1

Pap Smear

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OUTLINE

Introduction and a statement on adequacy
Reactive changes
Low-grade squamous intraepithelial lesions (LGSIL)
Arias-Stella change of pregnancy
Atypical squamous cells
High-grade squamous intraepithelial lesions (HGSIL)
High-grade squamous intraepithelial lesions involving endocervical glands
Keratinizing squamous cell carcinoma
Nonkeratinizing squamous cell carcinoma
Papillary squamous transitional cell or papillary squamous cell carcinoma of the cervix
Adenocarcinoma-in-situ of endocervix
Endocervical adenocarcinoma
Endometrial cells
Endometrial adenocarcinoma
Extrauterine malignancies
  Papillary serous adenocarcinoma from either ovary, fallopian tube, and endometrium
  Small cell, undifferentiated or "oat cell" carcinoma
  Malignant mixed mullerian tumors
  High-grade sarcomas
  Malignant lymphoma
Metastases by direct extention and distant spread
  Breast Adenocarcinoma
  Paget's disease of the vulva
  Gastric adenocarcinoma
  Colon adenocarcinoma
  Urothelial cell carcinoma
  Malignant melanoma
Microorganisms in Pap smear
  Candida
  Herpes
  Cytomegalovirus
  Trichomonads
  Leptothrix
  Gardnerella vaginalis
  Actinomyces
  Schistosoma haematobium

- Chlamydia trachomatis
- Neisseria gonorrhea
- Syphilis
- Pubic louse or "crab"

Contaminants in Pap smear
- Alternaria alternata
- Asterosclereids
- Plant matter
- Trichomes
- Carpet beetle part
- Pollen

INTRODUCTION

Although this chapter on Pap smears will closely follow the recommendations set forth by the current Bethesda System, it is not by any means a replacement of the standard text on Pap smear cytology. It, however, should be a good resource for the experienced cytomorphologist for possibilities of various differential diagnoses, which may present in Pap smears

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 8,000 and 12,000 cells and between 5,000 and 20,000 cells in liquid-based media (Bethesda 2001). The presence of at least ten well-preserved endocervical or squamous metaplastic cells, which implies adequate sampling of the transformation zone, is also important. This criteria is only negated if the woman has had 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: (i) the type of specimen obtained; (ii) a statement on the specimen adequacy and any reason for an unsatisfactory specimen; (iii) the general category of lesions; and (iv) interpretations of results. The reader is referred to the current publication of Bethesda 2001 for a more complete listing of the classification system

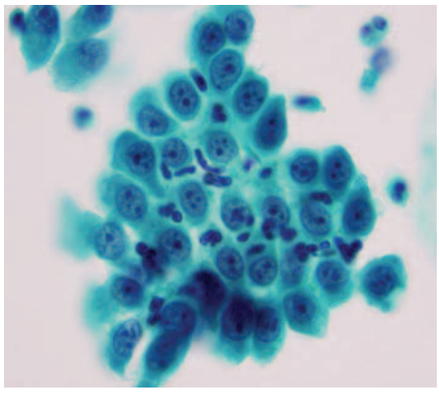
REACTIVE CHANGES

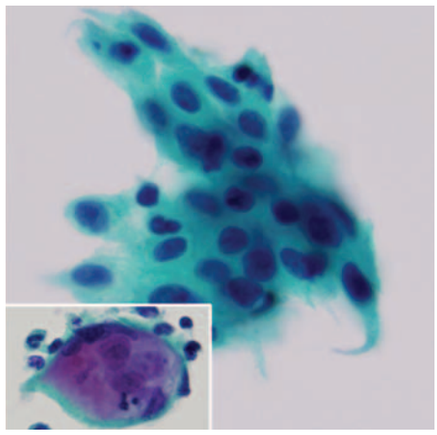
Clinical Features

- Common cytologic pattern, which can be due to repair, atrophy, prolapsed uterus, radiation changes, and infectious processes
- Could be due to numerous infectious process 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 is a presenting concern to the patient

Cytologic Features

- A prominent or subtle neutrophilic exudate
- Maybe associated with common infectious processes: candida, trichomonas, Gardnerella vaginosis, etc.
- Cells are commonly found in cohesive sheets and tile-like or honeycomb configuration
- Nuclei are enlarged (1 to 1.5× the size of an intermediate cell nucleus), can be binucleated, nuclear outlines are round, smooth and uniform, vesicular, and hypochromatic to mildly hyperchromatic
- Multiple or single nucleoli in most of the cells in the sheets





