

## Chapter

## 1

## The PAP smear

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### Outline

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- Endometrial adenocarcinoma
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- Microorganisms in the PAP smear
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  - Actinomyces
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  - *Neisseria gonorrhoea*
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  - *Alternaria alternata*
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### Introduction

This chapter on Papanicolaou (PAP) smears will follow the recommendations set forth by the most current Bethesda system (second edition, 2001) closely. However, it is not by any means a replacement of the standard text on PAP smear cytology. However, it should be a good resource for the experienced cytomorphologist for thinking of the differential diagnoses that may present in PAP smears.

### New screening guidelines and human papillomavirus (HPV) testing

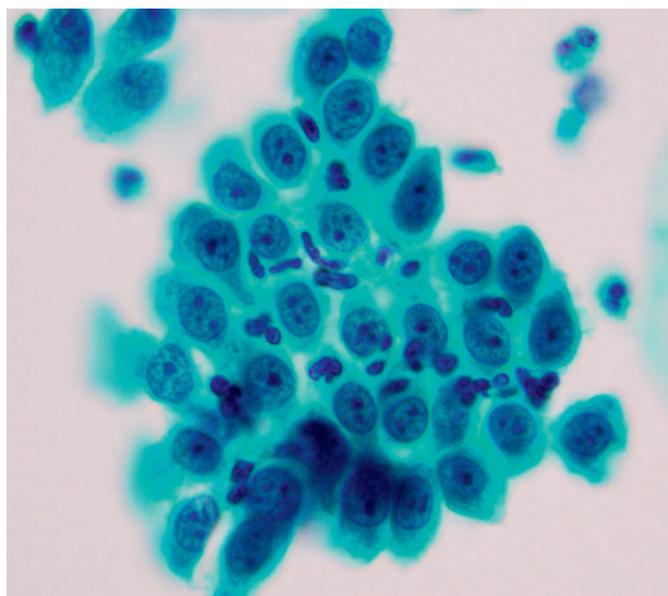
With the advent of the American Cancer Society (ACS), American Society of Colposcopy and Cervical Pathology (ASCCP), and the American Society of Clinical Pathology

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(ASCP) change in recommendations for PAP smear cytology screening with increasing intervals published in 2011 [1], PAP smear cytology will play an even more pivotal role in the detection of dysplastic lesions. As HPV testing (or co-testing) becomes more common in women every five years in the appropriate age groups, an apparent increase in prevalence of squamous intraepithelial lesions will likely be seen by most laboratories of PAP smear cytology. Hence, the morphologic assessment and detection of these cells will become more important in the ensuing years as a surrogate for precursor cervical cancerous lesions. Consensus guideline recommendations are included in an Appendix at the end of this chapter for quick reference.

### Adequacy

Any interpretation of cytologic material, including PAP smears, starts with the procurement, processing, and presentation of the criteria for an adequate sample. The minimum cellularity of squamous cells on conventional PAP smears is anywhere between 8000 and 12 000 cells and between 5000 and 20 000 cells on liquid-based media (Bethesda system, 2001). The presence of at least 10 well-preserved endocervical or squamous metaplastic cells, which implies adequate sampling of the transformation zone, is also important. This criterion is negated only if the woman has a known history of a hysterectomy. Having satisfied all criteria for an adequate sample, the 2001 Bethesda system suggests a uniformity in laboratory reporting of PAP smears by adhering to a proscribed and standardized method of reporting, which includes: the type of specimen obtained; a statement on the specimen adequacy and any reason for an unsatisfactory specimen; the general category of lesions; and interpretations of results. The reader is referred to the current publication of Bethesda (2001) for a more complete listing of the classification system.

