasal-type cells, with preserved nuclear polarity

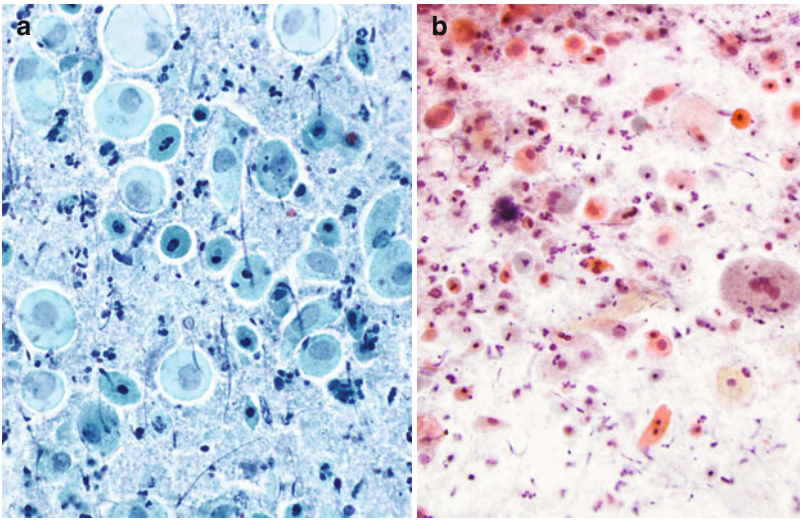


**Fig. 2.24** Atrophy with inflammation ("atrophic vaginitis") (*CP*). Note the classic finding of granular debris in background, degenerating parabasal cells, and polymorphonuclear leukocytes. (**a**) "Blue blobs" and pseudoparakeratosis are also seen in atrophic vaginitis, the former being more prominent in conventional preparations (**b**)

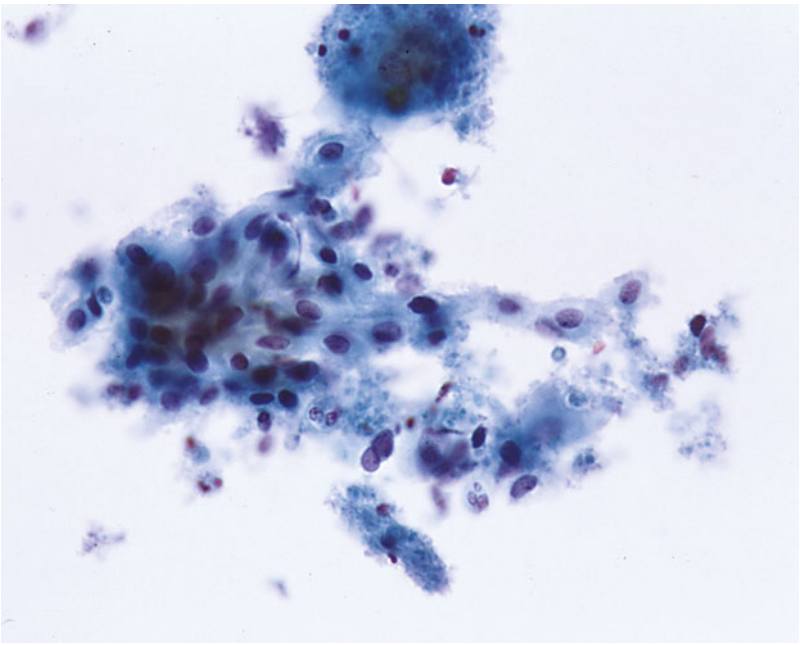

**Fig. 2.25** Atrophy with inflammation (atrophic vaginitis) (*LBP*, *ThinPrep*). In liquid-based preparations, the granular debris is often clumped and adheres to atrophic cell clusters in a pattern that may mimic "clinging tumor diathesis" (see Fig. 5.58). Attention to cellular features is crucial to avoid overinterpretation

Globular collections of basophilic amorphous material (blue blobs) reflect either degenerated parabasal cells or inspissated mucus.

Degenerated orangeophilic or eosinophilic parabasal cells with nuclear pyknosis resembling "parakeratotic" cells may be present ("pseudoparakeratosis") (Fig. 2.26).

Histiocytes varying in size and shape and containing multiple, round to epithelioid nuclei and foamy or dense cytoplasm may be seen (Fig. 2.27).

### Preparation-Specific Criteria

*Liquid-Based Preparations*:

Less nuclear enlargement than in conventional preparations due to immediate fixation, rounding up, and a lack of flattening on the slide.

Naked nuclei from autolysis may be reduced in number.

Granular background material tends to clump rather than be dispersed, yielding a "cleaner" background (Fig. 2.26); however, the clumps may "cling" to the cells and make it difficult to visualize individual cells (Fig. 2.25).

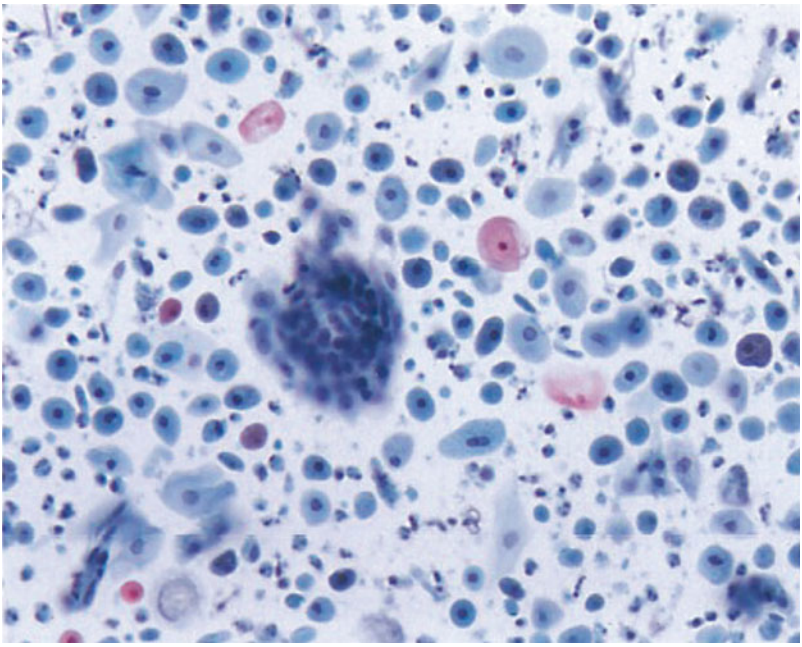

**Fig. 2.26** Atrophy (*LBP*, *SurePath*). Note more dissociation of parabasal cells in a relatively clean background

*Conventional Preparations*:
Air-drying artifact may result in more prominent cellular enlargement.
Granular basophilic "dirty" background of debris, with more "blue blobs" (Fig. 2.24).

### 2.4.4.3 Explanatory Notes

Atrophic changes are due to decreased hormonal support of epithelial tissues. The degree of atrophic change is thus highly variable, reflecting the differing levels of hormonal support that may be present. Cytomorphology can range from intermediate cell predominant to parabasal predominant to deeply atrophic (atrophic vaginitis) patterns in postmenopausal women. These differences may reflect alternate sources of endogenous estrogen or the presence of exogenous estrogenic substances.

Reporting of atrophic changes is variable and poorly reproducible [18]. Atypical cellular changes associated with atrophy warrant an interpretation of atypical squamous cells (ASC). Although cytology should be judged on its own morphologic merits, a patient is more likely to have significant disease in face of a history of previous cervical abnormality or a prior positive high-risk HPV test. In addition, atrophy may coexist with dysplasia or neoplasia, and the diffusely increased

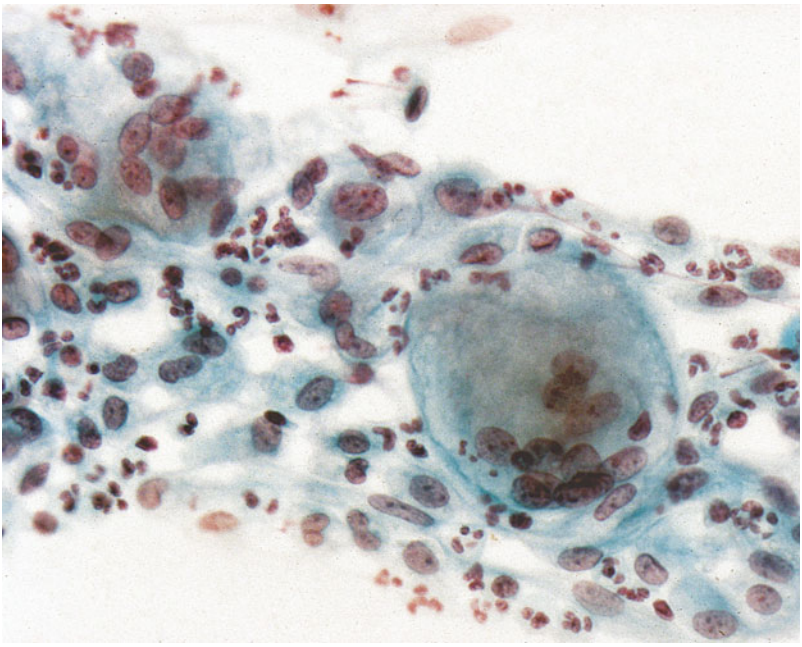

**Fig. 2.27** Atrophy with multinucleated giant cells (*CP*). Multinucleated histiocytic giant cells are a nonspecific finding and are often seen in postmenopausal and postpartum specimens. They differ from other giant cells such as syncytiotrophoblast (Fig. 2.29b) and multinucleated cells in herpes infection (Fig. 2.63)

nuclear to cytoplasmic ratio of background parabasal/basal squamous cells can make identification of true abnormalities more challenging. As such, these cases should be reviewed with care. "Atrophic" changes may also be seen for weeks after parturition and other situations where estrogen and progesterone levels have decreased.

In postmenopausal and postpartum states, multinucleated histiocytes (giant cells) are often found in cervical samples associated with chronic inflammatory processes [19] (Fig. 2.27).

### 2.4.5 Pregnancy-Related Cellular Changes (Figs. 2.28–2.30)

During pregnancy, a variety of epithelial and non-epithelial cell changes can be identified in cervical cytology specimens. These changes can be misinterpreted as representing neoplastic abnormalities.

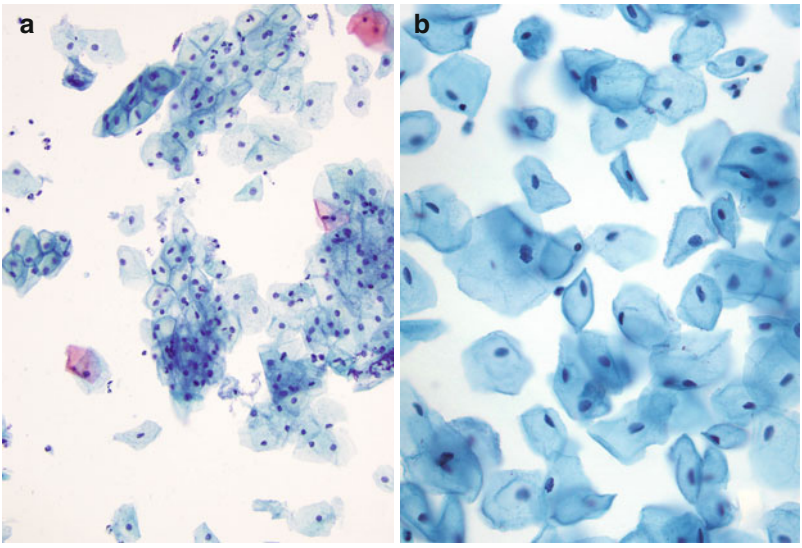


**Fig. 2.28** Pregnancy-related hormonal changes – navicular cells. In pregnant patients, squamous cells become laden with glycogen, and have a vaguely "boatlike" shape referred to as "navicular" cells (**a**) *left*, *LBP*, *ThinPrep*, and (**b**) *right*, *LBP*, *SurePath*

### 2.4.5.1 Hormonal Changes (Fig. 2.28)

The altered hormonal stimulation in pregnancy leads to incomplete maturation of the squamous epithelium resulting in an intermediate cell – dominant pattern. In association with this pattern, a particular appearance of the intermediate squamous cell showing prominent glycogen with a flattened "boatlike" appearance is common. This appearance is referred to as "navicular" cells. When progesterone secretion is prolonged (as in pregnancy), the navicular cells have greatly thickened borders and can form dense clusters (Fig. 2.28).

#### 2.4.5.1.1 Criteria

Boat-shaped intermediate cells.
Abundant basophilic to clear cytoplasm, rich in glycogen.
Nuclei are vesicular and have a delicate chromatin structure.

### 2.4.5.2 Decidua (Fig. 2.29a)

Decidual cells are present in pregnancy and during the postpartum period. These cells are derived from hormonally stimulated endocervical or endometrial stroma.