1-1A. Reactive changes *(opposite, left)*. With neutrophils (Papanicolaou stain)

1-1B. Reactive changes due to atrophy *(opposite, right)*. (Papanicolaou stain)

1-1C. Reactive changes due to Radiation *(below, top)*. (Papanicolaou stain)

1-1D. Reactive changes due to IUD *(below, middle)*. (Insets are reactive endometrial cells) (Papanicolaou stain)

1-1E. Tubal metaplasia *(below, bottom)*. (Papanicolaou stain)

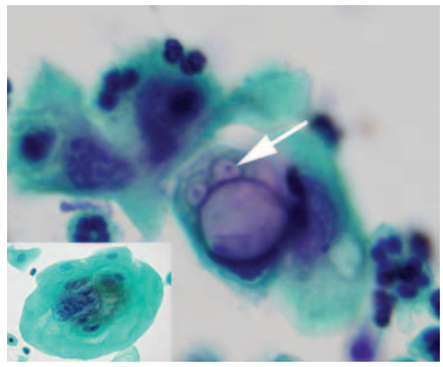
- Cytoplasm could be polychromatic, vacuolated with a small perinuclear halo

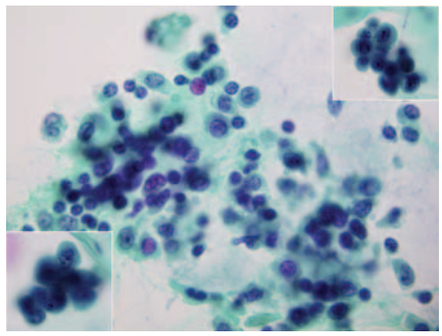
Special Stains and Immunohistochemistry

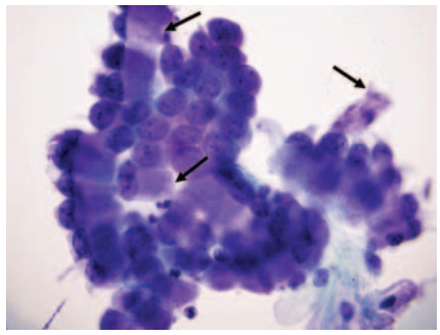
- The cytologic features of most infectious agents are well-defined and usually do not need special stains in a Papanicolaou-stained slide

Modern Techniques for Diagnosis

- PCR testing for both chlamydia and gonorrhea

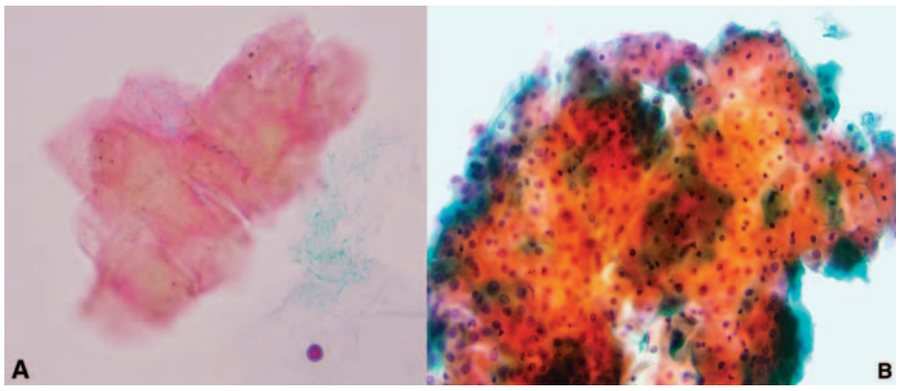




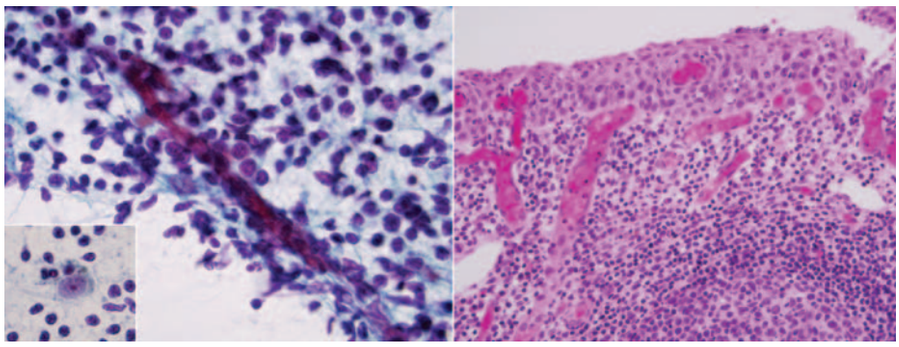


Differential Diagnosis

- 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 (arrow)
- Reactive cellular changes associated with 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