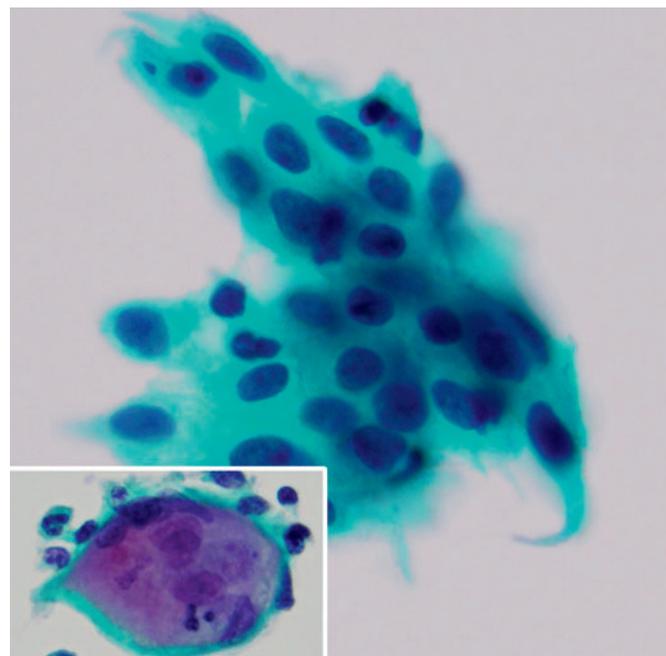


**Figure 1-1A.** Reactive changes with neutrophils (PAP stain).

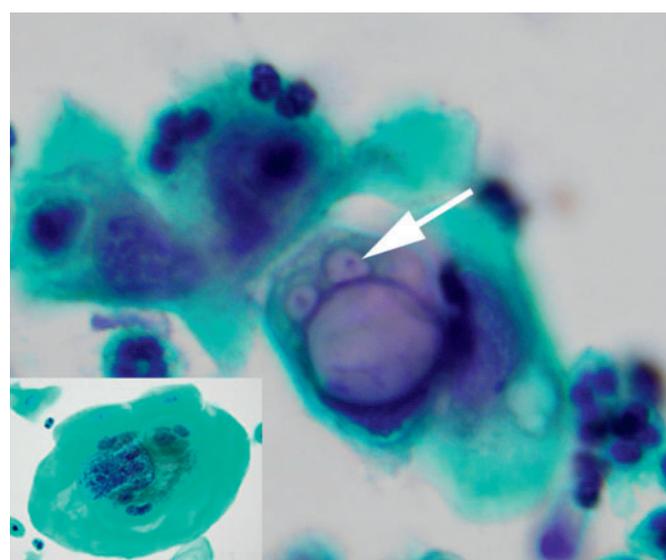
### Reactive changes

#### Clinical features

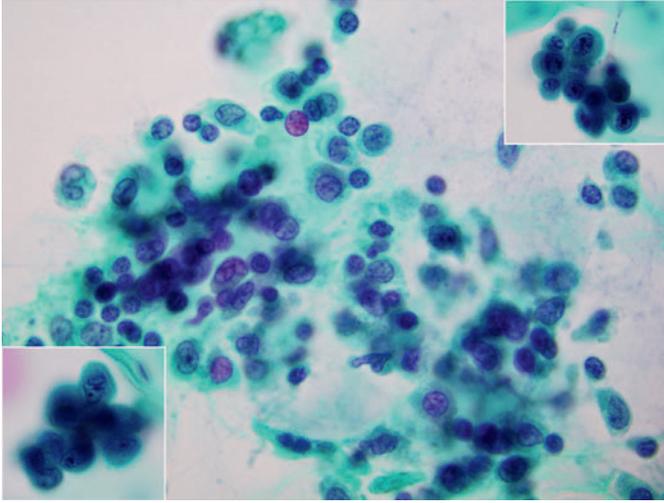
- Common cytologic pattern, which can be due to repair, atrophy, prolapsed uterus, radiation changes, and infectious processes
- Could be due to numerous infectious processes such as trichomonas, bacterial vaginosis, *Chlamydia*, gonorrhea, HPV, candida, and herpes
- Commonly clinically treated and PAP smear repeated after treatment
- Most cases are symptomatic and a presenting concern to the patient



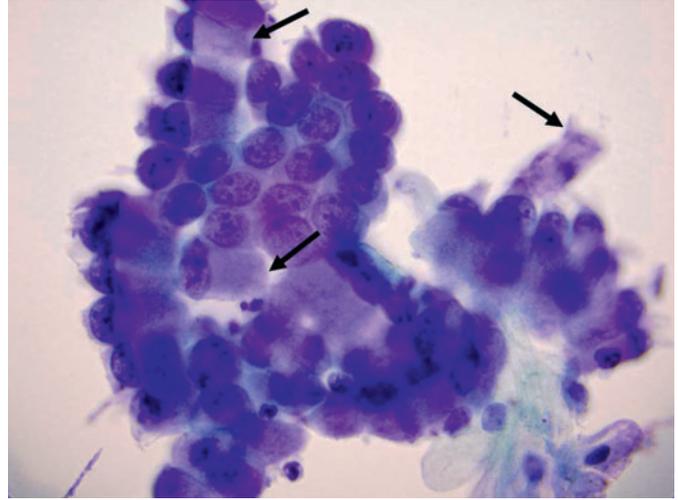
**Figure 1-1B.** Reactive changes due to atrophy (inset: high power) (PAP stain).



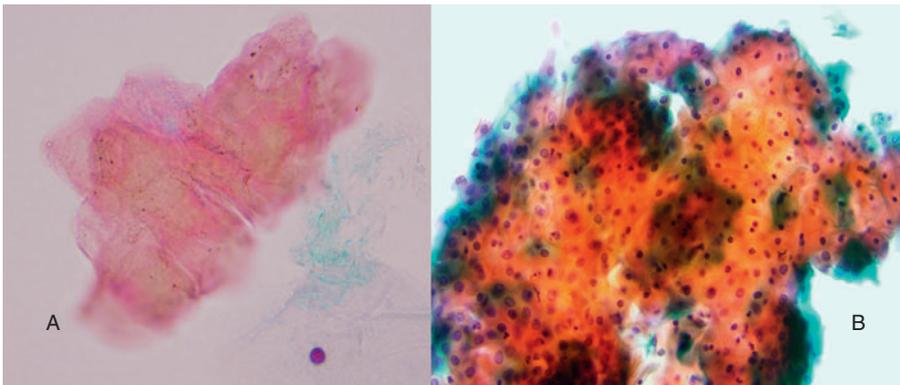
**Figure 1-1C.** Reactive changes due to radiation (arrow and inset) (PAP stain).



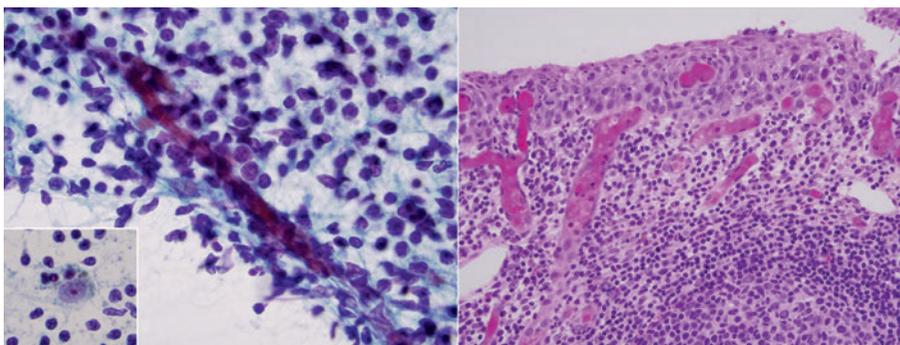
**Figure 1-1D.** Reactive changes due to intrauterine device (IUD) (insets are reactive endometrial cells) (PAP stain).