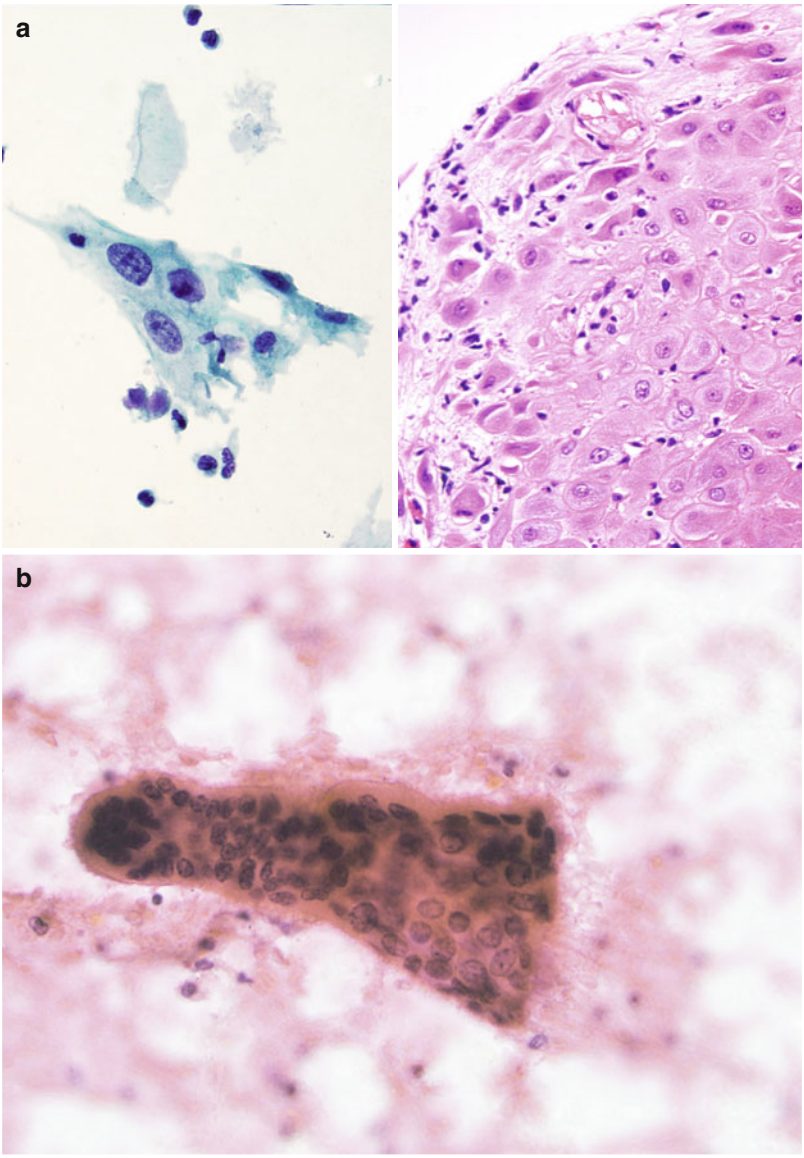


**Fig. 2.29** (**a**) Pregnancy-related cellular changes, decidua. Decidual change involving the cervical stroma can be sampled and resemble epithelial cell abnormalities, both LSIL and HSIL (See Fig. 5.53). *On the upper left* (*LBP*, *ThinPrep*) are cells that are loosely cohesive, approximately the size of mature squamous cells, with soft, ill-defined cytoplasm, and nuclei that have nucleoli and pale, finely granular, evenly distributed chromatin. They can be misinterpreted as reactive squamous cells or LSIL if one is not aware of the history of pregnancy or recent delivery. On the upper right (histology, H&E) is the corresponding histology showing decidual change. Note the resemblance to the cytology *on the upper left*. Pregnancy-related cellular changes, syncytiotrophoblast (*CP*). (**b**) The placental-derived syncytiotrophoblast is a unique cell that can have 50 or more nuclei and tends to be elongated with granular cytoplasm. Other multinucleated cells that can be seen in cervical cytology include multinucleated histiocytes in postmenopausal and postpartum women and cells infected with herpes virus

#### 2.4.5.2.1 Criteria

Cells occur singly and rarely in small clusters.
Cytoplasm is abundant, granular, or finely vacuolated and there may be cytoplasmic processes.
Nuclei are 35–50 $\mu m^2$ in area and may be lobulated or multinucleated.
Chromatin is fine, evenly distributed, and normochromatic to hyperchromatic.
Nuclear membranes are generally smooth.
Nucleoli are usually prominent and basophilic [20, 21].

### 2.4.5.3 Cytotrophoblast

Cells of cytotrophoblastic origin are derived from the placenta in late pregnancy and in the postpartum period. Rarely, they can be present for months after delivery. Cytotrophoblast are rarely identified as such. They may resemble small squamous metaplastic or endometrial cells, as well as high-grade squamous intraepithelial lesion cells. When recognized, the background often has either findings of exodus or other elements of pregnancy, e.g., decidua or syncytiotrophoblasts, which gave a clue to their identity [20].

#### 2.4.5.3.1 Criteria

Typically single cells, occasionally in small clusters.
Cells are small with enlarged nuclei, high nuclear to cytoplasmic ratios, and hyperchromasia. Chromatin is evenly distributed.
Cytoplasm is scant and may have prominent vacuoles.
Background often highly inflamed and sometimes bloody.

### 2.4.5.4 Syncytiotrophoblast (Fig. 2.29b)

Syncytiotrophoblastic cells are derived from fusion of cytotrophoblastic cells. They can be identified in cervical cytology specimens in late pregnancy and postpartum periods. They can rarely be present for months after delivery.

#### 2.4.5.4.1 Criteria

Large, multinucleated cells with up to 50 or more nuclei (Fig. 2.29b).
Nuclei are normochromatic with even chromatin distribution but often have irregular nuclear contours.
Tapering of granular cytoplasm at one end of cell.

### 2.4.5.5 Arias-Stella Reaction (Fig. 2.30)

Arias-Stella reaction is a benign process which involves glandular epithelial cells (either endocervical or endometrial) and is found in association with pregnancy or occasionally

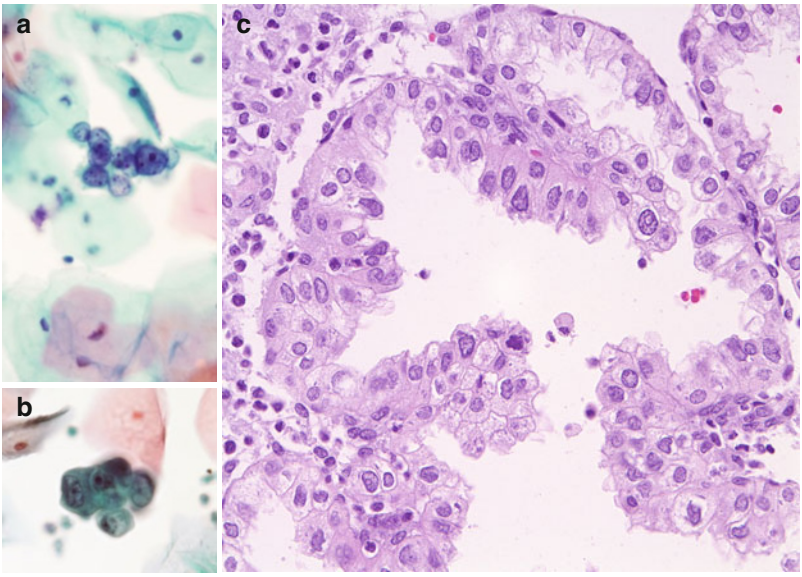


**Fig. 2.30** Pregnancy-related cellular changes, Arias-Stella reaction. The upper and lower left images (**a**, **b**, *LBP*, *SurePath*) show groups of stimulated endometrial glandular epithelium that could be mistaken for a glandular epithelial abnormality. The histology (**c**, *right*, H&E) demonstrates the exuberant variation in epithelial nuclear morphology due to hormonal stimulation during pregnancy

in nonpregnant hormonally stimulated individuals. In histologic specimens, Arias-Stella reaction manifests as pleomorphism of size and shape in glandular cell nuclei, often with bizarre forms, in association with a characteristic smudgy chromatin pattern.

#### 2.4.5.5.1 Criteria

Glandular cells, singly or in clusters.
Cytoplasm is of variable quantity and may be vacuolated.
Nuclear to cytoplasmic ratio variable, but often high.
Nuclei are large, hyperchromatic with contour irregularities (grooves and pseudoinclusions), and granular to smudgy chromatin.
Multiple prominent nucleoli.
Background is usually inflammatory, often with leukophagocytosis [22].

### 2.4.5.6 Explanatory Notes

The changes seen in pregnancy can be misinterpreted as being of preneoplastic or neoplastic origin, primarily because they may show concerning nuclear features [20]. It is important to be aware of the patient's pregnant or postpartum status to avoid overinterpretation of these findings. Even if the clinician does not provide this

information, the finding of one or more of the characteristic features noted above should elicit query regarding pregnancy or postpartum state, particularly if only a few cells with the changes are present and if the features noted are not classic for epithelial neoplasia.

Squamous alterations are common during pregnancy. Reactive and metaplastic squamous changes are often present. In addition, increased glycogenation can result in cytoplasmic clearing in intermediate (navicular) cells that may mimic koilocytic change; however, the clearing due to glycogenation is typically diffuse, involving all or most of the cell, and lacks the sharp "cookie cutter" edges of koilocyte vacuoles (See Figs. 5.4, 5.5, and 5.6). More importantly, the cells lack nuclear atypia, necessary for the interpretation of a squamous preneoplastic abnormality. Reactive glandular cell alterations are also commonly encountered in cervical cytology specimens from pregnant women and have features similar to reactive/reparative endocervical alterations from other causes.

Decidual cells can be misinterpreted as ASC-US or LSIL when cytoplasm is abundant or ASC-H or HSIL when there is a high nuclear to cytoplasmic ratio. However, at low magnification, these cells are typically larger than dysplastic squamous cells, particularly those of high-grade lesions. Additionally, the nuclear contours are typically smooth, the chromatin is finely granular and evenly distributed, and nucleoli are usually prominent [20, 21].

Cytotrophoblast cells most commonly resemble reactive squamous cells but can occasionally be mistaken for HSIL or ASC-H, as the nuclei are large and hyperchromatic and the nuclear to cytoplasmic ratios are often high. However, chromatin texture is fine and evenly distributed. Nucleoli, when present, also support a benign interpretation. Syncytiotrophoblast is most likely to be mistaken for herpes infection, but the nuclei lack the ground-glass inclusions seen in herpetic cytopathic effect and show some heterochromatin. The tapering of the cytoplasm at one end (where the cell was attached to the placenta) and "bunching up" of nuclei may be helpful in distinguishing syncytiotrophoblast from other multinucleated cells.

## 2.5 Other Nonneoplastic Findings

### 2.5.1 Reactive/Reparative Cellular Changes

#### 2.5.1.1 Definition

Reactive cellular changes which are associated with inflammation, physical or chemical trauma, radiation, IUD irritation, or other nonspecific causes.

### 2.5.2 Reactive Cellular Changes Associated with Inflammation (Includes Typical Repair) (Figs. 2.31–2.40)

#### 2.5.2.1 Criteria

Nuclear enlargement of a variable degree (Figs. 2.31).
Nuclei are typically nonoverlapping.
Endocervical cells may show greater nuclear enlargement (Figs. 2.32 and 2.33).
Occasional binucleation or multinucleation may be observed.
Nuclear outlines are smooth, round, and uniform.
Nuclei may appear vesicular and hypochromatic (Figs. 2.34).
Mild hyperchromasia may be present, but the chromatin structure and distribution remain uniformly finely granular (Fig. 2.35).
Prominent single or multiple nucleoli may be present.
Cytoplasmic boundaries are well defined.
Cytoplasm may show polychromasia, vacuolization, or perinuclear halos but without peripheral thickening (Figs. 2.36 and 2.37).
Enlarged cells often form cohesive sheets that interdigitate in a classic "school of fish" architecture or may be mechanically distorted by sampling and elongate to form "taffy pull" cytoplasmic appendages (Figs. 2.38, 2.39, and 2.40).

**Preparation-Specific Criteria**

*Liquid-Based Preparations:*

Both squamous and endocervical reparative groups are more rounded and three-dimensional and thus darker due to light having to pass through more cytoplasmic and nuclear material. The edges of cells are better fixed and show less streaming relative to conventional preparations (Fig. 2.38).

*Conventional Preparations:*

Reparative changes may be more pronounced as cells flatten out against the slide. Inflammatory background tends to be more pronounced.

#### 2.5.2.2 Explanatory Notes

Reparative changes ("typical repair") may involve mature squamous, squamous metaplastic, or columnar epithelium. Cognizance of criteria for reactive/reparative changes is important for stratifying the boundaries between NILM and epithelial abnormalities. Reactive and reparative processes can show wide variation in nuclear area. This size variability can range from the normal area of squamous or endocervical cell nuclei to markedly enlarged, often within the same cellular

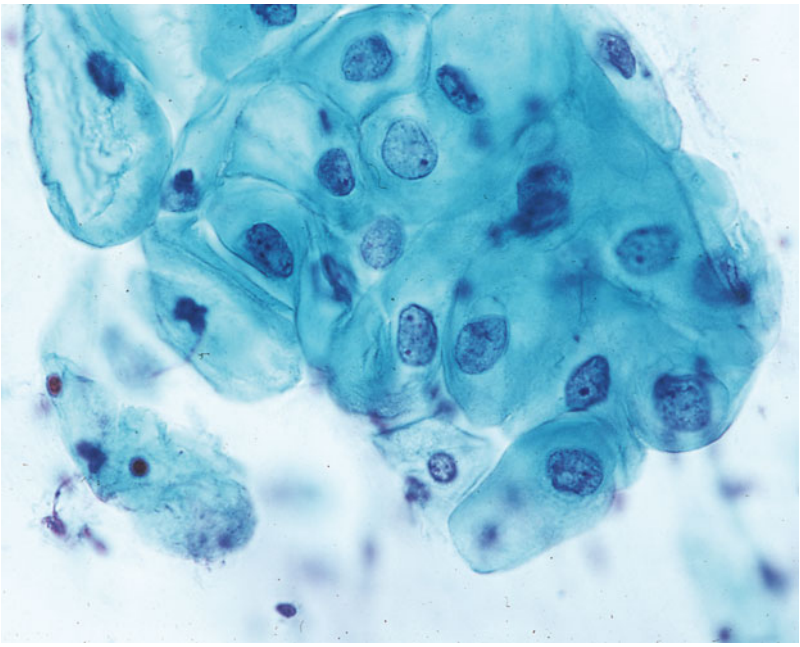

**Fig. 2.31** Reactive-reparative cellular changes (*CP*). These reactive squamous epithelial cells display mild nuclear enlargement without any significant chromatin abnormalities (Reprinted with permission from Kurman RJ. [39])

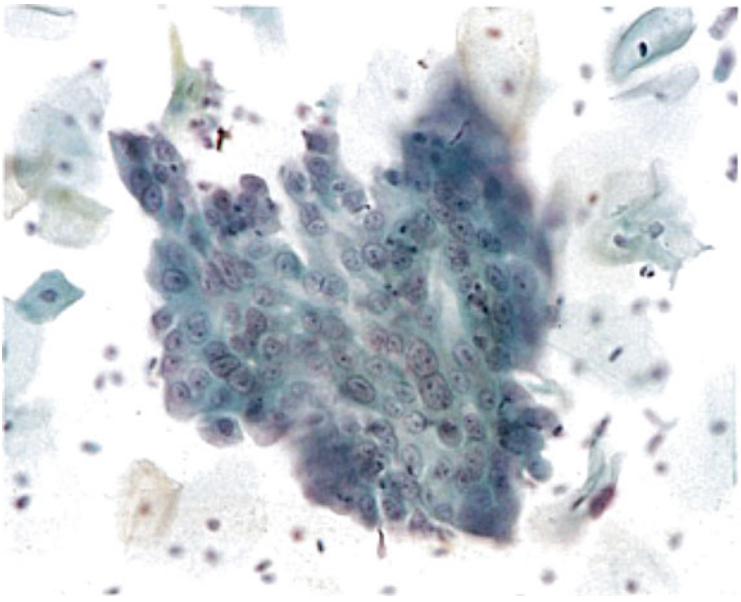

**Fig. 2.32** Reactive-reparative cellular changes: reactive endocervical cells (*LBP*, *SurePath*). Thirty-two-year-old woman. Variation in nuclear size, prominent nucleoli, and rare intracytoplasmic polymorphonuclear leukocytes are seen; these features are consistent with endocervical repair. Follow-up cytology was NILM