1-1F. Hyperkeratosis and Parakeratosis. (A) and (B) (Papanicolaou stain)



1-1G. Lymphocytic cervicitis. (Papanicolaou stain)

■ Other nonneoplastic or metaplastic changes that 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 (arrows)

➤ Hyperkeratoses and parakeratoses

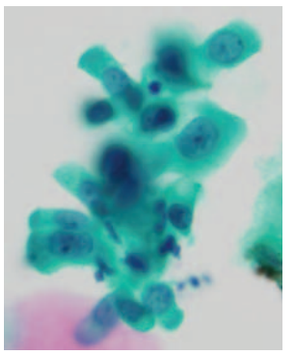
Commonly associated with prolapse, but could also harbor a squamous intraepithelial lesion (SIL)

➤ Lymphocytic cervicitis

Commonly associated with Chlamydia trachomatis *infection than any other venereal infection*

➤ Reactive endocervical cells

Commonly have sheets of endocervical cells with multiple nucleoli or prominent chromocenters



1-1H. Endocervical cell atypia. (Papanicolaou stain)

PEARLS

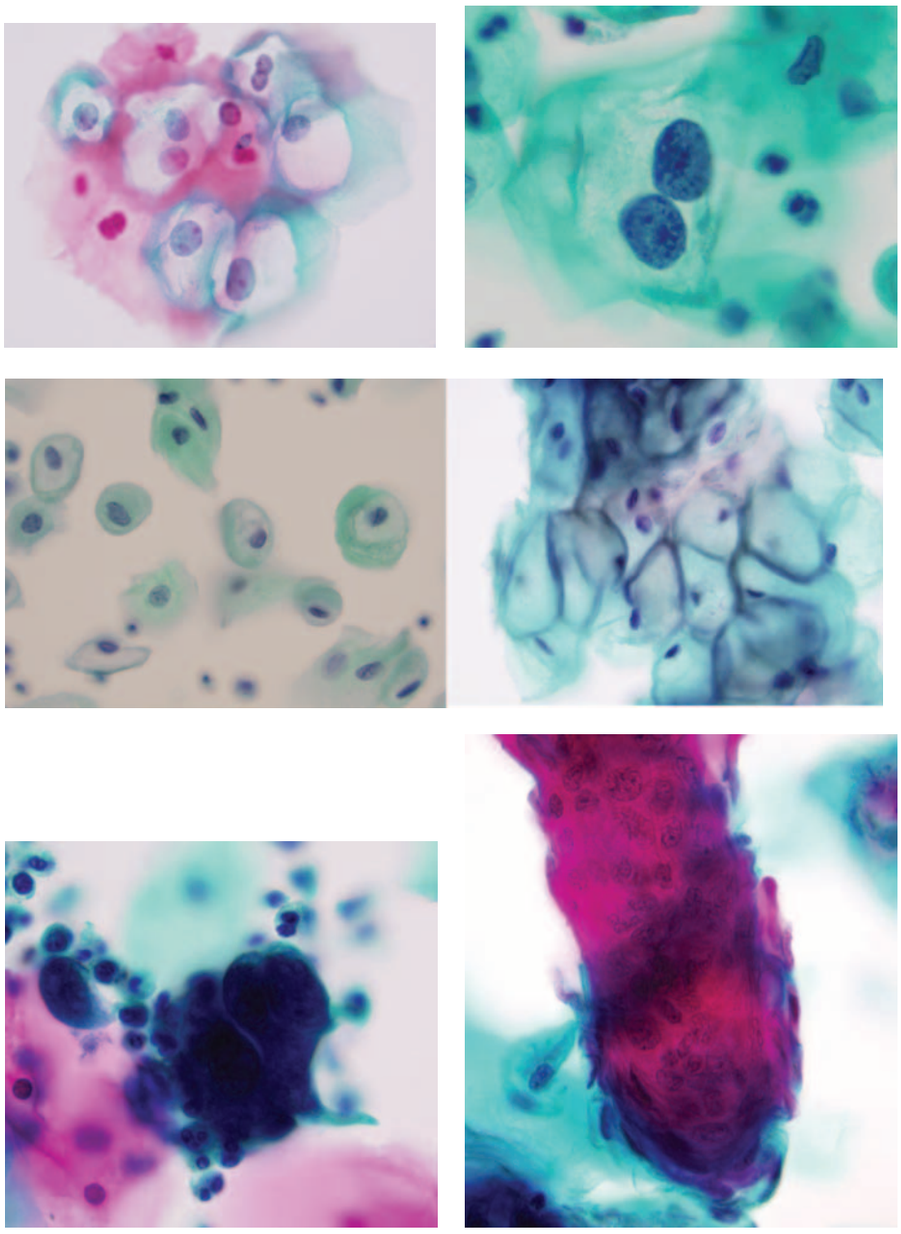
★ The search for an infectious agent and an adequate correlation with clinical history is imperative in the diagnosis