**Figure 1-1E.** Tubal metaplasia (arrows) (PAP stain).



**Figure 1-1F.** Hyperkeratosis (A) and parakeratosis (B) (PAP stain).

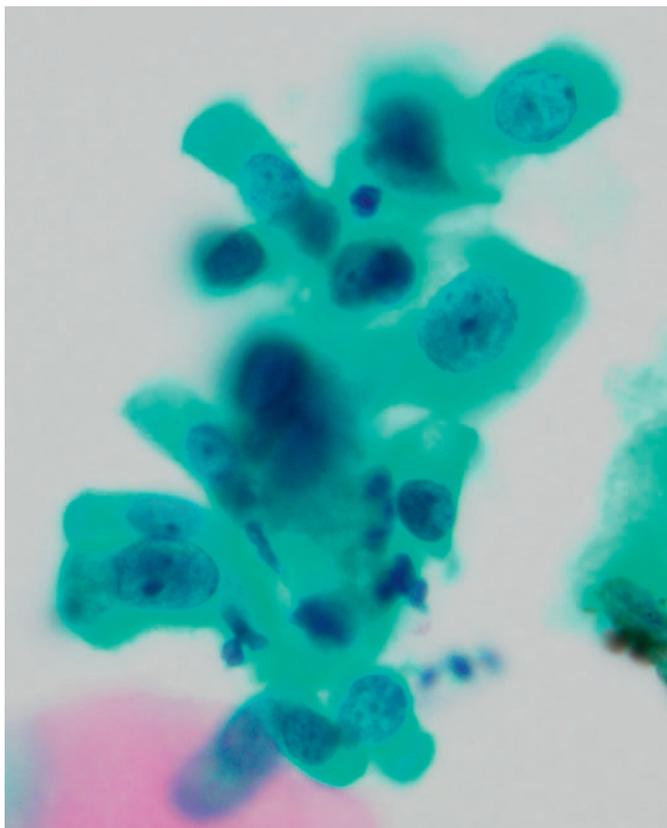


**Figure 1-1G.** Lymphocytic cervicitis (PAP stain).

#### Cytologic features

- A prominent or subtle neutrophilic exudate
- May be associated with common infectious processes: candida, trichomonas, *Gardnerella vaginalis*, etc.
- Cells are found commonly in cohesive sheets and tile-like or honeycomb configurations
- Nuclei are enlarged (1–1.5 × the size of an intermediate cell nucleus); can be binucleated; nuclear outlines are

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**Figure 1-1H.** Endocervical cell atypia (PAP stain).

round, smooth, and uniform; vesicular and hypochromatic to mildly hyperchromatic

- Multiple or single nucleoli in most of the cells in the sheets
- Cytoplasm could be polychromatic, vacuolated, with a small perinuclear halo

### Special stains and immunohistochemistry

- The cytologic features of most infectious agents are well defined and usually do not need special stains in a PAP-stained slide

### Modern techniques for diagnosis

- Polymerase chain reaction (PCR) testing for both *Chlamydia* and gonorrhea

### Differential diagnosis

- Typical repair associated with atrophy with and without inflammation
  - Age >50 years, anovulatory syndromes or history of bilateral oophorectomy for treatment of breast cancers
  - Presence of squamous metaplastic cells or parabasal cells are more common
  - Presence of naked nuclei secondary to autolysis called “blue blobs”
  - Presence of granular or degenerated background

- +/- parakeratotic cells and histiocytes
- Lack of maturing squamous epithelium
- Reactive cellular changes associated with radiation
  - History of cervical or endometrial malignancy, status post-radiation
  - Markedly enlarged cells with preserved nuclear to cytoplasmic ratio
  - Presence of bizarre cell shapes with multinucleation and polychromasia
  - Presence of cytoplasmic and nuclear vacuolization
- Reactive cellular changes associated with intrauterine device (IUD)
  - History of IUD placement or recent removal
  - Presence of endometrial cells
  - Presence of small vacuolated cells or histiocytes
  - Presence of nuclear degeneration and prominent nucleoli
- Other non-neoplastic or metaplastic changes, which could be seen in association with these reactive changes
- Tubal metaplasia
  - Very common in reactive endocervices and is composed of endocervical cells with well-defined terminal bars and ciliated borders
- Hyperkeratoses and parakeratoses
  - Commonly associated with prolapse, but could also harbor a squamous intraepithelial lesion (SIL)
- Lymphocytic cervicitis
  - Commonly associated with *Chlamydia trachomatis* infection, more than any other venereal infection
- Reactive endocervical cells
  - Commonly have sheets of endocervical cells with multiple nucleoli or prominent chromocenters

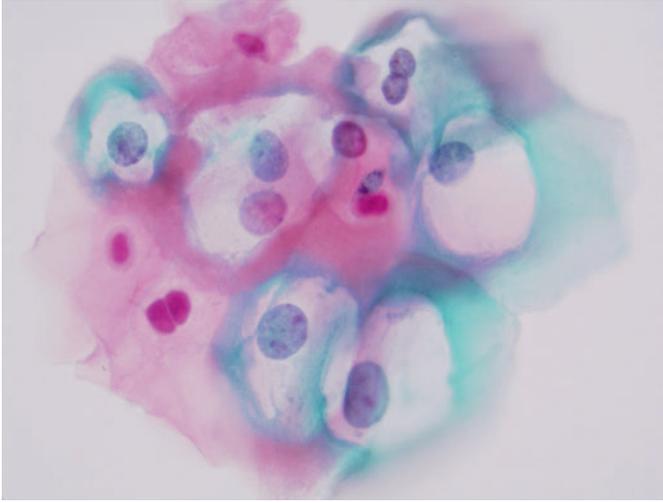
### Pearls

- The search for an infectious agent and an adequate correlation with the clinical history is imperative in the diagnosis