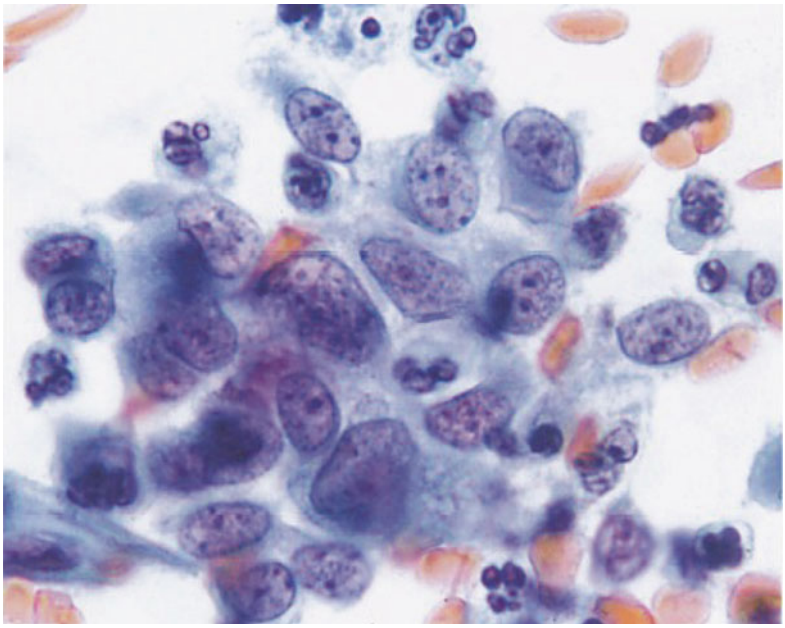

**Fig. 2.33** Reactive-reparative cellular changes: reactive endocervical cells (*CP*). A 22-year-old woman status post loop electrosurgical excision procedure (LEEP) 6 months earlier for high-grade cervical intraepithelial neoplasia (CIN). Endocervical cells show variable increase in nuclear size, prominent nucleoli, and fine chromatin. Concurrent biopsy was benign

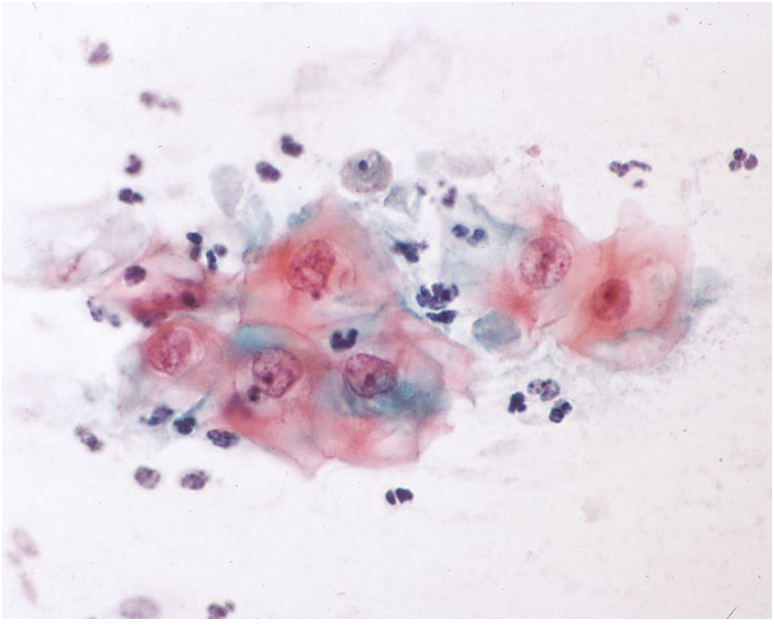

**Fig. 2.34** Reactive-reparative cellular changes: reactive squamous cells (*CP*). A 26-year-old woman, day 14 of menstrual cycle with mild vaginal discharge. Squamous cells show mild nuclear enlargement with nuclear hypochromasia, perinuclear halos, and cytoplasmic polychromasia resulting in a "moth-eaten" appearance. Trichomonads are seen in the background. Follow-up was NILM

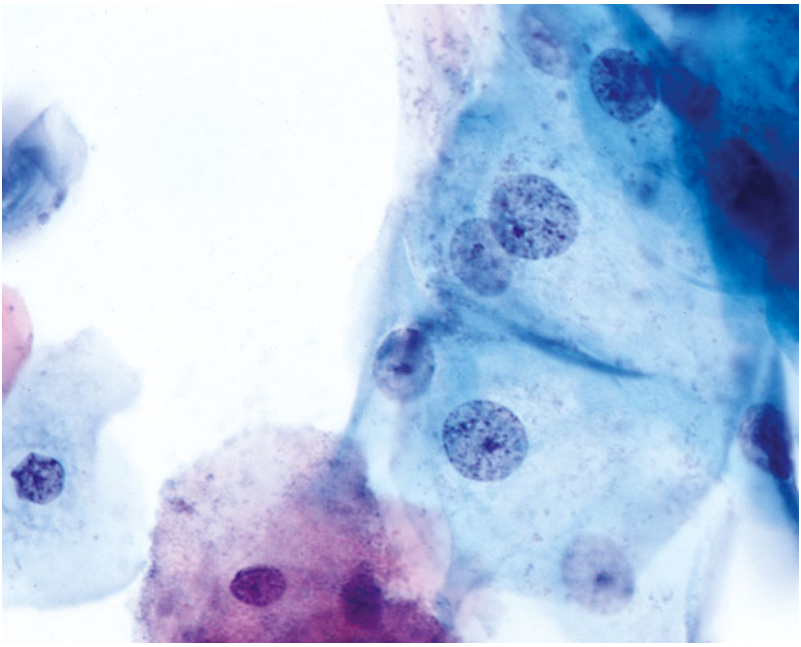

**Fig. 2.35** Reactive-reparative cellular changes: reactive squamous cells (*LBP*, *ThinPrep*). Routine screen of a 32-year-old woman. Although there is nuclear enlargement in the cells on the *right side*, the smooth nuclear contours and finely distributed chromatin favor reactive change over ASC-US

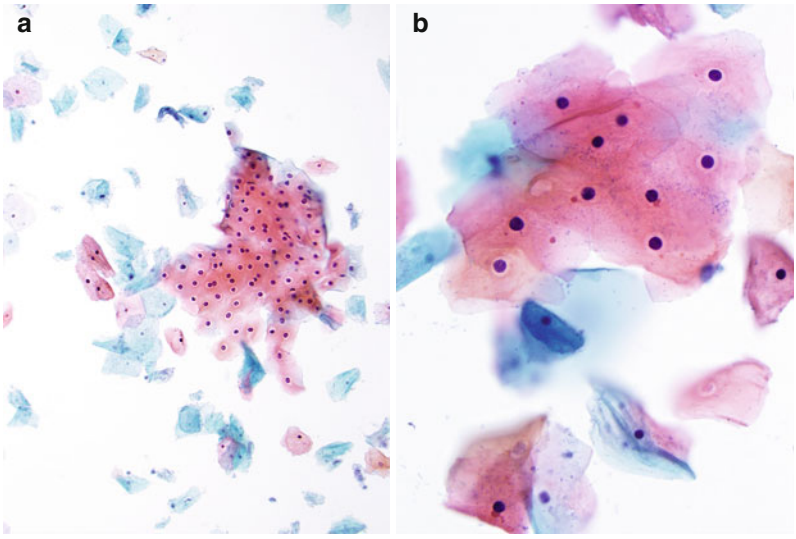


**Fig. 2.36** Reactive-reparative cellular changes: inflammatory halos. Examples of reactive perinuclear halos induced by organisms/inflammation such as seen in trichomonas infection. The images demonstrate reactive squamous cells showing small perinuclear halos that should be differentiated from koilocytic clearing seen in HPV cytopathic effect. *On the left* (**a**) is a low power from an *LBP*, *ThinPrep* and *on the right* (**b**) is a higher-power image obtained from a conventional preparation

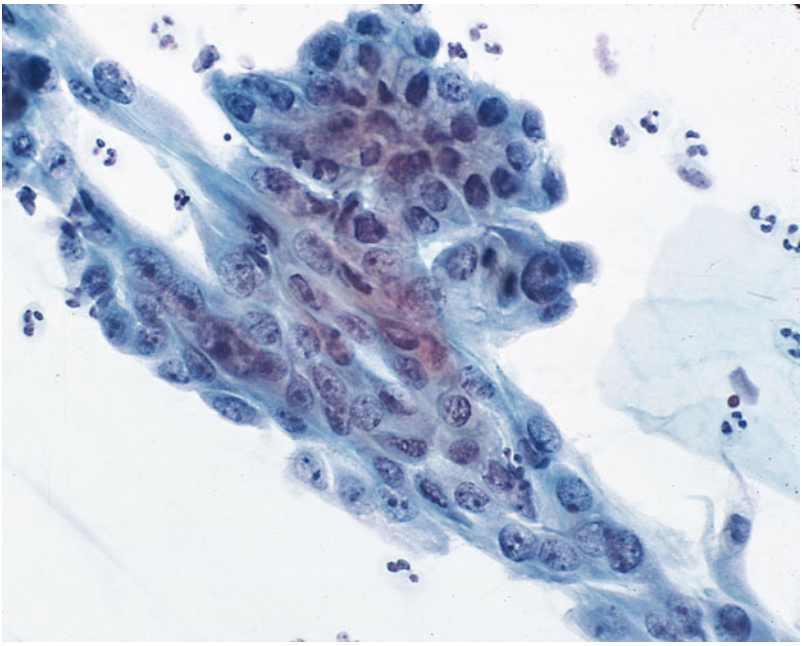

**Fig. 2.37** Reactive-reparative cellular changes: repair (*CP*). A 67-year-old woman with uterine prolapse. Flat, monolayer sheet of reparative cells with distinct cytoplasmic borders, streaming nuclear polarity, and a prominent nucleolus in almost every cell. Reactive group of endocervical cells seen at top center

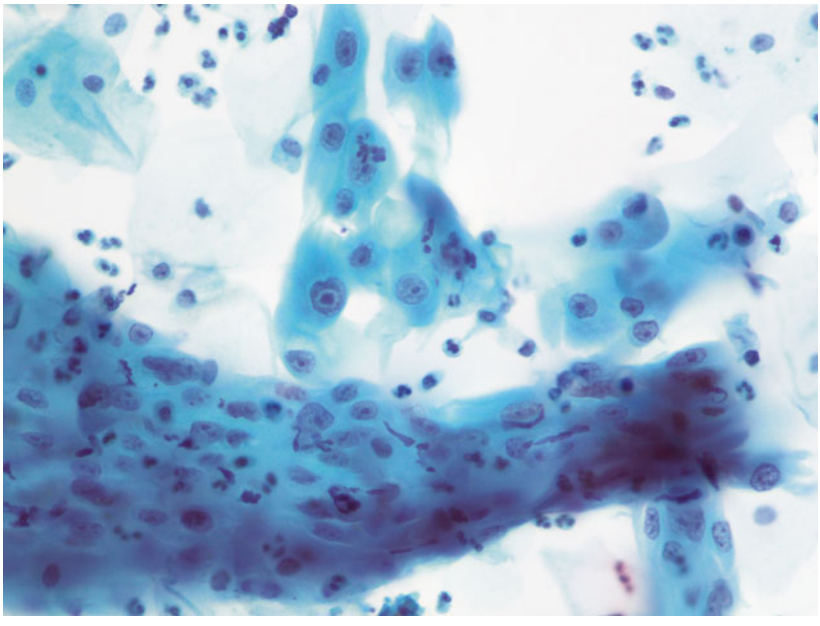

**Fig. 2.38** Reactive-reparative cellular changes: repair (*LBP*, *SurePath*). Thirty-two-year-old woman. Changes are similar to those seen on *CPs*, but cell streaming may be less apparent due to rounding of cell clusters. Note the intracytoplasmic polymorphonuclear leukocytes, another feature seen in repair. Compare to Figs. 2.39 and 2.40

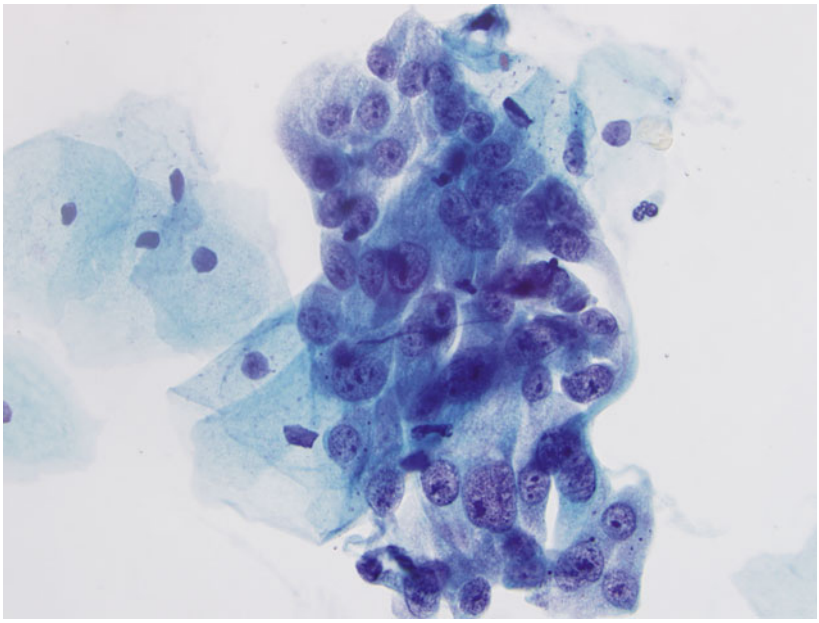

**Fig. 2.39** Reactive-reparative cellular changes: repair (*LBP*, *ThinPrep*). Cohesive group of reactive endocervical cells stimulated by factors related to inflammation and infection. Nucleoli are prominent

