

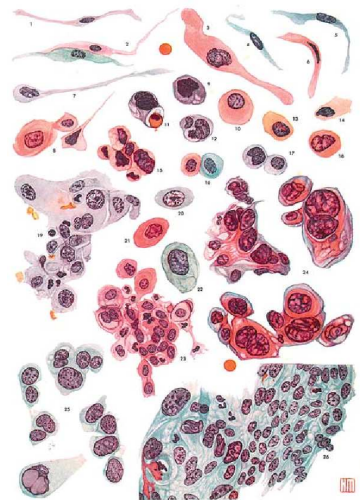
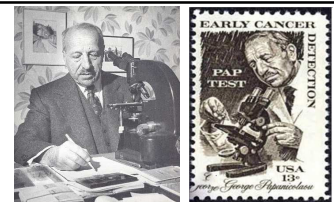
HSIL and its Mimics

Vanda Torous, MD

Assistant Professor, Harvard Medical School
Director, Cytopathology Quality Assurance and Improvement
Program Director, Massachusetts General Hospital Cytopathology Fellowship

History of the Pap Test

- Drastic decline in cervical cancer seen in the 20th century
 - Most common cause of cancer deaths in US women in 1930's, now not even in top 10
- Pap test is the “most successful cancer reduction program ever devised”
 - Correlation between implementation and decrease in cervical cancer mortality as well as intensity of screening and decreased mortality
 - Screening history a highly significant risk factor independent of other factors
- Not yet eradicated!
 - In US: 13,000 new cases each year and 4,000 deaths
 - Worldwide: 500,000 new cases each year and 300,000 deaths



Squamous Intraepithelial Lesions

- Precursors to invasive SCC
- Divided into two grades:
 - Low grade (LSIL): Transient infection with low risk of progression to cancer (0.15%)
 - High grade (HSIL): Persistent infection with increased risk of progression (1.4%)
- Associated with human papillomavirus (HPV) infection
 - Over 200 HPV genotypes, but only a minority cause invasive cancer
 - Low-risk, high-risk types based on frequency of association
 - HPV typing plays no role in grading of SIL
 - High-risk HPV predominates in both LSIL and HSIL

Low-risk HPV $\neq$ LSIL
High-risk HPV $\neq$ HSIL

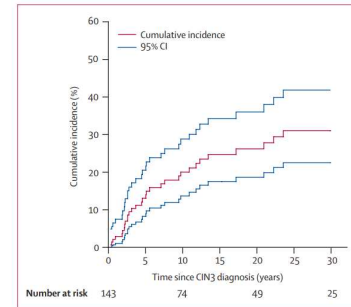
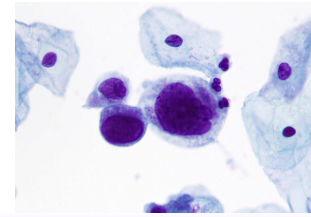


Figure 3: Cumulative incidence of cancer of the cervix or vaginal vault in women with minimum disturbance of the CIN3 lesion (no more than a punch or wedge biopsy)

Melnikow J. Obstet Gynecol 1998
McCredie MRE et al, Lancet Oncol 2001

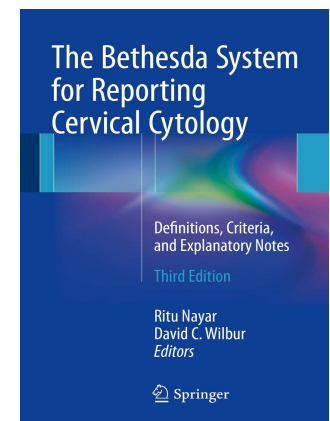
Reporting Squamous Lesions using The Bethesda System

Three general classification categories:

- Negative for intraepithelial lesion or malignancy
- Other: See Interpretation/Result
- Epithelial cell abnormality: See Interpretation/Result

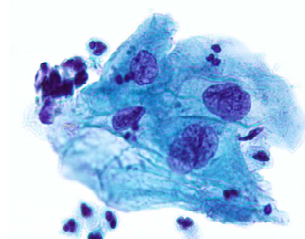
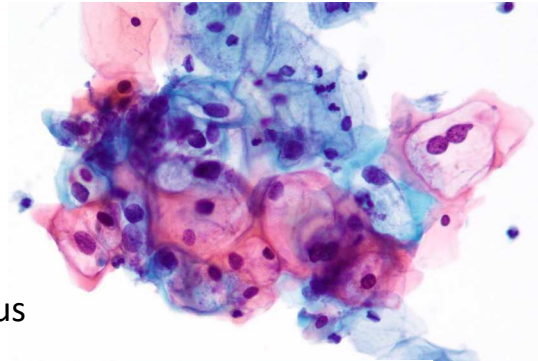
Epithelial cell abnormalities: Squamous cell