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



1-2A. Koilocytes (LGSIL) *(top, left).*

1-2B. Low-grade squamous intraepithelial lesion *(top, right).* (Papanicolaou stain)

1-2C. Navicular cells of pregnancy *(middle).* (Papanicolaou stain)

1-2D. Trophoblasts and Arias-Stella change of pregnancy *(bottom, left).* (Papanicolaou stain)

1-2E. Atypical parakeratosis *(bottom, right).* (Papanicolaou 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 to 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 above

Modern Techniques for Diagnosis

- Noncontributory

Differential Diagnosis

- Glycogenated or navicular cells of pregnancy
  - ➤ Lacks nuclear enlargement and hyperchromasia
  - ➤ 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 above, these lesions can harbor both low-and high-grade human papilloma viral types

ARIAS-STELLA CHANGE OF PREGNANCY

Clinical Features

- A history or pregnancy is present. Very early postpartum changes can also present with Arias-Stella changes in the epithelial and stromal cells that can be interpreted as atypical glandular cells, NOS

Cytologic Features

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

Special Stains and Immunohistochemistry

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

Modern Techniques for Diagnosis

- Noncontributory

Differential Diagnosis

- A clear cell adenocarcinoma of mullerian origin may be cytologically difficult to differentiate and may present in young women, therefore, a tissue biopsy and serum B-HCG levels would be necessary to exclude one from the other
- Radiation effect – a history of radiation or pregnancy are the main differentiating factors

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 B-HCG level may be necessary
★ May also be the presenting finding in women with molar pregnancies

ATYPICAL SQUAMOUS CELLS

Clinical Features

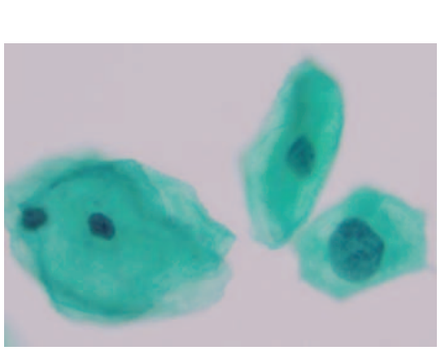
- Patients may not have a distinct or specific clinical symptom
- May represent the first indication of a smoldering squamous intraepithelial lesion

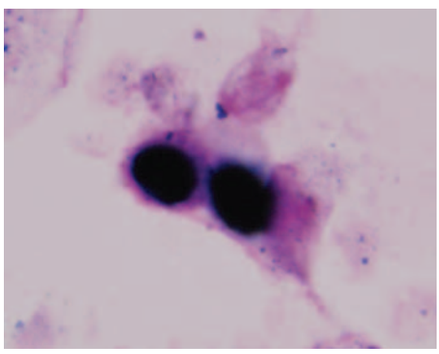
Cytologic Features

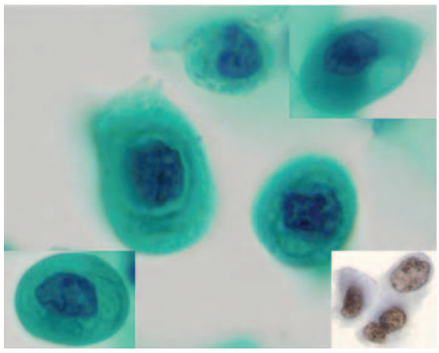
- ''ASC refers to cytologic changes suggestive of SIL, which are qualitatively and quantitatively insufficient for a definitive interpretation of an SIL lesion'' (Bethesda 2001)
- This is not a distinct diagnostic entity, but a ''waste-basket'' category reserved for those cases for which a definitive 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 United States 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)