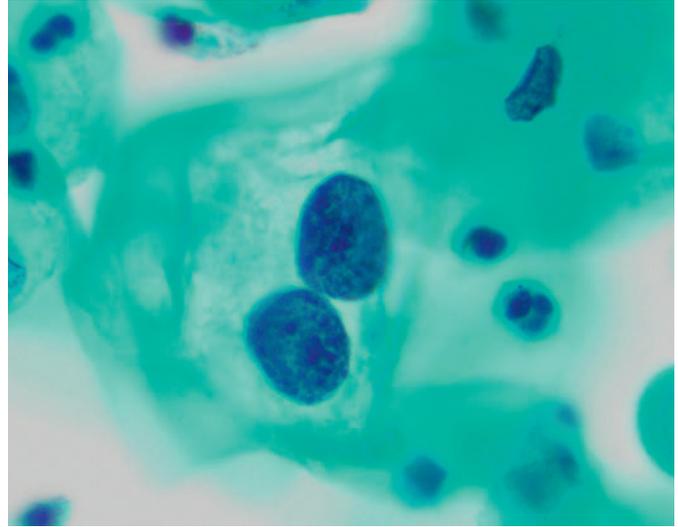
## Low-grade squamous intraepithelial lesions (LGSIL)

### Clinical features

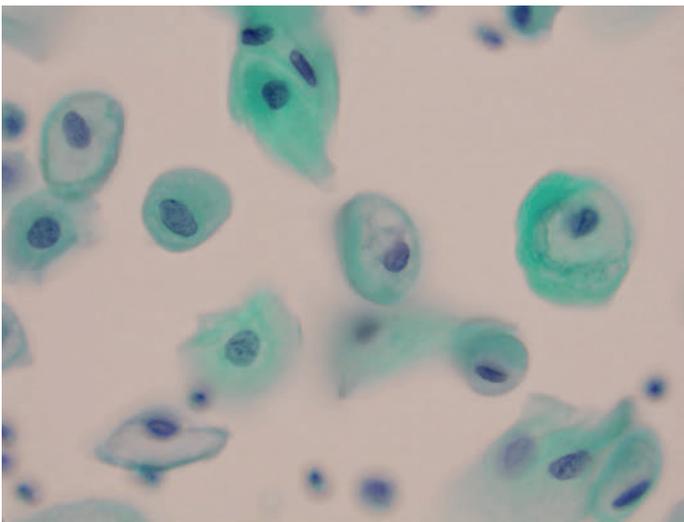
- Could be found as a raised warty or flat lesion in the vulva, vagina, or cervix that is acetowhite by acetic acid application upon colposcopy
- Includes histologic diagnoses of mild dysplasia (CIN 1) or koilocytic changes



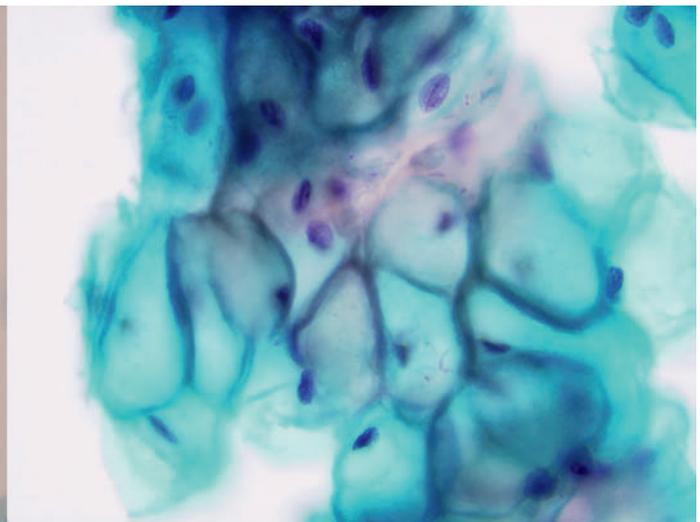
**Figure 1-2A.** Koilocytes (LGSIL) (PAP stain).



**Figure 1-2B.** Low-grade squamous intraepithelial lesion (LGSIL) (PAP stain).



**Figure 1-2C.** Navicular cells of pregnancy (PAP stain).



### Cytologic features

- Singly dispersed or groups of cells with abundant cytoplasm and a dense perinuclear clearing or halo around a nucleus
- Others may not have a distinct halo around the nucleus, but still have abundant cytoplasm
- Nucleus is at least 2–3 × the size of an intermediate cell nucleus, can be binucleated, with some variation in size from cell to cell
- Nucleus is hyperchromatic with irregularly granular chromatin and can have irregular nuclear contours
- Usually have no conspicuous nucleoli

### Special stains and immunohistochemistry

- Not necessary if the cytologic features are observed as given here

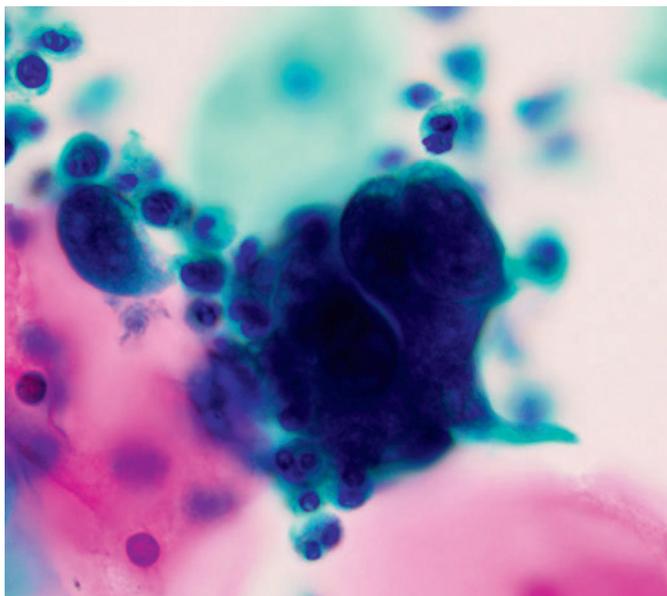
### Modern techniques for diagnosis

- Non-contributory

### Differential diagnosis

- Glycogenated or navicular cells of pregnancy
  - Lacks nuclear enlargement and hyperchromasia

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**Figure 1-2D.** Trophoblasts and Arias–Stella change of pregnancy (PAP stain).