- Atypical Squamous Cells
 - Of undetermined significance (ASC-US)
 - Cannot exclude HSIL (ASC-H)
- Low Grade Squamous Intraepithelial Lesion (LSIL)
- High Grade Squamous Intraepithelial Lesion (HSIL)
- Squamous cell carcinoma



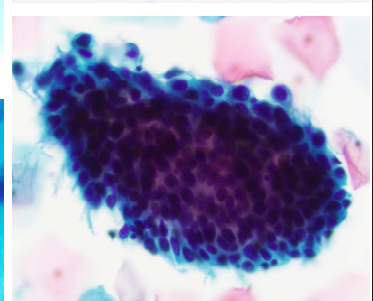
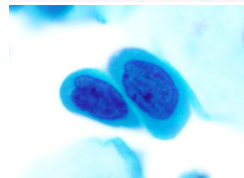
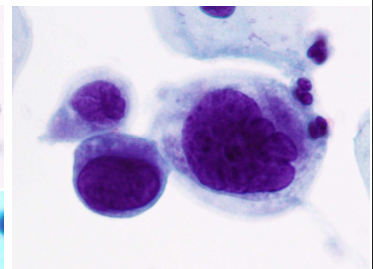
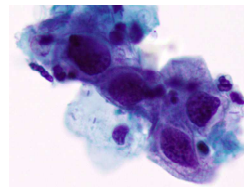
Low Grade Squamous Intraepithelial Lesions

- Cytologic changes in 'mature' type cells
- Nuclear atypia:
 - Enlargement >3x that of intermediate cell
 - Irregular nuclear contours
 - Hyperchromatic
 - Slight chromatin coarseness
- Nucleoli generally absent or inconspicuous if present
- Bi- and multinucleation common
- Perinuclear cytoplasmic cavitation (koilocytosis) may be seen
 - Non-koilocytic LSILs possible



High Grade Squamous Intraepithelial Lesions

- Lesion of immature squamous cells
- Architectural patterns:
 - Distinct individual cells or hyperchromatic crowded groups
- Nuclear atypia:
 - Enlargement
 - Marked nuclear contour irregularity
 - Marked hyperchromasia (usually)
 - Marked chromatin coarseness
- Variable appearance of cytoplasm:
 - Lacy/delicate
 - Cytoplasmic vacuolization
 - Densely metaplastic
 - Densely keratinized (keratinizing HSIL)