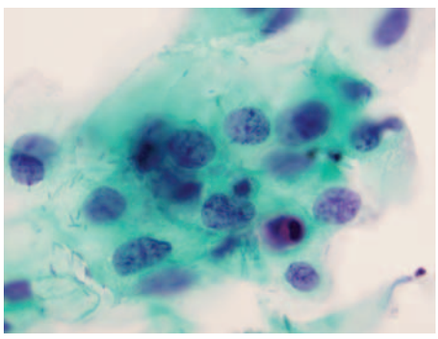
Special Stains and Immunohistochemistry

- 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
- ProExC® by Tripath imaging is a new antibody, which stains cells undergoing aberrant S-phase induction as would be seen in highly and inappropriately proliferative









1-3A. *(top, left).* Atypical squamous cells of undetermined significance (ASCUS) (Papanicolaou stain)

1-3B. *(top, right).* In situ hybridization technique for detecting high-risk HPV infection (Papanicolaou stain)

1-3C. *(bottom, left).* Atypical squamous cells – cannot exclude HGSIL (ASC-H) with ProExC® (Papanicolaou stain)

1-3D. *(bottom, right).* LGSIL without koilocytosis (Papanicolaou stain)

squamous epithelium. This could potentially 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. This test is based in Salt Lake City, Utah, to which all specimens are sent for testing

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 now 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