- Presence of small nuclei the same size as an intermediate cell nucleus
- A perinuclear halo without perinuclear cytoplasmic condensation around the nuclei
- Atypical squamous cells of undetermined significance (ASCUS)
  - Presence of nuclear enlargement up to 3 × the size of an intermediate cell nucleus with some degree of hyperchromasia
  - Lacks the irregular granular chromatin and nuclear contour irregularity of a LGSIL lesion

### Pearls

- Despite the morphologic changes described earlier, these lesions can harbor both low- and high-grade human papilloma viral types

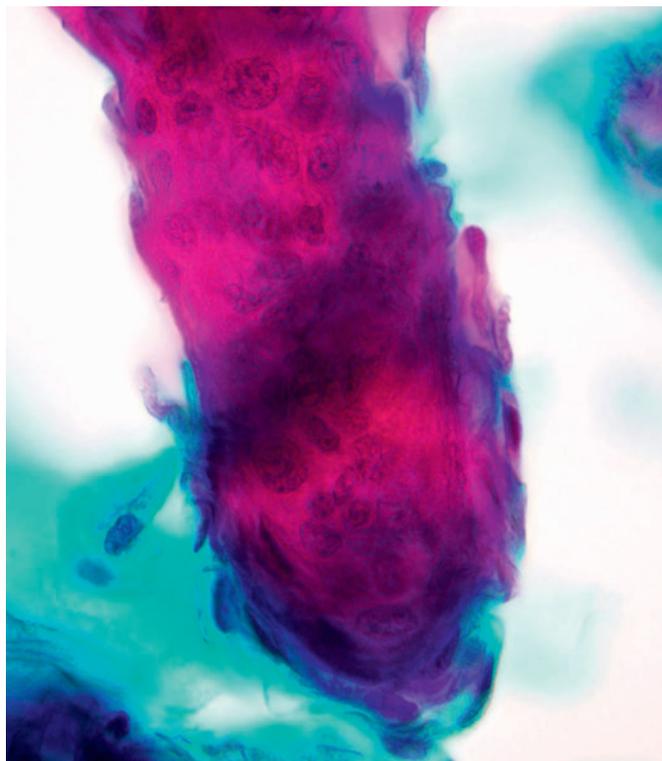
## Arias–Stella change of pregnancy

### Clinical features

- A history of pregnancy is present. Very early postpartum changes can also present with Arias–Stella changes in the epithelial and stromal cells, which can be interpreted as atypical glandular cells, not otherwise specified (NOS)

### Cytologic features

- Marked cellular enlargement, marked nuclear atypia including nuclear enlargement, pleomorphic nuclei, and prominent nucleoli. The nuclear to cytoplasmic ratio is unchanged



**Figure 1-2E.** Atypical parakeratosis (PAP stain).

### Special stains and immunohistochemistry

- CD10 may be helpful in the differentiation between an Arias–Stella cell of trophoblastic origin versus a clear cell carcinoma that was found to be negative in one study
- Cyclin E expression, on the other hand, has been seen mostly in clear cell adenocarcinoma of Müllerian origin and would be negative in clear cell tumors of a renal primary. A combination of these markers would be useful

### Modern techniques for diagnosis

- Non-contributory

### Differential diagnosis

- A clear cell adenocarcinoma of Müllerian origin – may be cytologically difficult to differentiate and may present in young women; therefore, a tissue biopsy and serum  $\beta$ -human chorionic gonadotropin (hCG) levels would be necessary to exclude one
- Radiation effect – a history of radiation or pregnancy is the main differentiating factor

### Pearls

- A history of recent pregnancy is of paramount importance

- May be the first presenting finding in a young woman with a tubal or extrauterine pregnancy, such that a serologic  $\beta$ -hCG level test may be necessary
- May also be the presenting finding in women with molar pregnancies
- Can be seen in patients taking phytoestrogens

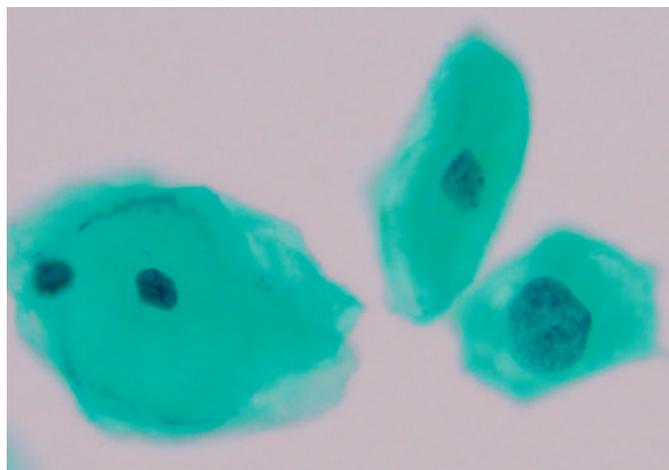
## Atypical squamous cells (ASC)

### Clinical features

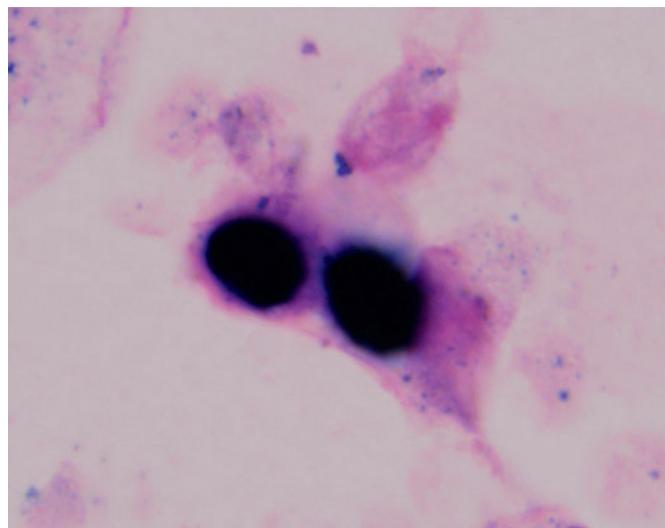
- Patients may not have a distinct or specific clinical symptom
- May represent the first indication of a smoldering squamous intraepithelial lesion