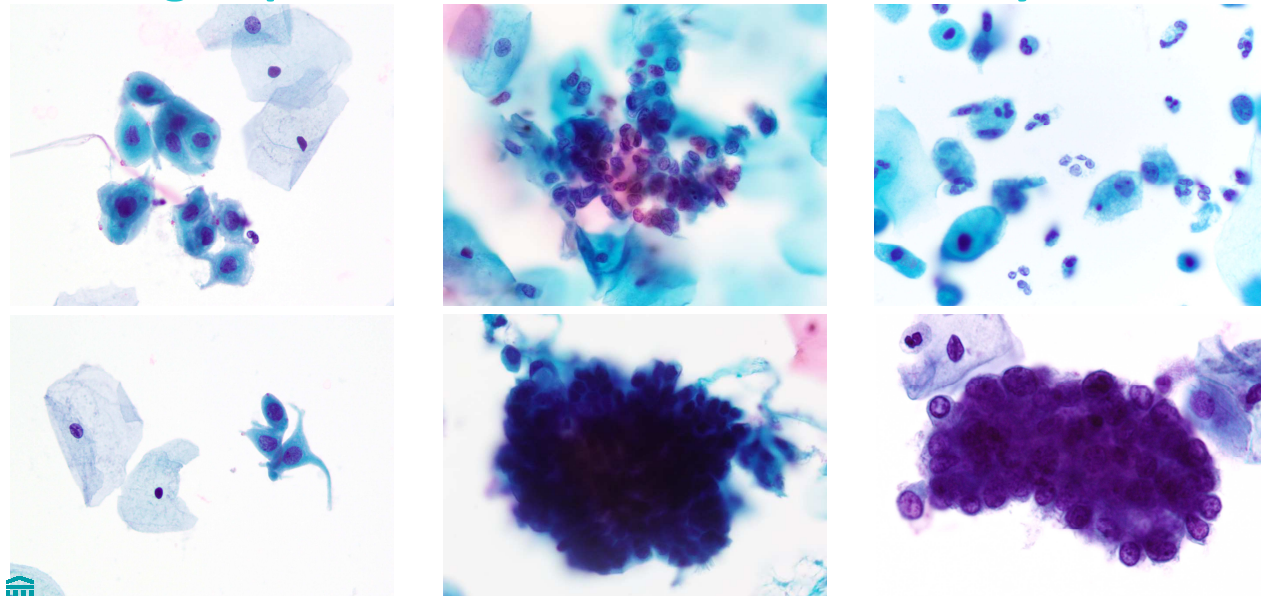


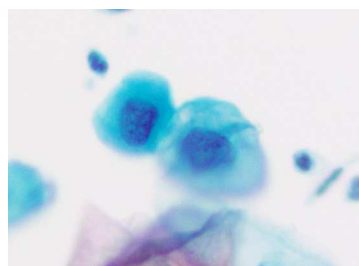
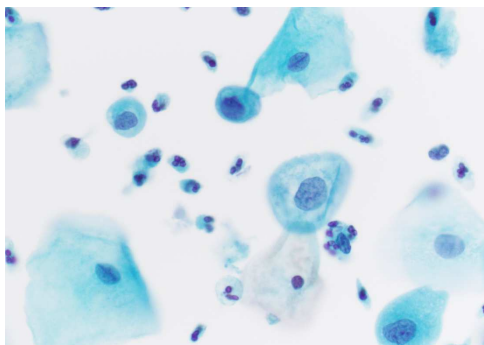
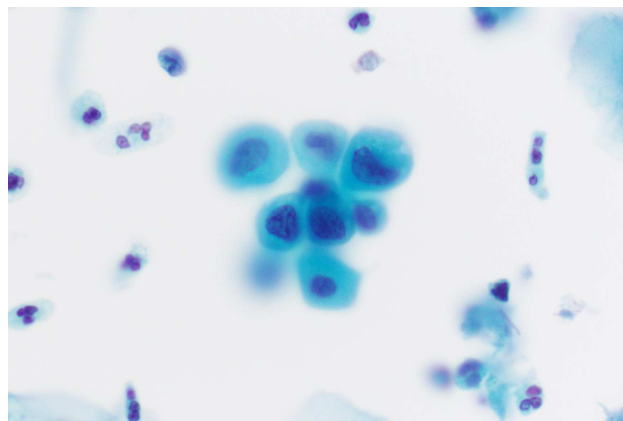
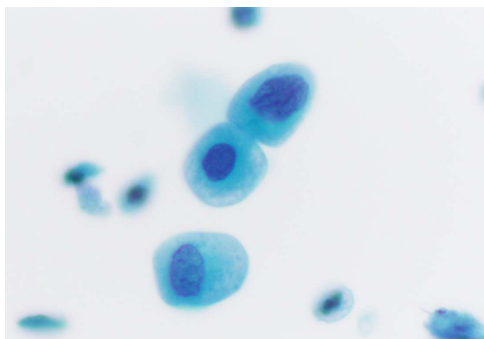
High Grade Squamous Intraepithelial Lesions

- Benign mimics
 - Benign epithelial cells (squamous / tubal metaplastic cells, endometrial cells)
 - Inflammatory cells (histiocytes, lymphocytes)
 - Immature squamous metaplasia
 - Transitional cell metaplasia
 - Herpes
 - IUD effect
 - Endocervical polyp
- Neoplastic mimics
 - LSIL, SCC, AIS
- Problematic patterns:
 - Syncytial aggregates
 - SIL with endocervical gland involvement
 - HSIL resembling endometrial cells
 - Single and rare small HSIL cells
 - Abnormal stripped nuclei
 - Keratinizing lesions
 - HSIL in atrophy
 - LSIL with some features suggestive of concurrent HSIL