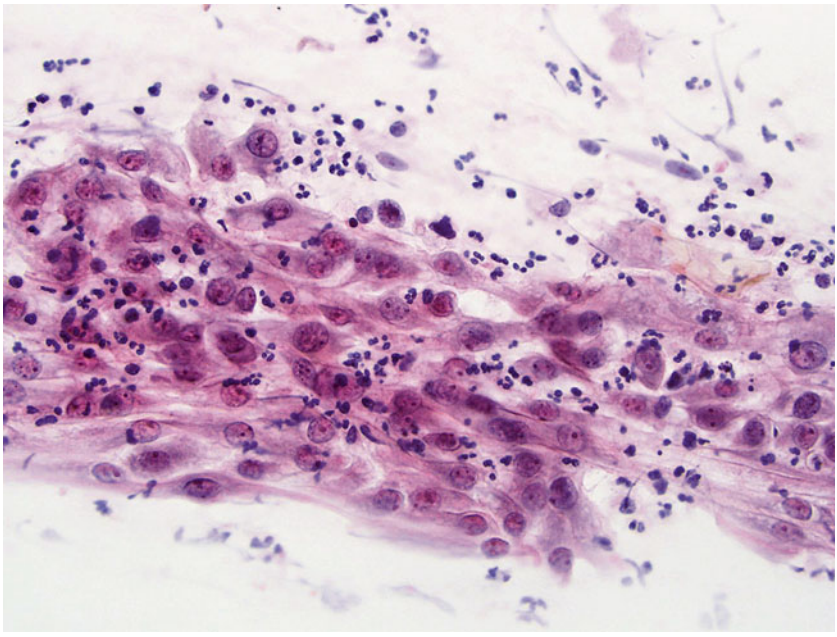

**Fig. 2.40** Reactive-reparative cellular changes: repair (*CP*). Example of cytoplasmic cohesion and streaming in repair. Note intracytoplasmic polymorphonuclear leukocytes. The streaming and interdigitation of cells has been likened to a "school of fish." Also seen in Fig. 2.37

group. In some instances, the nuclear size may even fall into the range noted in SIL or cancer. In general, round nuclear contours, even chromatin distribution, nucleoli, cellular cohesion with "school of fish" or "taffy pull" cytoplasmic features, and overall uniform cellular morphology favor a nonneoplastic process. In any preparation type, repair should have a paucity of isolated cells. When a combination of anisonucleosis, irregularities in chromatin distribution, nuclear contour irregularities, or variation in size and shape of nucleoli are present – features of so-called atypical repair – the differential diagnosis widens to include not only reactive conditions but also squamous intraepithelial lesions and even invasive cancers. When present such changes may be better categorized as "atypical glandular cells" (AGC) or "atypical squamous cells" (ASC-US or ASC-H) (see Figs. 5.66, 4.17 and 4.18).

### 2.5.3 Lymphocytic (Follicular) Cervicitis (Figs. 2.41 and 2.42)

Lymphocytic cervicitis (follicular cervicitis) is a form of chronic cervicitis that results in the formation of mature lymphoid follicles in the subepithelium of the cervix. These subepithelial lymphocytes may be sampled in the course of obtaining a cervical specimen.

#### 2.5.3.1 Criteria

Polymorphous population of lymphocytes with or without tingible body macrophages.

**Preparation-Specific Features**

*Liquid-Based Preparations*:

Lymphocytes more often appear as loosely aggregated clusters or scattered single lymphocytes in the background due to separation during processing (Fig. 2.41).

*Conventional Preparations*:

Lymphocytes are seen in clusters or streaming in strands of mucus (Fig. 2.42).

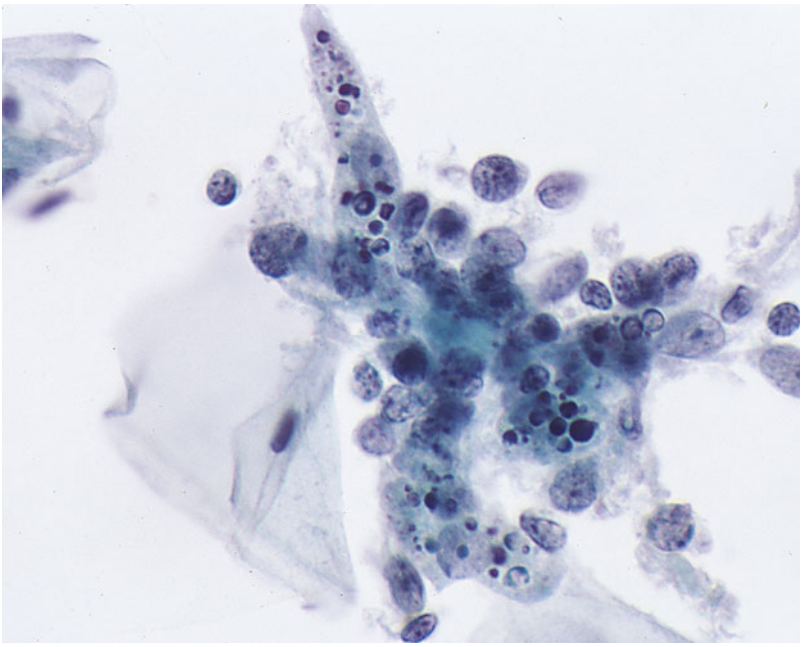

**Fig. 2.41** Reactive-reparative cellular changes: lymphocytic (follicular) cervicitis (*LBP*, *ThinPrep*). Note polymorphous population of lymphoid cells and tingible body macrophages; the lymphoid cells may clump on liquid-based preparations

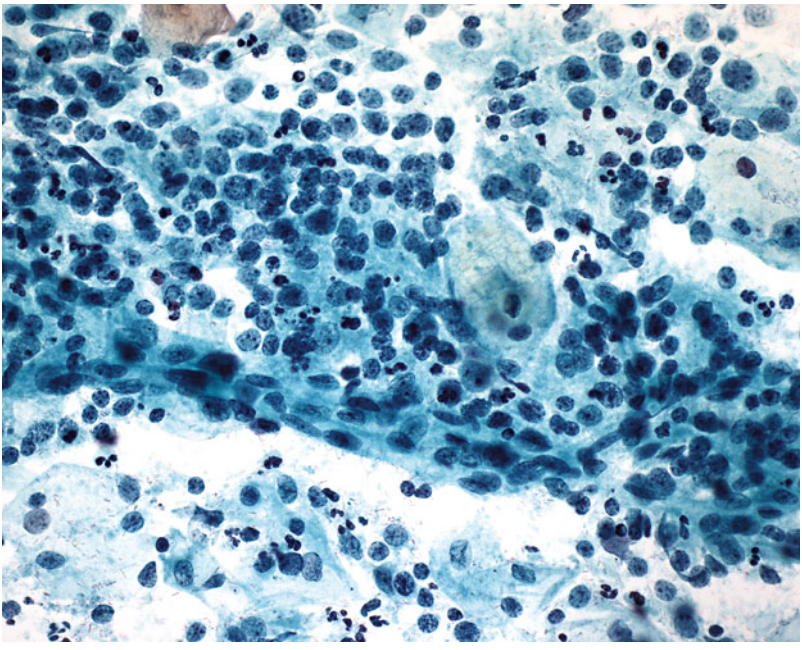

**Fig. 2.42** Reactive-reparative cellular changes: lymphocytic (follicular) cervicitis (*CP*). Abundant lymphoid cells with a tingible body macrophage located centrally

### 2.5.4 Reactive Cellular Changes Associated with Radiation (Figs. 2.43 and 2.44)

The effects of ionizing radiation on cells can lead to cytologic features which may be mistaken for neoplastic or preneoplastic conditions.

#### 2.5.4.1 Criteria

Cell size is markedly increased without a substantial increase in the nuclear to cytoplasmic ratio (Figs. 2.43 and 2.44).

Bizarre cell shapes may occur.

Nuclei may vary in size, with some cell groups having both enlarged and normal-sized nuclei.

Binucleation or multinucleation is common.

Mild nuclear hyperchromasia may be present.

Enlarged nuclei may show degenerative changes including nuclear pallor, wrinkling or smudging of the chromatin, and nuclear vacuolization.

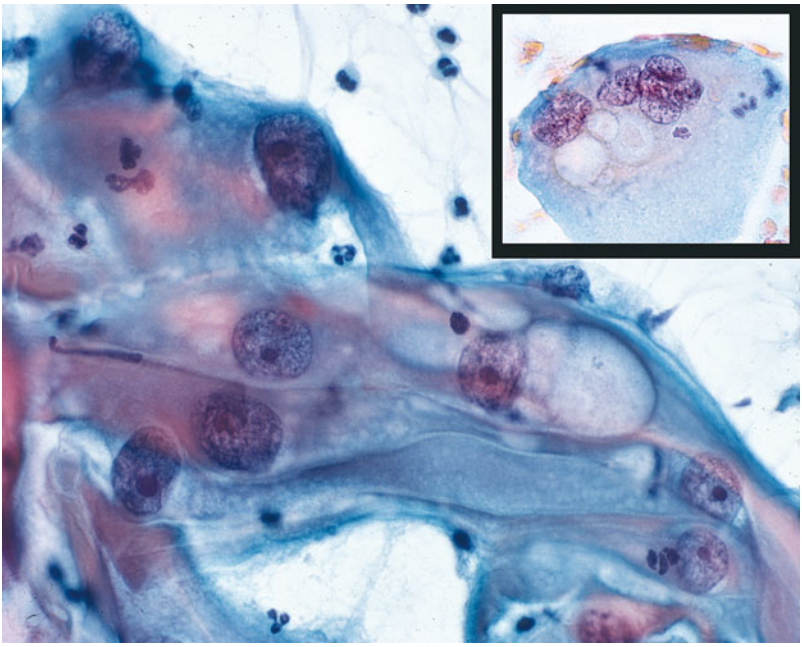

**Fig. 2.43** Reactive-reparative cellular changes: radiation (*CP*). Reactive cellular changes associated with radiation (*CP*). A 40-year-old woman with history of squamous cell carcinoma of the cervix who completed radiation therapy 8 weeks earlier. Cells with enlarged nuclei, abundant vacuolated polychromatic cytoplasm, mild nuclear hyperchromasia without coarse chromatin, and prominent nucleoli. Note multinucleation (*upper right corner inset*)

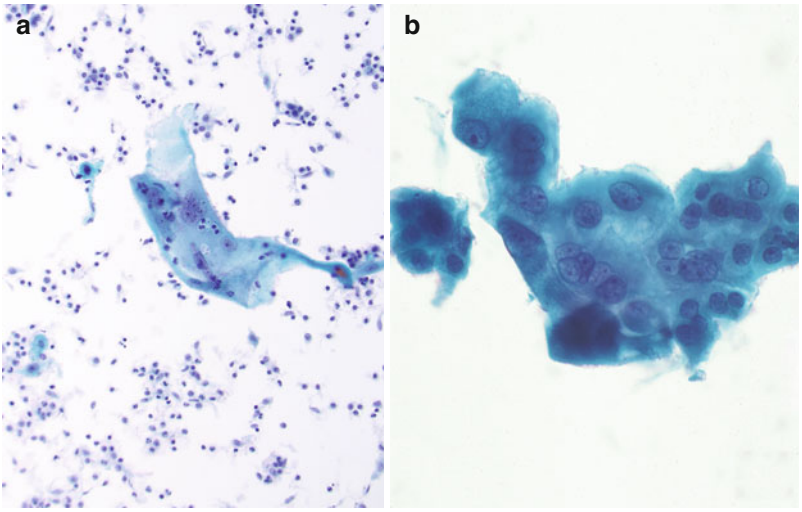


**Fig. 2.44** Reactive-reparative cellular changes: radiation. Low-power image of radiation changes in a squamous cell (**a**, *CP*). Note the irregularly shaped abundant cytoplasm and the streaming or "windblown" edges of the cell in a conventional preparation. Nuclei are typically enlarged and may be pale or become hyperchromatic as nuclear material condenses. Nucleoli are typically seen. In this case, numerous polymorphonuclear leukocytes are seen in the background. On the *left* (**b**, *LBP*, *ThinPrep*) radiated cells in liquid-based preparations do not tend to show the streaming and the cytoplasm is typically more dense. Nuclear degeneration and cytoplasmic vacuolization are common in both preparation types

Prominent single or multiple nucleoli may be seen if coexisting repair is present.
Cytoplasmic vacuolization and/or cytoplasmic polychromatic (two-color, amphophilic) staining and intracytoplasmic polymorphonuclear leukocytes may be seen.

#### Preparation-Specific Criteria

*Liquid-Based Preparations*
Cytoplasmic rounding, with less streaming.
Better preservation may attenuate the finding of bizarre cytoplasmic morphology.
Nucleoli may be more prominent.
Nuclei are often degenerated and may resemble the findings of low-grade squamous intraepithelial lesion [23].

### 2.5.4.2 Explanatory Notes

Acute radiation-induced changes, consisting of degenerated blood, bizarre cell forms, and cellular debris, generally resolve within 6 months following therapy. However, in some patients, chronic radiation-induced cellular changes may persist indefinitely. These chronic changes can include increases in cytoplasmic amount (cytomegaly), nuclear enlargement (karyomegaly) without nuclear to cytoplasmic ratio alteration, mild hyperchromasia, neutrophil invasion of cytoplasm (so-called engulfment), and

persistent polychromatic cytoplasmic staining. Certain chemotherapeutic agents may produce changes in cervical epithelial cells similar to those seen with acute and chronic radiation effects. It is important to note that bona fide squamous intraepithelial lesions in patients who have received pelvic radiation therapy will appear identical to such lesions in non-radiated patients. Care must be taken to not overinterpret specimens from radiated patients, especially in the face of perceived low-grade lesions associated with degenerated cells. Pelvic examinations and colposcopic procedures are more difficult in an irradiated pelvis which can complicate overall management.

### 2.5.5 Reactive Cellular Changes Associated with Intrauterine Contraceptive Device (Figs. 2.45–2.47)

The reactive glandular cell clusters occasionally seen in women with intrauterine devices (IUD) may represent either endometrial or endocervical columnar cells exfoliated as a result of chronic irritation by the device.

#### 2.5.5.1 Criteria

Glandular cells may be present singly or in clusters, usually of 5–15 cells, present in a clean background (Fig. 2.45, see Fig. 6.5).

The amount of cytoplasm varies, and frequently large vacuoles may displace the nucleus, creating a signet-ring appearance (Fig. 2.46).

Occasional single epithelial cells with increased nuclear size and high nuclear to cytoplasmic ratio may be present, which can be mistaken for HSIL/ASC-H (Fig. 2.47).