### Cytologic features

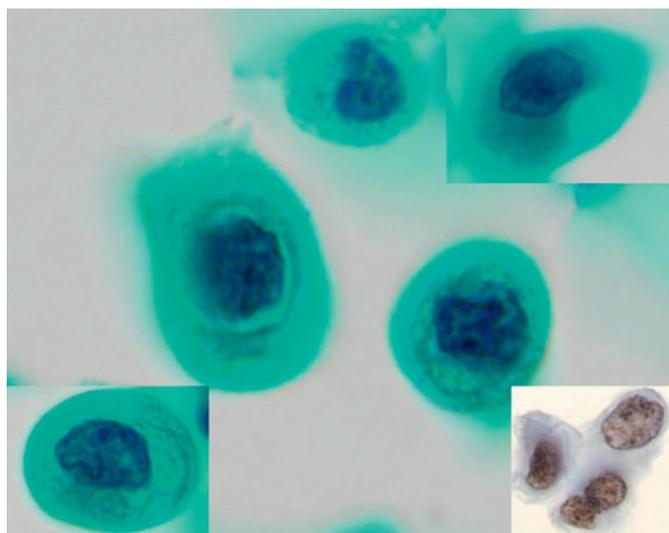
- “ASC refers to cytologic changes suggestive of SIL, which are qualitatively and quantitatively insufficient for a definitive interpretation of an SIL lesion” (Bethesda, 2001)
- This is not a distinct diagnostic entity, but a “waste-basket” category reserved for those cases for which a definitive diagnosis of SIL could not be reached. An ASCUS rate of 5.2% (mean) and 4.5% (median) was seen in 768 participating cytology laboratories across the US in 1996. This entity includes both ASCUS and ASC-H for cases in which atypical squamous metaplastic cells have features that fall short of a high-grade squamous intraepithelial lesion (HGSIL)



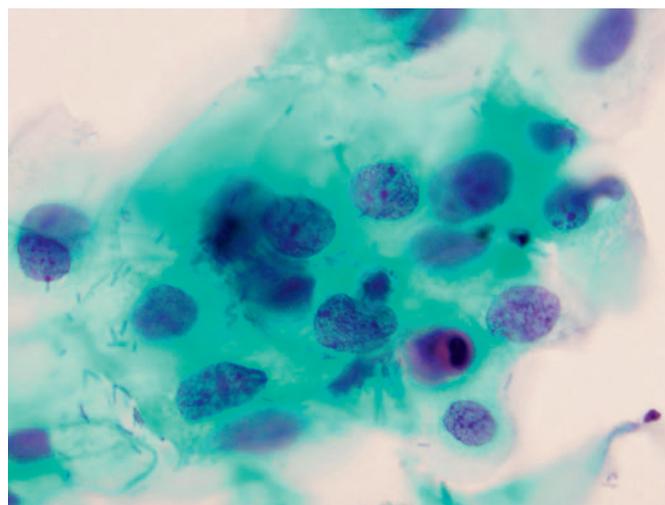
**Figure 1-3A.** Atypical squamous cells of undetermined significance (ASCUS) (PAP stain).



**Figure 1-3B.** *In situ* hybridization technique for detecting high-risk HPV infection (PAP stain).



**Figure 1-3C.** Atypical squamous cells (ASC) – cannot exclude HGSIL (ASC-H) with ProExC® (lower right inset) (PAP stain).



**Figure 1-3D.** LGSIL without koilocytosis (PAP stain).

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### Special stains and immunohistochemistry

- *In situ* hybridization techniques for the detection of episomic and integrated HPV DNA. A positive test indicates the presence of one or more high-risk HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 68
- Genotyping for HPV 16/18 can be done in women >30 years of age who have negative cytologies, but have a positive high-risk HPV before colposcopy is done, as per ASCCP guidelines
- ProExC® by Tripath imaging is a new antibody that stains cells undergoing aberrant S-phase induction as would be seen in highly and inappropriately proliferative squamous epithelium. This potentially could differentiate HGSIL from ASC-H lesions, which are similar in size to squamous metaplastic cells

### Modern techniques for diagnosis

- Digene hybrid capture method for the detection of high-risk HPV viral types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 68. A positive test indicates the presence of one or more high-risk HPV types. This does not test for the presence of low-risk HPV DNA
- High-risk HPV testing can be done with genotyping for HPV 16/18 in women >30 years of age when performed every five years with co-testing, as per ASCCP guidelines

### Differential diagnosis

- LGSIL without koilocytosis
  - Squamous cells considered ASCUS lack the hyperchromasia and irregular granularity of chromatin present in most LGSIL lesions. However, when in doubt, some type of HPV DNA testing is mandatory

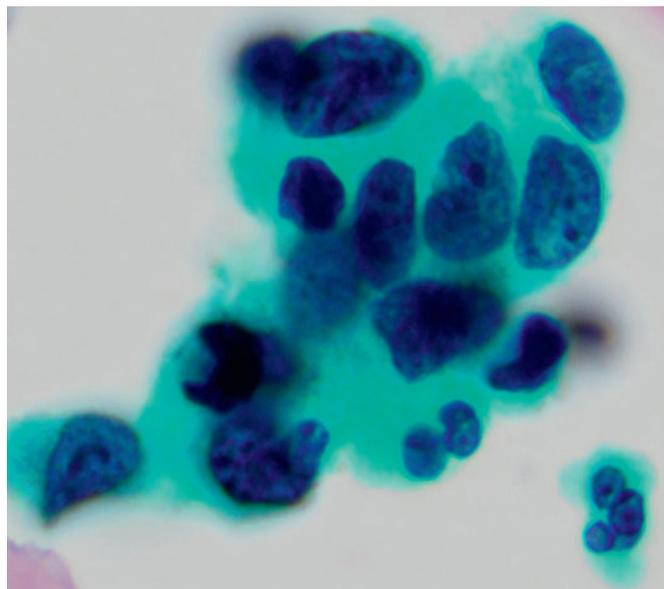
### Pearls

- NOT a specific diagnostic entity and should only be reserved for those cases for which a diagnosis of SIL cannot be made morphologically