Cytologic Features

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

Special Stains and Immunohistochemistry

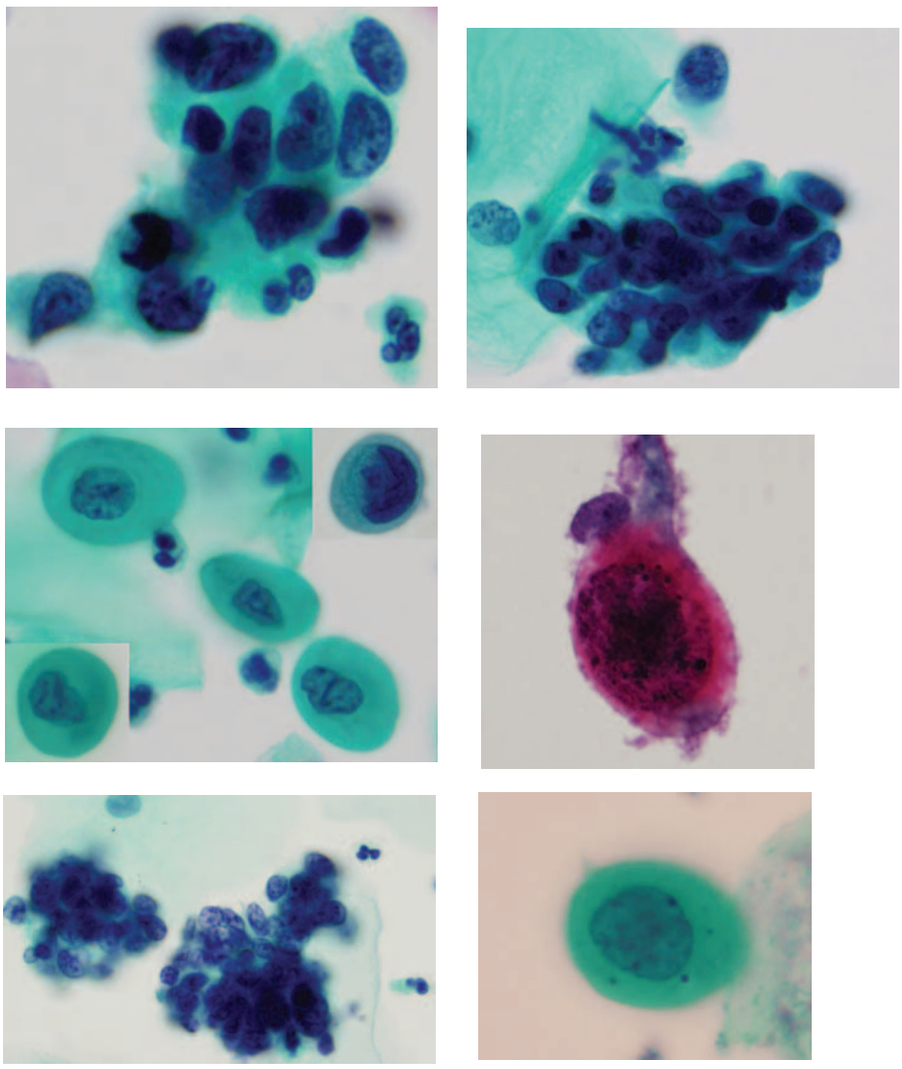
- Noncontributory

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 found its usefulness in Pap smear cytologic screening

Differential Diagnosis

- Endometrial cells
  - ➤ Cells are usually found in clusters or as in a ball, typically called ‘‘exodus’’ cells and may be seen during the first fourteen days of menstrual cycle



1-4A. High-grade squamous intraepithelial lesion (HGSIL) *(top, left).* (Papanicolaou stain)

1-4B. HGSIL with lacy cytoplasm *(top, right).* (Papanicolaou stain)

1-4C. HGSIL with metaplastic-appearing cytoplasm *(middle, left).* (Papanicolaou stain)

1-4D. HGSIL with densely keratinized cytoplasm *(middle, right).* (Papanicolaou stain)

1-4E. Benign endometrial cells *(bottom, left).* (Papanicolaou stain)

1-4F. ASC-H *(bottom, right).* (Papanicolaou stain)

➤ Cytoplasm is usually lacy and lacks the metaplastic or orangeophilic and keratinized cytoplasm of HGSIL

■ ASC-H

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

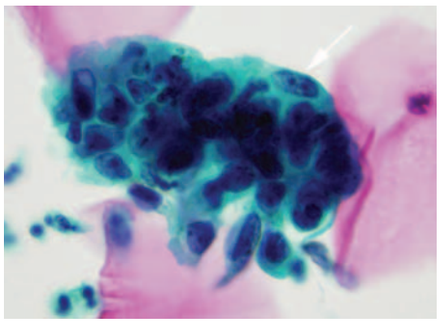
PEARLS

★ HGSIL maybe associated with an endocervical adenocarcinoma in situ

HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS INVOLVING ENDOCERVICAL GLANDS

Clinical Features

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



1-5. HGSIL involving endocervical glands. (Papanicolaou stain)

Cytologic Features

- Cellular features similar to that 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 adenocarcinoma in situ of the endocervix

Special Stains and Immunohistochemistry

- Not necessary if the cytologic features are observed as above

Modern Techniques for Diagnosis

- Noncontributory

Differential Diagnosis

- Adenocarcinoma in situ of the endocervix
  - ➤ Presence of abundant feathery borders in the hyperchomatic 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 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

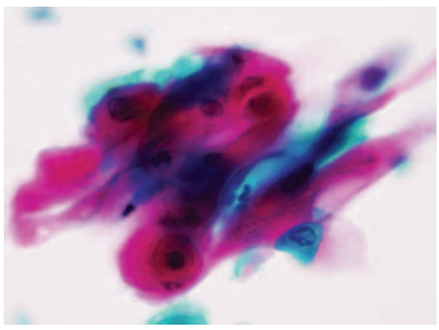
Cytologic Features

- Singly dispersed orangeophilic cells in varying sizes and shapes including caudate and spindled cells colloquially called ‘‘tadpole cells’’
- Nuclei can be variable in size, but generally dense and hyperchomatic or pyknotic with densely packed chromatin
- Inconspicuous nucleoli
- Can be seen with a background tumor diathesis and marked acute inflammation

Special Stains and Immunocytochemistry

- Noncontributory in cytology

1-6. Keratinizing squamous cell carcinoma. (Papanicolaou stain)



Modern Techniques for Diagnosis

- Noncontributory

Differential Diagnosis

- Keratinizing squamous cell carcinoma from other sites such as skin, but the cytologic features are the same

PEARLS

★ The morphologic feature commonly used to identify these lesions is the presence of keratin ‘‘pearls’’ or more commonly ‘‘tadpole cells’’ in cytology

★ Tumor diathesis