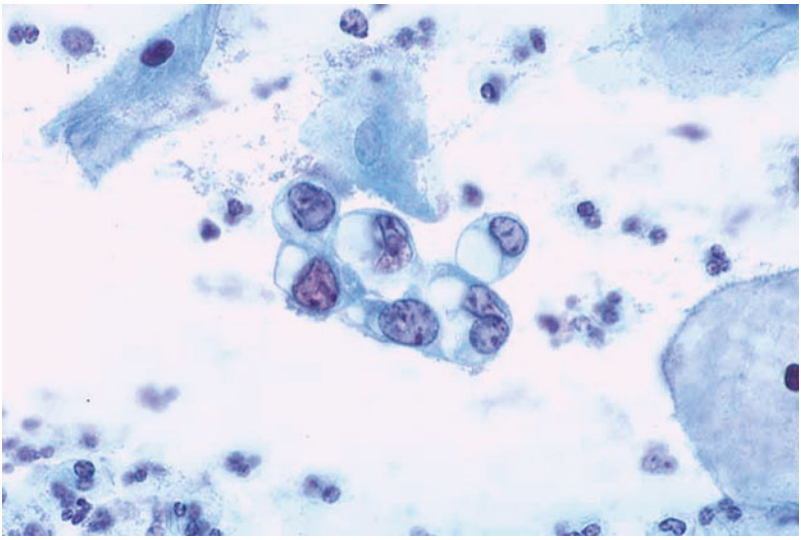

**Fig. 2.45** Reactive-reparative cellular changes: IUD (*CP*). Reactive cellular changes associated with intrauterine contraceptive device (IUD). Note small cluster of glandular cells with cytoplasmic vacuoles displacing nuclei

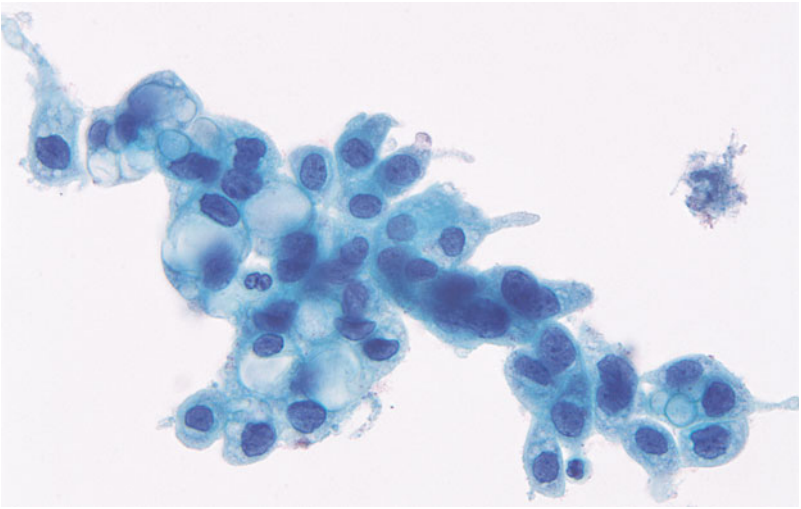

**Fig. 2.46** Reactive-reparative cellular changes: IUD (*LBP*, Thin Prep). In liquid-based preparations, cellular groups tend to be tighter but the same features of cytoplasmic vacuolization and reactive nuclear changes are present as are noted in *CP*

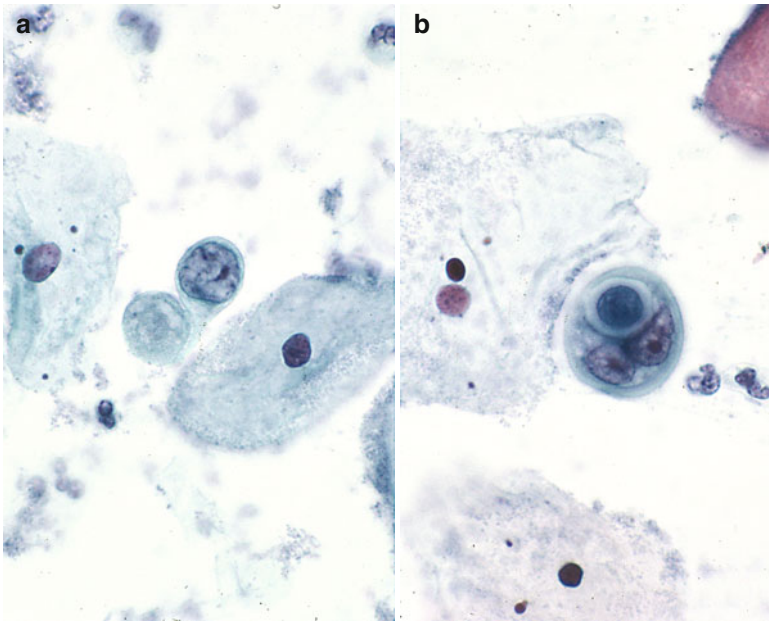


**Fig. 2.47** Reactive-reparative cellular changes: IUD (*CP*). Epithelial cells with a high nuclear to cytoplasmic ratio may mimic high-grade squamous intraepithelial lesion (HSIL) (*left*, **a**); however, the morphologic spectrum of abnormalities usually present with squamous intraepithelial lesions is absent. Presence of nucleoli in isolated cells with a high N/C ratio as seen in this cell (*right*, **b**) is not typical of HSIL. Obtaining a history of the presence of an IUD is important in the face of this type of abnormal morphology

Nuclear degeneration with a "wrinkled" chromatin appearance or nuclear "cracking" may be present.
Nucleoli may be prominent.
Calcifications resembling psammoma bodies are sometimes present.
Actinomyces-like organisms may be present in up to 25 % of cases (see Figs. 2.60 and 2.61).

#### 2.5.5.2 Explanatory Notes

Cells associated with the presence of an IUD may persist for several months after removal of the device. The characteristic changes fall into two distinct patterns. When present as three-dimensional clusters with vacuolated cytoplasm and nuclear changes, IUD-associated cells may resemble clusters of cells derived from adenocarcinoma of the endometrium, fallopian tube, or ovary (see Figs. 6.46, 6.47, 6.48, 6.49, 6.50, 6.51, 6.55, 6.56, and 6.57). When present as single atypical cells with higher nuclear to cytoplasmic ratios, IUD-associated cells mimic a high-grade squamous intraepithelial lesion. In general, the interpretation of adenocarcinoma should be made only with great caution in the presence of an IUD. In cases where the differential diagnosis includes HSIL or ASC-H, hrHPV testing may be helpful. If there is any doubt as to the significance of the cellular abnormalities, the cytopathologist should consider recommending removal of the IUD followed by repeat cervical cytology sampling.

## 2.6 Glandular Cells Status Post Hysterectomy (Figs. 2.48 and 2.49)

Occasionally benign-appearing glandular cells can be present in cervical cytology specimens from women who have undergone prior hysterectomy. While the origin of these benign cells may be obscure, the morphology should not be of concern for neoplasia [24].

### 2.6.1 Criteria

Benign-appearing endocervical-type glandular cells that cannot be differentiated from those routinely sampled from the endocervix (Figs. 2.48 and 2.49).
Goblet cell or mucinous metaplasia may be noted.
Round to cuboidal cells may resemble endometrial-type cells.

#### Preparation-Specific Criteria

In liquid-based preparations, there is more rounding up, formation of three-dimensional groups, and a hyperchromatic appearance.

### 2.6.2 Explanatory Notes

There are a number of explanations for this phenomenon, including the existence of glandular rests adjacent to vaginal mucosa, development of adenosis after

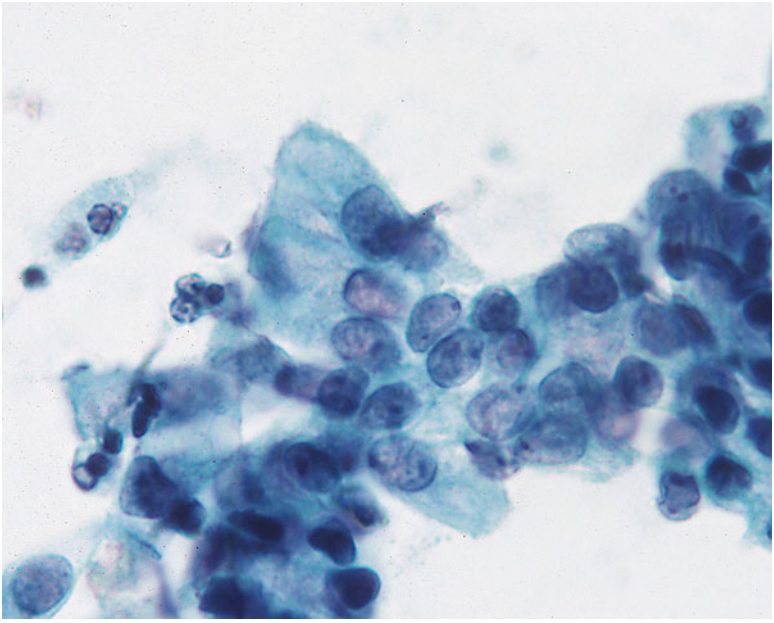

**Fig. 2.48** Glandular cells status post hysterectomy (*CP*). Vaginal smear from a 49-year-old woman status post total hysterectomy for squamous cell cancer of the cervix, showing benign, endocervical-like cells. If benign-appearing, these are of no clinical consequence and reporting is optional

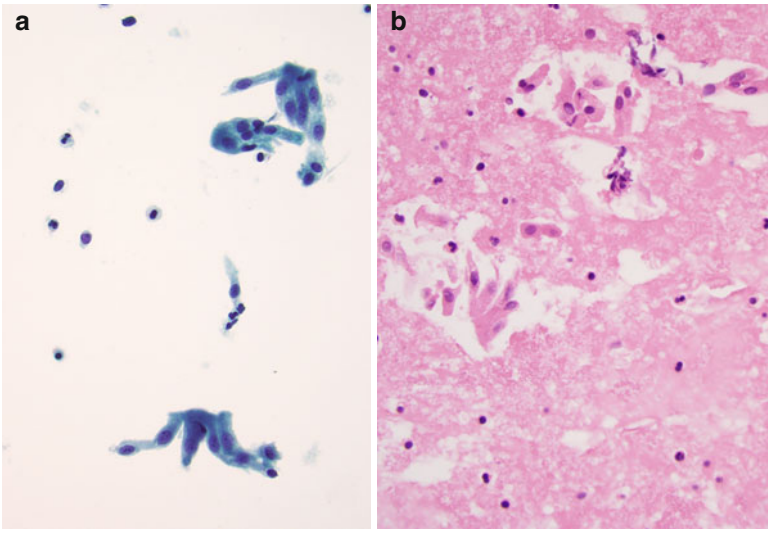


**Fig. 2.49** Glandular cells status post hysterectomy (*LBP*, *ThinPrep*). Columnar glandular cells are seen in a vaginal sample from a 68-year-old woman status post hysterectomy (**a**). She had a recto-vaginal fistula; however, a cell block (**b**) was made and the glandular cells were negative for CDX2 immunostain, making colonic origin unlikely

trauma [25, 26], mucinous or goblet cell metaplasia in response to atrophy [27], or prolapse of the remaining fallopian tube after simple hysterectomy. Following supracervical hysterectomy, an increasingly common procedure, benign endocervical-type glandular cells should be expected. The most important task is to exclude adenocarcinoma, particularly when the hysterectomy was performed for glandular neoplasia. If not atypical, post hysterectomy glandular cells have no clinical significance and reporting them is optional, since they do not change management [28].

## 2.7 Organisms

In the evaluation of reports of cervical samples with organisms, clinical management is dictated by signs and symptoms in most instances, rather than the mere presence of an organism. Clinicians and laboratories should communicate with one another about their expectations for reporting organisms and the format in which they would like to see such reports. In the absence of specific communication regarding this issue, the organisms listed in TBS should generally be reported, if identified.

Cervical cytology has relatively high specificity for most of the organisms discussed in the following sections, thus reporting them can be helpful in alerting clinicians to a potential new diagnosis, although a confirmatory test is often merited. The literature indicates that the Papanicolaou test has low sensitivity for most organisms, so it is rarely the ideal method for primary screening or diagnosis [29]. On the other hand, some laboratories are using the same liquid-based cytology vial for both morphology and microbiologic testing. Testing menus currently include *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in addition to well-established hrHPV tests.

### 2.7.1 *Trichomonas vaginalis* (Figs. 2.50–2.53)

#### 2.7.1.1 Criteria

Pear-shaped, oval, or round cyanophilic organism ranging in area from 15 to 30 $\mu m^2$ (Fig. 2.50).
Nucleus is pale, vesicular, and eccentrically located.
Eosinophilic cytoplasmic granules are often evident.
Flagella are sometimes observed.
Leptothrix may be seen in association with *T. vaginalis* (Fig. 2.51).
Associated background changes include mature squamous cells with small perinuclear halos ("trich change") and 3-dimensional clusters of neutrophils ("polyballs") (Fig. 2.52).

#### Preparation-Specific Criteria

*Liquid-Based Preparations*
Organisms tend to be smaller due to fixation in solution and rounding.
Nuclei and cytoplasmic eosinophilic granules are often better visualized.

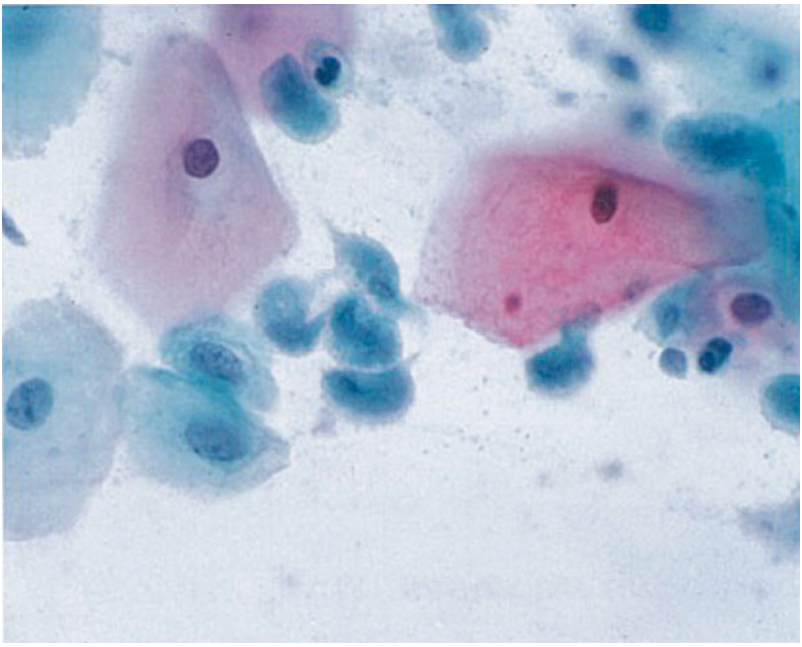

**Fig. 2.50** *Trichomonas vaginalis* (*CP*): trichomonads. Pear-shaped organism with eccentrically located nucleus and eosinophilic cytoplasmic granules. Presence of a nucleus and cytoplasmic granules distinguishes trichomonads from cytoplasmic fragments

Flagella may be better preserved and therefore identified more readily.
Occasional kite-shaped forms may be seen, especially on SurePath preparations (Fig. 2.53).