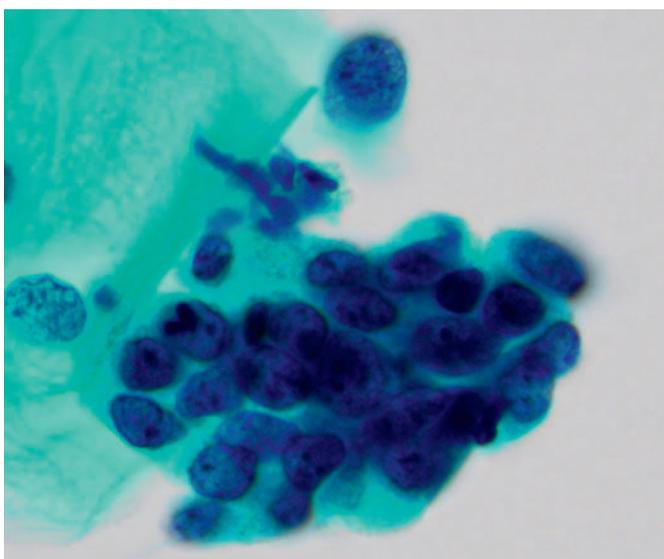
## High-grade squamous intraepithelial lesions (HGSIL)

### Clinical features

- May present as raised or flat excoriated lesions with punctuate hypervascularity and mosaicism on colposcopy in the vulva, vagina, or cervix
- Includes histologic diagnoses of moderate (CIN 2) or severe dysplasia (CIN 3) lesions



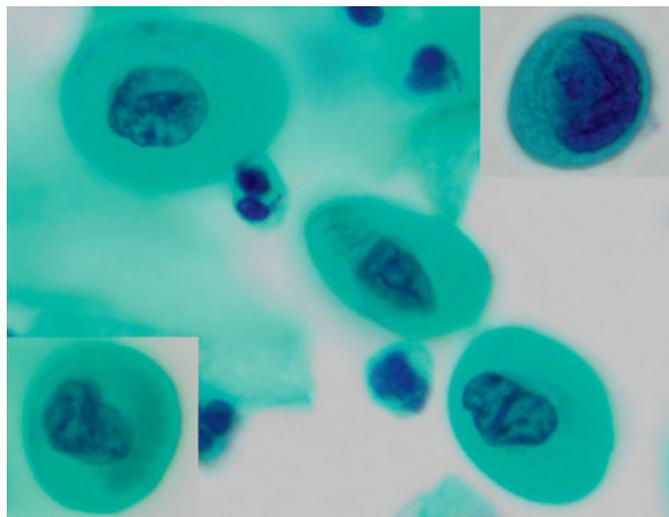
**Figure 1-4A.** High-grade squamous intraepithelial lesion (HGSIL) (PAP stain).



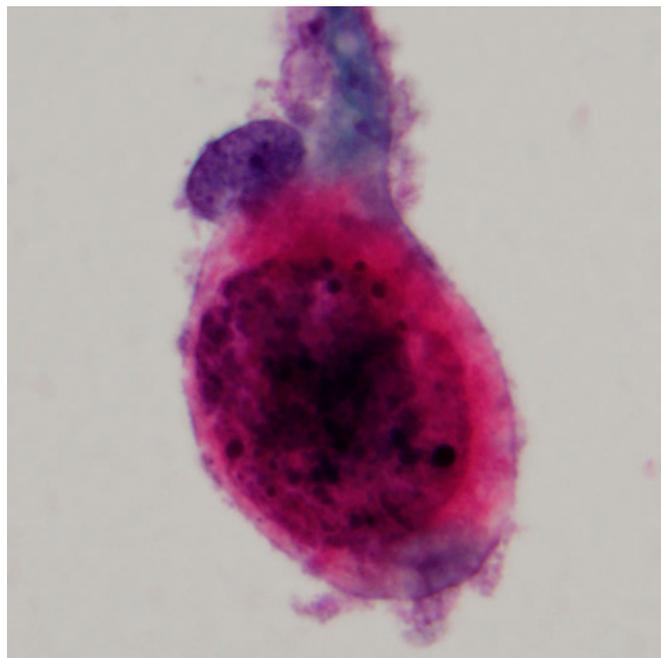
**Figure 1-4B.** HGSIL with lacy cytoplasm (PAP stain).

### Cytologic features

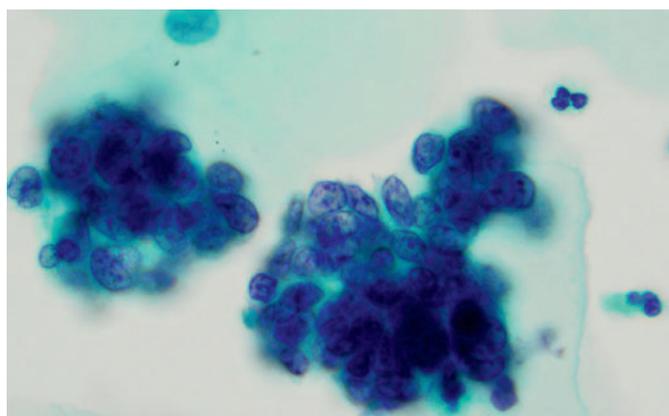
- Singly dispersed, linearly arranged sheets, and syncytial aggregates of cells with high nuclear to cytoplasmic ratios
- Cells are considerably smaller in size and can be 1–1.5 × the size of an intermediate cell nucleus
- Cytoplasm can vary from delicate to polychromatic, and metaplastic to densely keratinized