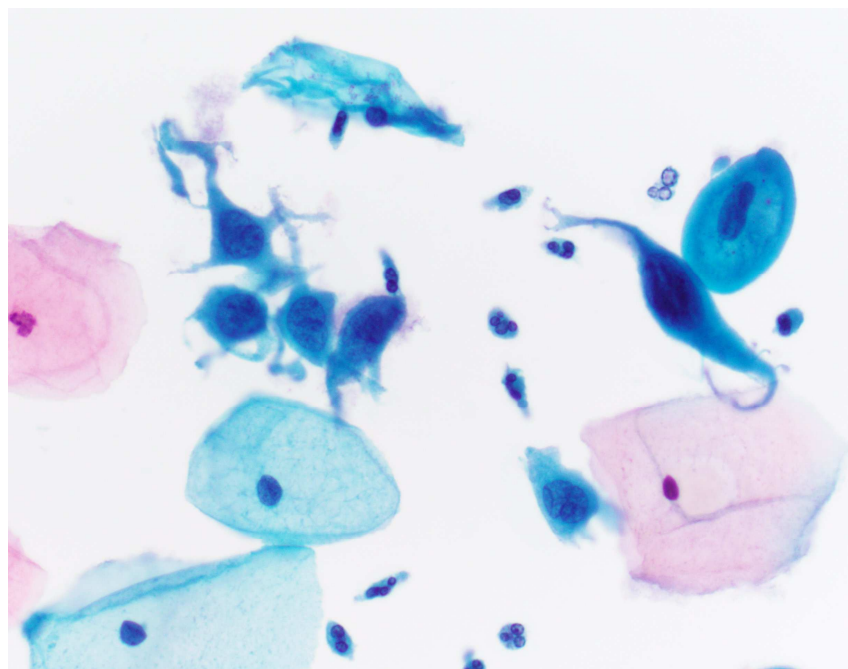


Benign Epithelial Cells and Inflammatory Cells



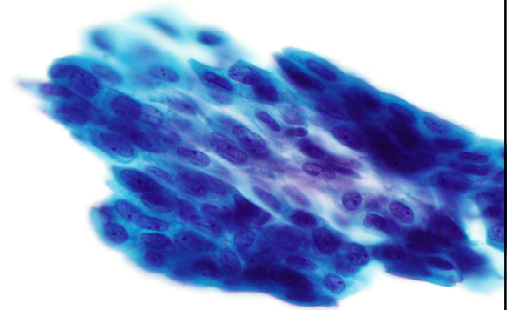
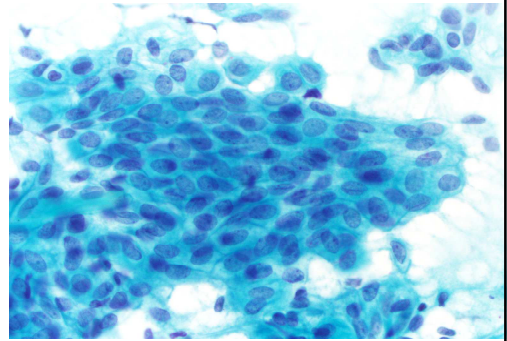


HSIL with
metaplastic-type
cytoplasm



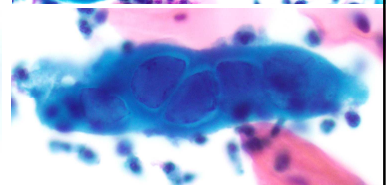
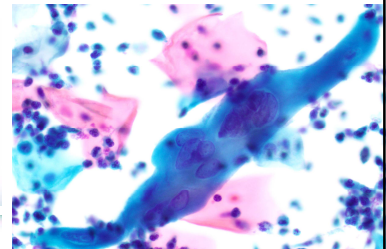
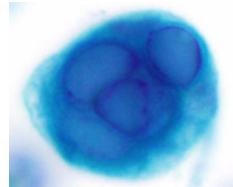
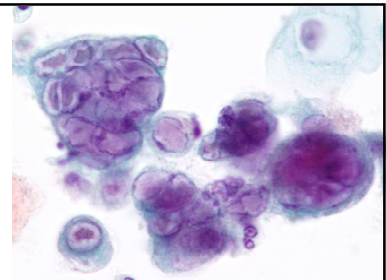
Transitional Cell Metaplasia

- Atrophic epithelium resembling transitional cell epithelium
- Characteristic cytologic finding frequently encountered in FTM transgender patients on testosterone therapy
- Flat sheets of cells
- Wrinkled, oval to spindle-shaped nuclei, powdery chromatin, small nucleoli, small perinuclear halos
- Prominent longitudinal nuclear grooves (“coffee bean nuclei”)