*Conventional Smears*
Increased neutrophilic infiltrate is common.
Flagella are less often identifiable.

### 2.7.1.2 Explanatory Notes

At times degenerated fragments of cytoplasm (especially in cytolysis) or inflammatory cells can be mistaken for trichomonads. Therefore, at least one of the following – good nuclear detail, eosinophilic cytoplasmic granules, or flagella – should be present to make an accurate interpretation of trichomonas. In most cases, trichomonad organisms are plentiful. Therefore, a rare fragment of cyanophilic debris is not likely to be a true trichomonad. When cervical *Leptothrix* (a gram-positive anaerobic rod, which is longer than lactobacilli, but shorter and thinner than *Candida pseudohyphae*) are present, one should search for the possible presence of trichomonads.

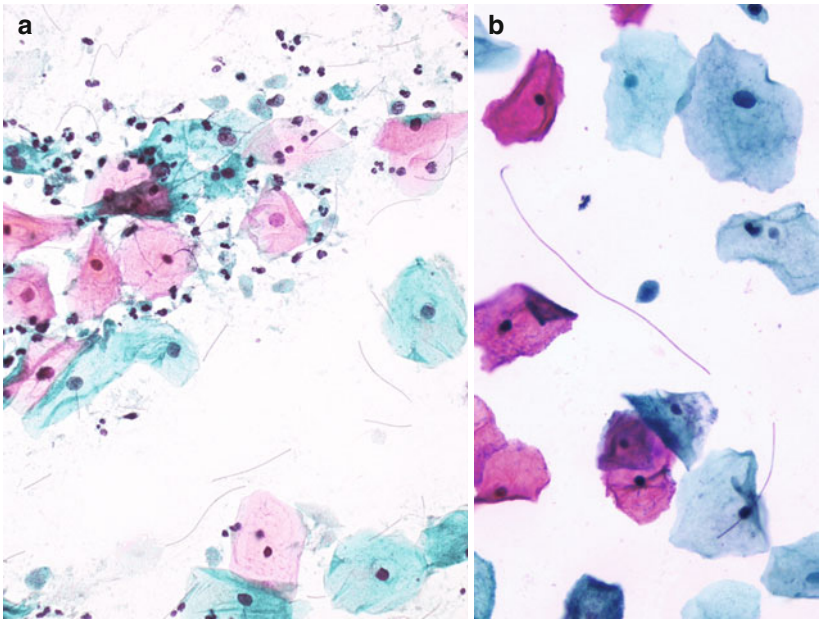


**Fig. 2.51** *Trichomonas vaginalis* and *Leptothrix. Leptothrix* (***a***, *left*, *CP*) may be seen in association with *T. vaginalis*; finding *Leptothrix* alone is not sufficient for a diagnosis of *Trichomonas,* but suggests the presence of trichomonads. On the right (**a**) is an example from a liquid-based (*SurePath*) preparation

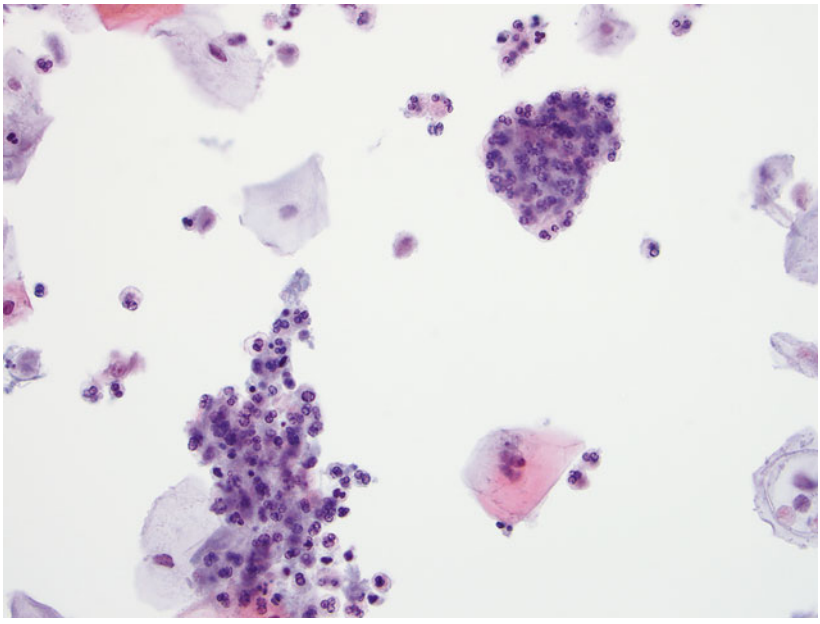

**Fig. 2.52** *Trichomonas vaginalis* (*LBP*, *ThinPrep*): "polyballs." A clue to the presence of trichomonads in a sample is the presence of aggregates of neutrophils or "polyballs." These are seen here along with a few trichomonads in the background

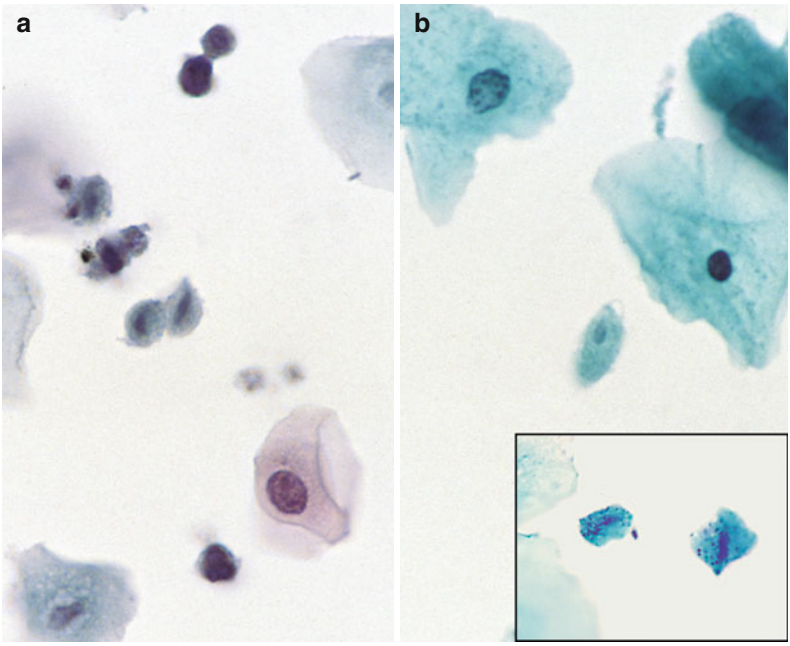


**Fig. 2.53** *Trichomonas vaginalis* (*LBP*, *SurePath*): a 32-year-old woman with vaginal discharge. The organism's nucleus, cytoplasmic granules, and flagella (*right*) may be better visualized on liquid-based preparations. Note the kite shape and granules (*bottom right inset*)

## 2.7.2 Fungal Organisms Morphologically Consistent with *Candida* Species (Figs. 2.54–2.56)

### 2.7.2.1 Criteria

Budding yeast (3–7 μm) and/or pseudohyphae; pseudohyphae can be quite long, spanning many cells, and are eosinophilic to gray brown on the Papanicolaou stain.

Pseudohyphae, formed by cytoplasmic extension of budding yeasts, lack true septations but show complete constrictions along their length that indicate the formation of new cells (Fig. 2.54).

Fragmented leukocyte nuclei and groups of squamous epithelial cells "speared" by pseudohyphae and held together in a rouleaux are often seen (Fig. 2.55).

#### Preparation-Specific Criteria

*Liquid-Based Preparations*

"Spearing" of epithelial cells is more common and can be seen at low power even if the pseudohyphae are not prominent ("shish kebab" effect) (Fig. 2.55).

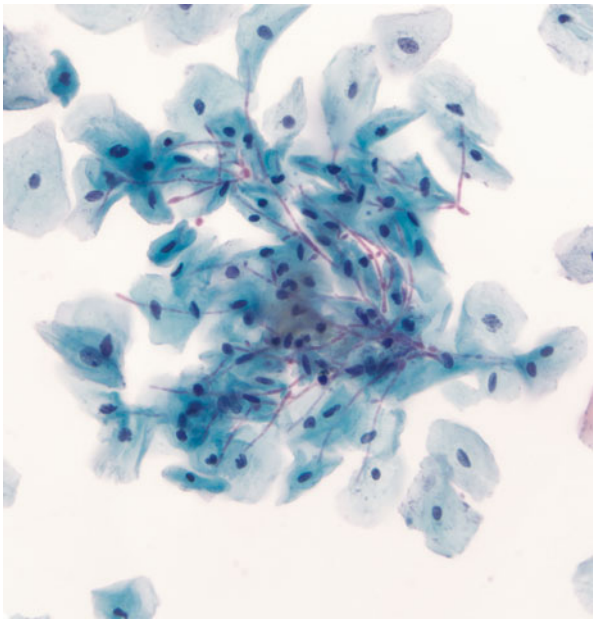

**Fig. 2.54** *Candida species*. (*LBP*, *ThinPrep*): pseudohyphae. Fungal organisms morphologically consistent with *Candida* spp. Note pseudohyphae and modest number of yeast forms

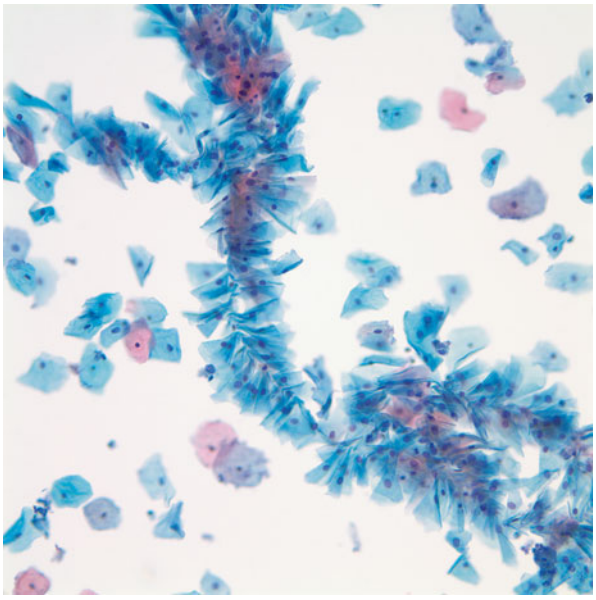

**Fig. 2.55** *Candida species*. (*LBP*, *ThinPrep*): spearing. Fungal organisms morphologically consistent with *Candida* spp. Forty-five-year-old woman. Note "spearing" or a "shish kebab" appearance of squamous cells. This feature is readily appreciated at low power, even when the pseudohyphae are not prominent. Follow-up cytology was NILM

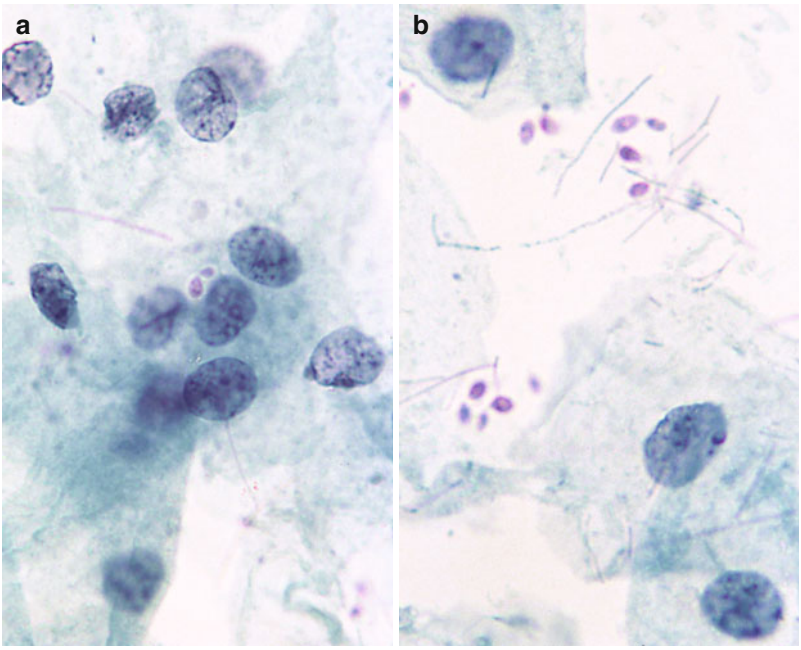


**Fig. 2.56** Candida species (*CP*): *Torulopsis*. Routine screening of a 63-year-old woman. Fungal organisms morphologically consistent with *Candida glabrata* (previously known as *Torulopsis glabrata*). Note clear halos surrounding the yeast forms (*left*). Bacteria, not pseudohyphae, are also seen in the background. This organism does not form pseudohyphae and may be pathogenic in immunocompromised individuals

#### 2.7.2.2 Explanatory Notes

*Candida (Torulopsis) glabrata* shows small uniform, round budding yeast forms surrounded by clear halos on Papanicolaou stain. Unlike other *Candida* species, it does not form pseudohyphae in vivo or in culture (Fig. 2.56).

### 2.7.3 Shift in Flora Suggestive of Bacterial Vaginosis (Figs. 2.57 and 2.58)

#### 2.7.3.1 Criteria

Individual squamous cells are covered by a layer of coccobacilli that obscure the cell membrane, forming the so-called clue cells (Fig. 2.57). Large numbers of inflammatory cells indicate a vaginitis rather than a vaginosis. There is a conspicuous absence of lactobacilli.