**Figure 1-4C.** HGSIL with metaplastic-appearing cytoplasm (PAP stain).



**Figure 1-4D.** HGSIL with densely keratinized cytoplasm (PAP stain).



**Figure 1-4E.** Benign endometrial cells (PAP stain).

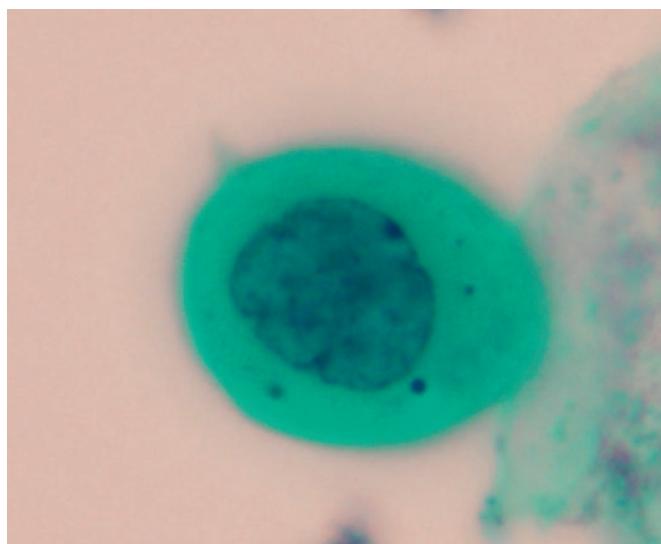
- Hyperchromatic nuclei with irregular granular chromatin and prominent nuclear convolutions
- Inconspicuous nucleoli

#### Special stains and immunohistochemistry

- Non-contributory

#### Modern techniques for diagnosis

- Biomarkers of proliferation and cell cycle dysregulation such as p16INK4, cyclin E, and Ki-67 have been used in histologic specimens to gauge the thickness of dysplastic epithelium, but these antibodies have not been found to be useful in PAP smear cytologic screening



**Figure 1-4F.** ASC-H (PAP stain).

#### Differential diagnosis

- Endometrial cells
  - Cells are usually found in clusters or in a ball, typically called “exodus” cells, and may be seen during the first 14 days of the menstrual cycle
  - Cytoplasm is usually lacy and lacks the metaplastic or orangeophilic and keratinized cytoplasm of HGSIL

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- ASC-H
  - Typically, the cells in ASC-H are few and far between and may not have all of the features of an HGSIL lesion

### Pearls

- HGSIL may be associated with an endocervical adenocarcinoma-*in situ* (AIS)

## High-grade squamous intraepithelial lesions (HGSIL) involving endocervical glands

### Clinical features

- Usually does not have a clinically visible lesion, but generally is associated with an HGSIL lesion with punctuate hypervascularity and mosaicism in the cervix on colposcopy

### Cytologic features

- Cellular features similar to those seen in HGSIL lesions, but typically have a flattening of the nuclei at the edge of the cluster (arrow)
- Columnar cells at the edge of the clusters may mimic an AIS of the endocervix

### Special stains and immunohistochemistry

- Not necessary if the cytologic features are observed as given here

### Modern techniques for diagnosis

- Non-contributory

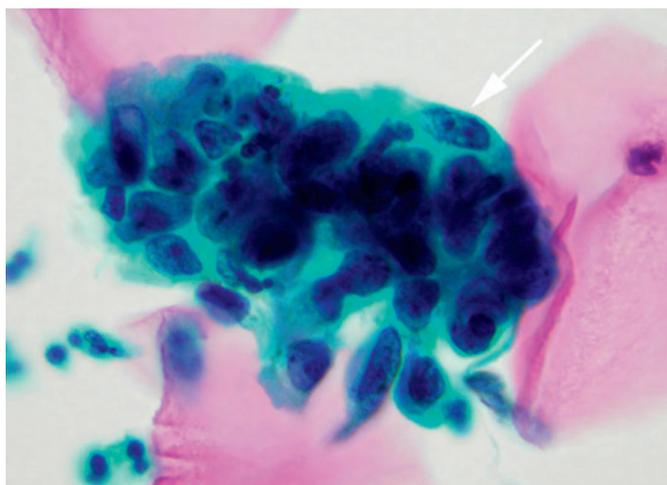


Figure 1-5. HGSIL involving endocervical glands (PAP stain).

### Differential diagnosis

- AIS of the endocervix
  - Presence of abundant feathery borders in the hyperchromatic crowded groups of an AIS lesion

### Pearls

- Although not a commonly diagnosed entity in cytology, it can be seen in liquid-based cytologic preparations

## Keratinizing squamous cell carcinoma

### Clinical features

- Clinical history of SIL lesions without follow-up biopsy or patient lost to follow-up, patients with a clinical history of HIV, or patients who did not have a regular PAP smear screening test
- Clinically presents with irregular bleeding
- May present as large fungating lesions of the vulva, vagina, or cervix

### Cytologic features

- Singly dispersed orangeophilic cells in varying size and shapes including caudate and spindle-shaped cells colloquially called “tadpole cells”
- Nuclei can be variable in size, but generally are dense and hyperchromatic or pyknotic with densely packed chromatin
- Inconspicuous nucleoli
- Can be seen with a background tumor diathesis and marked acute inflammation

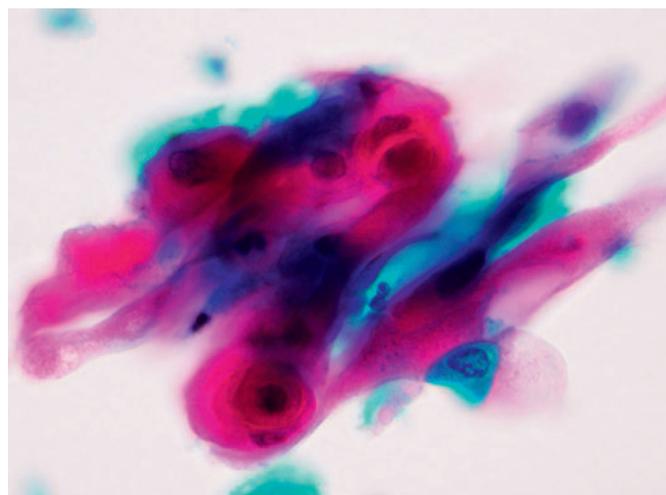


Figure 1-6. Keratinizing squamous cell carcinoma (SCC) (PAP stain).