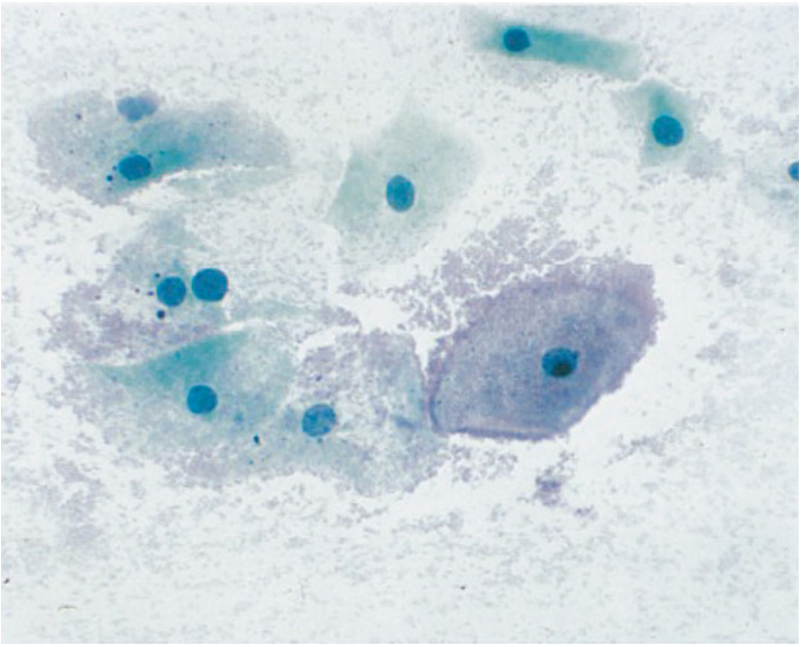

**Fig. 2.57** Bacteria – coccobacilli (*CP*). Shift in flora suggestive of bacterial vaginosis. Note the "clue cell" and filmy background due to large numbers of coccobacilli

### Preparation-Specific Criteria

*Liquid-Based Preparations*:

Squamous cells are covered with coccobacilli; however, the background is clean (Fig. 2.58).

*Conventional Preparations*:

A generalized film of coccobacilli covers cells and the background, usually without a significant neutrophilic response.

## 2.7.3.2 Explanatory Notes

*Lactobacillus* spp. (Döderlein's bacilli) are gram-positive facultative anaerobic rod-shaped bacteria that constitute a major component of the normal vaginal flora (see Fig. 2.59). Predominance of coccobacilli represents a shift in vaginal flora from lactobacilli to a polymicrobial process involving several types of obligate and facultative anaerobic bacteria, including but not limited to *Gardnerella vaginalis*, *Peptostreptococcus*, *Bacteroides*, and *Mobiluncus* spp. [30, 31]. This shift in flora, with or without accompanying clue cells, is not sufficient for the clinical diagnosis of bacterial vaginosis because specimens obtained from any single site are not necessarily representative of the entire flora of the cervix and vagina [32]. However,

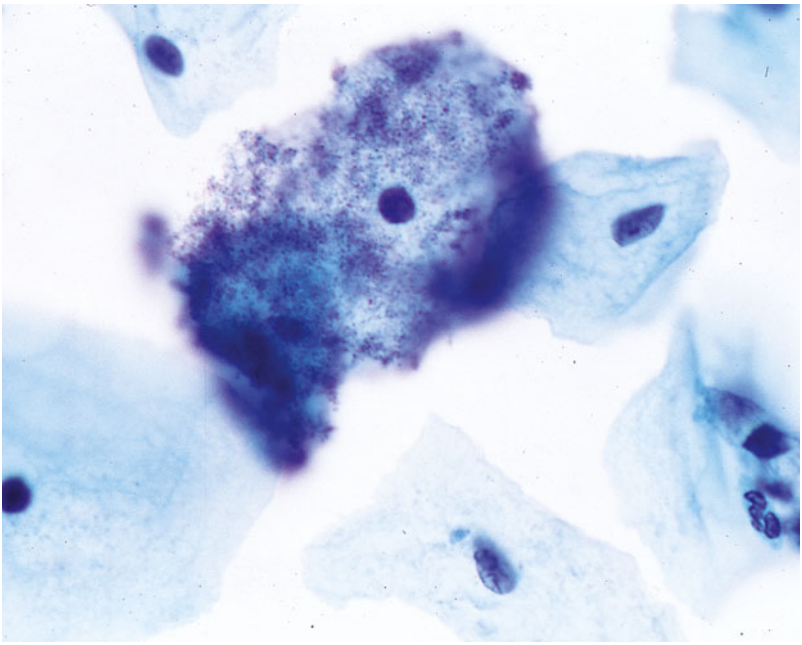

**Fig. 2.58** Bacteria – coccobacilli (*LBP*, *SurePath*). Shift in flora suggestive of bacterial vaginosis. Twenty-five-year-old woman. Note clue cell and the relatively clean background compared to that in *CPs* (see also Fig. 2.57)

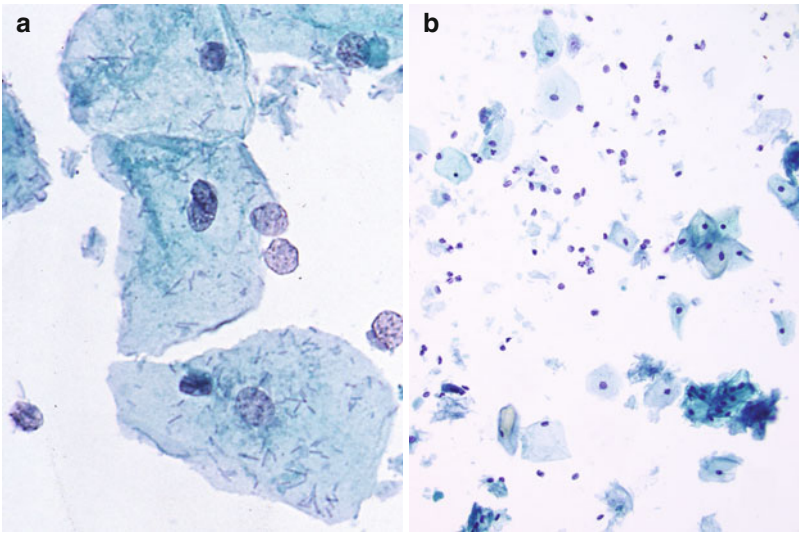


**Fig. 2.59** Bacteria: lactobacilli and cytolysis (**a**, *left*, *CP*). Lactobacilli are typically seen on the cell surfaces in liquid-based preparations and not dispersed in the background as in conventional preparations. Contrast with coccobacilli in Figs. 2.57 and 2.58 in **b**, *right*, *LBP*, *ThinPrep*) note the presence of a cytolytic background with cell debris and numerous stripped nuclei of intermediate cells

the presence of coccobacilli and absence of lactobacilli do correlate with gram-stained smears of vaginal secretions and in the proper clinical context are suggestive of the clinical diagnosis of bacterial vaginosis [33]. Bacterial vaginosis has been associated with pelvic inflammatory disease, preterm birth, postoperative gynecologic infections, and abnormal cervical cytology [34, 35]. Consultation with clinical services is suggested before routinely reporting findings of vaginitis/vaginosis so as to tailor reports to meet clinical needs.

### 2.7.4 Bacteria Morphologically Consistent with *Actinomyces* (Figs. 2.60–2.62)

#### 2.7.4.1 Criteria

Tangled clumps of filamentous organisms, often with acute angle branching, are recognizable as "cotton ball" clusters on low power (Fig. 2.60).

Filaments sometimes have a radial distribution or have an irregular "woolly body" appearance.

Masses of leukocytes adherent to microcolonies of the organism with swollen filaments or "clubs" at the periphery may be identified.

An acute inflammatory response with polymorphonuclear leukocytes is often present.

**Preparation-Specific Criteria**

*Liquid-Based Preparations*:

The strands of actinomycotic organisms tend to be finer and more delicate since the coating proteinaceous material is washed away during processing (Fig. 2.61).

The number of background neutrophils is decreased.

*Conventional Preparations*:

Aggregation of proteinaceous material tends to form a coating or "club" at the periphery of actinomyces filaments.

#### 2.7.4.2 Explanatory Notes

The presence of *Actinomyces* species in cervical cytology has an association with the presence of an intrauterine contraceptive device (IUD) and may be associated with chronic endometritis (up to 25 % of IUD patients will have *Actinomyces* organisms in cervical specimens). Detection of *Actinomyces* in cervical cytology specimens along with clinical evidence of pelvic infection can help alert clinicians to the possibility of a significant Actinomycotic infection [36]. The mere presence of *Actinomyces* in a cervical smear in an asymptomatic IUD user does not appear to constitute grounds for IUD removal [37]. Therefore, the implications of finding *Actinomyces* on a cervical cytology specimen should be considered in conjunction with the clinical findings. In liquid-based preparations, lactobacilli may aggregate to form "clumps" and mimic *Actinomyces* (Fig. 2.62).

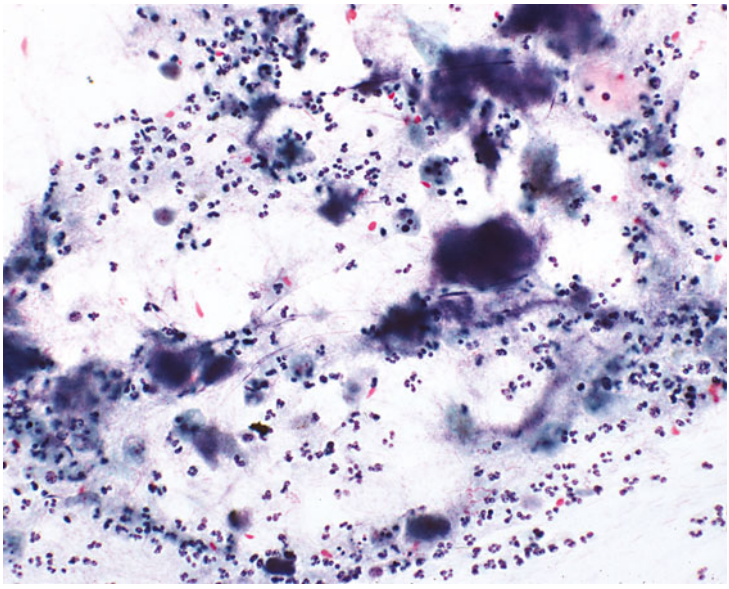

**Fig. 2.60** Bacteria morphologically consistent with *Actinomyces* (*CP*). Forty-one-year-old woman. Low power shows "cotton ball" appearance of tangled clumps of filamentous organisms. An acute inflammatory response is also apparent

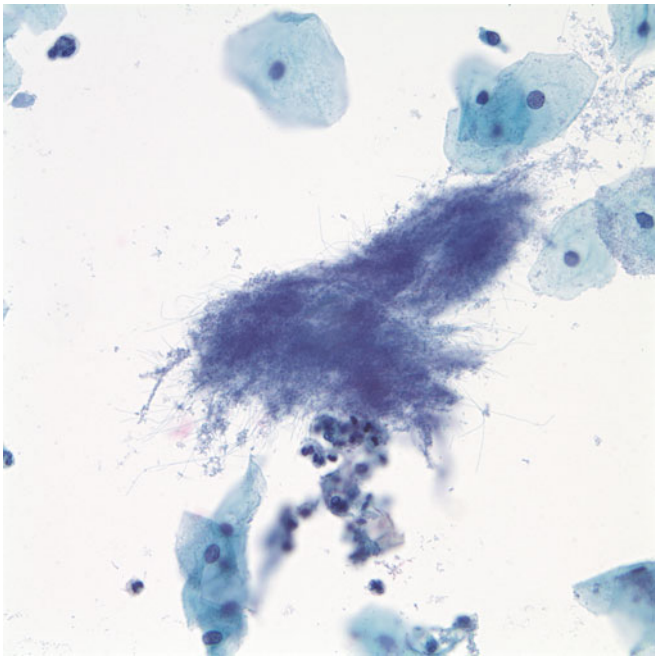

**Fig. 2.61** Bacteria morphologically consistent with *Actinomyces* (*LBP*, *ThinPrep*). Note that the clumps of protein usually seen in conventional preparations tend to be washed away in liquid-based preparations leaving only fine thin bacterial filaments. These are much thinner than the pseudohyphae of *Candida* spp

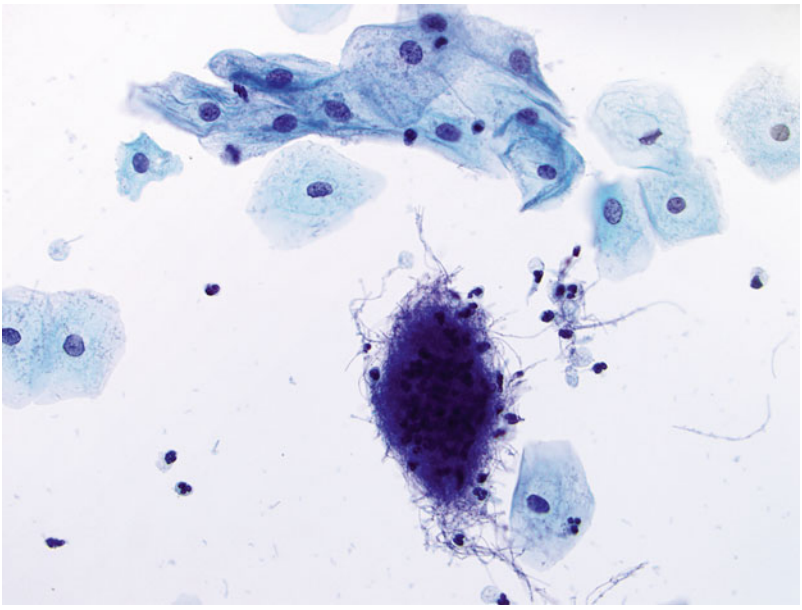

**Fig. 2.62** Bacteria: lactobacilli (*LBP*, *ThinPrep*). In liquid-based preparations, lactobacilli may aggregate to form "clumps" that may resemble *Actinomyces* species and should be distinguished by the presence of similar isolated bacilli in the background and absence of characteristic features of actinomyces

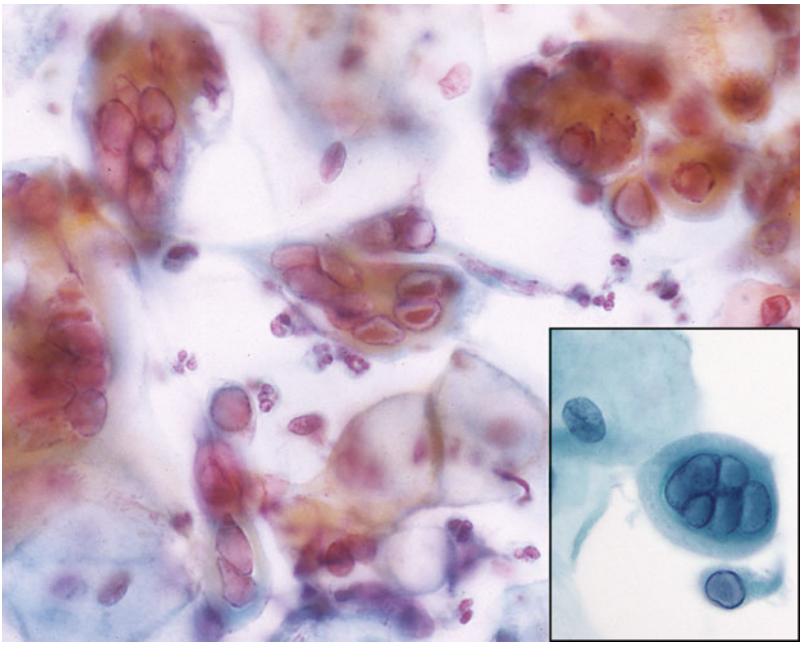

**Fig. 2.63** Cellular changes consistent with herpes simplex virus (*CP*). Note the eosinophilic intranuclear "Cowdry A-type" inclusions. The "ground-glass" appearance of the nuclei is due to accumulation of viral particles leading to peripheral margination of chromatin. The *inset* shows a *SurePath* liquid-based preparation with a typical multinucleated herpetic cell showing "ground-glass" appearance of the nuclei

### 2.7.5 Cellular Changes Consistent with Herpes Simplex Virus (Fig. 2.63)

#### 2.7.5.1 Criteria

Nuclei have a "ground-glass" appearance due to intranuclear viral particles and enhancement of the nuclear envelope caused by peripheral margination of chromatin.

Dense eosinophilic intranuclear (Cowdry) inclusions surrounded by a halo or clear zone are variably present and can be seen in both primary and recurrent infections.

Large multinucleated epithelial cells with molded nuclei are characteristic but may not always be present; mononucleate cells with the nuclear features described above may be the only finding.

#### 2.7.5.2 Explanatory Notes

Herpes cytopathic effect shows 3 "Ms" – multinucleation, molding, and margination of chromatin. Multinucleated cells have a limited differential diagnosis that includes multinucleated endocervical cells, multinucleated histiocytes, and syncytiotrophoblast cells. Herpes infection is distinguished from all of these by ground-glass (hyaline) intranuclear inclusions. The mononuclear cells of herpes infection have been shown to be overinterpreted as both LSIL and HSIL (see Fig. 5.12), especially in testing situations, although herpes is a relatively reproducible interpretation in clinical practice. Distinguishing the intranuclear herpetic inclusion from the hyperchromatic chromatin of SIL is the key to making this distinction [38].

### 2.7.6 Cellular Changes Consistent with Cytomegalovirus (Fig. 2.64)

The cytopathic effect of cytomegalovirus (CMV) affects mostly the endocervical glandular cells but can also be present in stromal cells.

#### 2.7.6.1 Criteria

Cellular and nuclear enlargement.

Large eosinophilic intranuclear viral inclusions with a prominent halo.

Small cytoplasmic, basophilic inclusions can also be present.

#### 2.7.6.2 Explanatory Notes

CMV cytopathic effect is most commonly seen in immunocompromised individuals. The large CMV infected cells may sometimes be confused with bizarre tumor cells; however, the inclusions have characteristic central eosinophilic bodies and marginated material, creating a prominent halo around the central inclusion. In contrast to herpes viral effect, CMV can also show cytoplasmic, in addition to nuclear, viral inclusions.

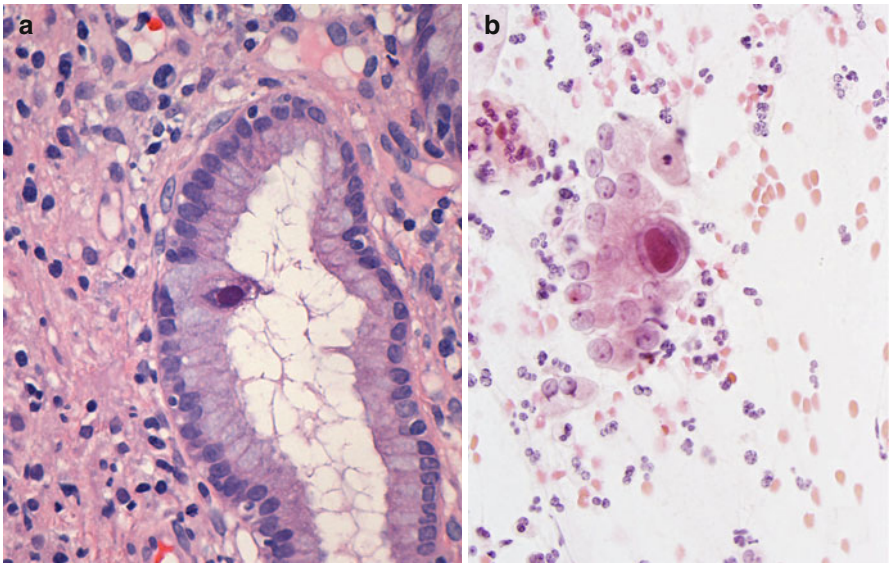


**Fig. 2.64** Cytomegalovirus (CMV). The histologic image on the left (**a**, H&E) shows CMV cytopathic effect in an endocervical cell with the typical lilac-red-colored large intranuclear inclusion. Smaller basophilic cytoplasmic inclusions adjacent to the nucleus are also apparent. On the right (**b**, *CP*) CMV inclusions are seen in an endocervical cell. CMV infection is usually not seen in squamous cells; however, it can infect a wide range of other epithelial, mesenchymal, lymphoid, and hematopoietic cells

## 2.8 Sample Reports

**Example 1**
*Specimen Adequacy:*
Satisfactory for evaluation; endocervical/transformation zone component present.
*Interpretation*:
Negative for intraepithelial lesion or malignancy.

**Example 2**
*Specimen Adequacy:*
Satisfactory for evaluation; endocervical/transformation zone component present; partially obscuring inflammation present.
*Interpretation*:
Negative for intraepithelial lesion or malignancy.
Trichomonas vaginalis identified.
Reactive squamous cells associated with inflammation (includes typical repair).

**Example 3**
*Specimen Adequacy:*
Satisfactory for evaluation; endocervical/transformation zone components absent.
*Interpretation*:
Negative for intraepithelial lesion or malignancy.
Reactive cellular changes associated with radiation.

**Example 4**
*Specimen Adequacy:*
Satisfactory for evaluation; endocervical/transformation zone component cannot be assessed because of severe atrophy.
*Interpretation*:
Negative for intraepithelial lesion or malignancy.
Fungal organisms morphologically consistent with Candida species.
Atrophy.

## References

1. Colgan TJ, Woodhouse SL, Styer PE, Kennedy M, Davey DD. Reparative changes and the false-positive/false-negative Papanicolaou test. Arch Pathol Lab Med. 2001;125:134–40.
2. Young NA, Naryshkin S, Atkinson BF, Ehya H, Gupta PK, Kline TS, et al. Interobserver variability of cervical smears with squamous-cell abnormalities: a Philadelphia study. Diagn Cytopathol. 1994;11:352–7.
3. Young NA, Kline TS. Benign cellular changes: allied ambiguity in CLIA'88 and the Bethesda System. Diagn Cytopathol. 1994;10(4):307–8 [editorial].
4. Davey DD, Nielsen ML, Frable WJ, Rosenstock W, Lowell DM, Kraemer BB. Improving accuracy in gynecologic cytology. Results of the College of American Pathologists Interlaboratory Comparison Program in Cervicovaginal Cytology. Arch Pathol Lab Med. 1993;117:1193–8 [see comments].
5. Young NA. Back to the negative Pap test: behind the scenes at Bethesda 2001. Diagn Cytopathol. 2002;26:207–8.
6. Jones BA. Rescreening in gynecologic cytology. Rescreening of 3762 previous cases for current high-grade squamous intraepithelial lesions and carcinoma: a College of American Pathologists Q-Probes study of 312 institutions. Arch Pathol Lab Med. 1995;119:1097–103.
7. Barr Soofer S, Sidawy MK. Reactive cellular change: is there an increased risk for squamous intraepithelial lesions? Cancer. 1997;81:144–7 [see comment].
8. Malik SN, Wilkinson EJ, Drew PA, Hardt NS. Benign cellular changes in Pap smears. Causes and significance. Acta Cytol. 2001;45:5–8.
9. Tlsty TD, Coussens LM. Tumor stroma and regulation of cancer development. Annu Rev Pathol. 2006;1:119–50.
10. Patten Jr SF. Diagnostic cytopathology of uterine cervix. In: Wied G, editor. Monographs in clinical cytology. 2nd ed. New York: Karger Press; 1978.
11. Heaton Jr RB, Harris TF, Larson DM, Henry MR. Glandular cells derived from direct sampling of the lower uterine segment in patients status post-cervical cone biopsy. A diagnostic dilemma. Am J Clin Pathol. 1996;106:511–6.

12. Sauder K, Wilbur DC, Duska L, Tambouret RH. An approach to post-radical trachelectomy vaginal-isthmus cytology. Diagn Cytopathol. 2009;37:437–42.
13. Feratovic R, Lewin SN, Sonoda Y, Park KJ, Abu-Rustum NR, Moreira AL, et al. Cytologic findings after fertility-sparing radical trachelectomy. Cancer. 2008;114:1–6.
14. Sorosky JI, Kaminski PF, Wheelock JB, Podczaski ES. Clinical significance of hyperkeratosis and parakeratosis in otherwise negative Papanicolaou smears. Gynecol Oncol. 1990;39: 132–4.
15. Williamson BA, DeFrias D, Gunn R, Tarjan G, Nayar R. Significance of extensive hyperkeratosis on cervical/vaginal smears. Acta Cytol. 2003;47:749–52.
16. Bibbo M, Wied GL. Look-alikes in gynecologic cytology. In: Wied GL, editor. Tutorials of cytology, vol. 12. 2nd ed. Chicago: Tutorials of Cytology Press; 1988.
17. Babkowski RC, Wilbur DC, Rutkowski MA, Facik MS, Bonfiglio TA. The effects of endocervical canal topography, tubal metaplasia, and high canal sampling on the cytologic presentation of non-neoplastic endocervical cells. Am J Clin Pathol. 1996;105:403–10.
18. College of American Pathologists 2013 Interlaboratory Comparison Program in Cervicovaginal Cytopathology (PAP) Year End Summary Report. Northfield, Illinois: College of American Pathologists; 2014.
19. Koss LG. Inflammatory processes and other benign disorders of the cervix and vagina. In: Koss LG, editor. Diagnostic cytology and its histopathologic bases. 4th ed. Philadelphia: Lippincott; 1992. p. 314–70.
20. Michael CW, Esfahani FM. Pregnancy-related changes: a retrospective review of 278 cervical smears. Diagn Cytopathol. 1997;17:99–107.
21. Hakima L, Kaplan RE, Guo M, Hoda RS. Decidual cells may be mistaken for glandular or squamous atypia on ThinPrep Pap test. Diagn Cytopathol. 2013;41:886–8.
22. Benoit JL, Kini SR. “Arias-Stella reaction”-like changes in endocervical glandular epithelium in cervical smears during pregnancy and postpartum states–a potential diagnostic pitfall. Diagn Cytopathol. 1996;14:349–55.
23. Shield PW, Daunter B, Wright RG. Post radiation cytology of cervical cancer patients. Cytopathology. 1992;3:167–82.
24. Ponder TB, Easley KO, Davila RM. Glandular cells in vaginal smears from posthysterectomy patients. Acta Cytol. 1997;41:1701–4.
25. Gondos B, Smith LR, Townsend DE. Cytologic changes in cervical epithelium following cryosurgery. Acta Cytol. 1970;14:386–9.
26. Sedlacek TV, Riva JM, Magen AB, Mangan CE, Cunnane MF. Vaginal and vulvar adenosis. An unsuspected side effect of $CO_2$ laser vaporization. J Reprod Med. 1990;35:995–1001.
27. Bewtra C. Columnar cells in posthysterectomy vaginal smears. Diagn Cytopathol. 1992;8: 342–5.
28. Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, Schiffman M, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis. 2013;17:S1–27.
29. Fitzhugh VA, Heller DS. Significance of a diagnosis of microorganisms on a Pap smear. J Low Genit Tract Dis. 2008;12:40–51.
30. Giacomini G, Paavonen J, Rilke F. Microbiologic classification of cervicovaginal flora in Papanicolaou smears. Acta Cytol. 1989;33:276–8.
31. Giacomini G, Schnadig VJ. The cervical Papanicolaou smear: bacterial infection and the Bethesda System. Acta Cytol. 1992;36:109–10.
32. Bartlett JG, Moon NE, Goldstein PR, Goren B, Onderdonk AB, Polk BF. Cervical and vaginal bacterial flora: ecologic niches in the female lower genital tract. Am J Obstet Gynecol. 1978;130:658–61.
33. Prey M. Routine Pap smears for the diagnosis of bacterial vaginosis. Diagn Cytopathol. 1999;21:10–3.

34. Donders GG, Van Bulck B, Caudron J, Londers L, Vereecken A, Spitz B. Relationship of bacterial vaginosis and mycoplasmas to the risk of spontaneous abortion. Am J Obstet Gynecol. 2000;183:431–7.
35. Schwebke JR. Bacterial vaginosis. Curr Infect Dis Rep. 2000;2:14–7.
36. Fiorino AS. Intrauterine contraceptive device-associated actinomycotic abscess and actinomyces detection on cervical smear. Obstet Gynecol. 1996;87:142–9.
37. Matsuda K, Nakajima H, Khan KN, Tanigawa T, Hamaguchi D, Kitajima M, et al. Preoperative diagnosis of pelvic actinomycosis by clinical cytology. Int J Womens Health. 2012;4:527–33.
38. Crothers BA, Booth CN, Darragh TM, Zhao C, Souers RJ, Thomas N, et al. False-positive Papanicolaou (PAP) test rates in the College of American Pathologists PAP education and PAP proficiency test programs: evaluation of false-positive responses of high-grade squamous intraepithelial lesion or cancer to a negative reference diagnosis. Arch Pathol Lab Med. 2014;138:613–9.
39. Sherman ME, Cytopathology, Blaustein's Pathology of the Female Genital Tract. Kurman RJ, editor. 4th ed. New York: Springer, 1994; p. 1099.