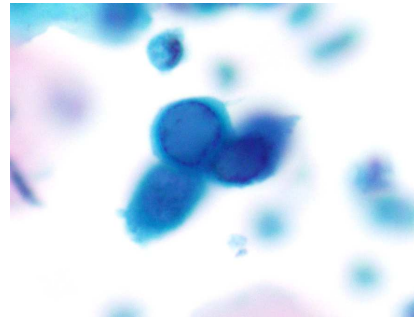
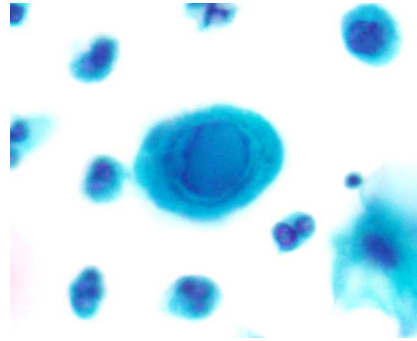
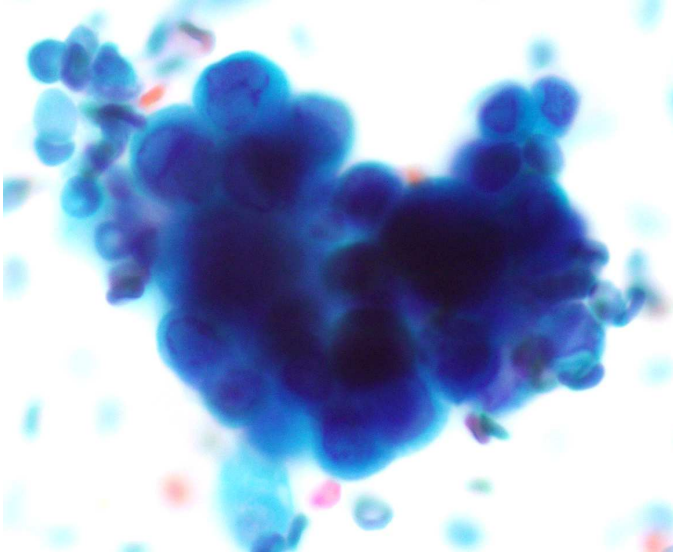


Herpes Simplex Virus

- Cytopathic effect
 - 3 M's: multinucleation, molding, margination
 - Ground-glass nuclei
 - Dense eosinophilic intranuclear (Cowdry) inclusions
- Relatively reproducible
- DDX LSIL and HSIL
 - Homogenous (ground-glass) appearance versus hyperchromatic nuclei of SILs

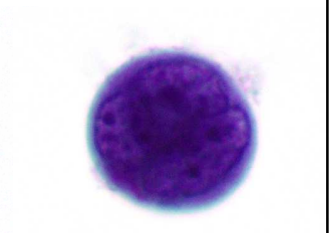
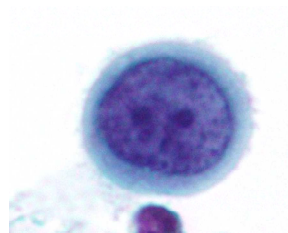
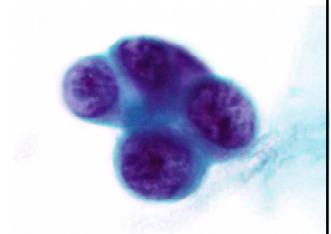
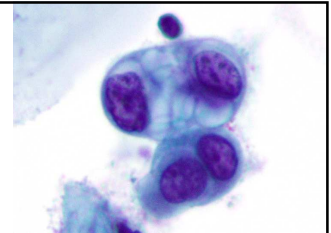


Herpes Viral Cytopathic Effect mimicking HSIL

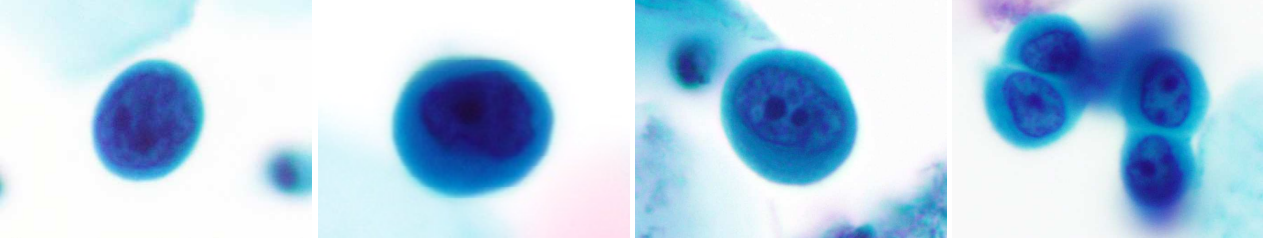


Intrauterine Device Effect

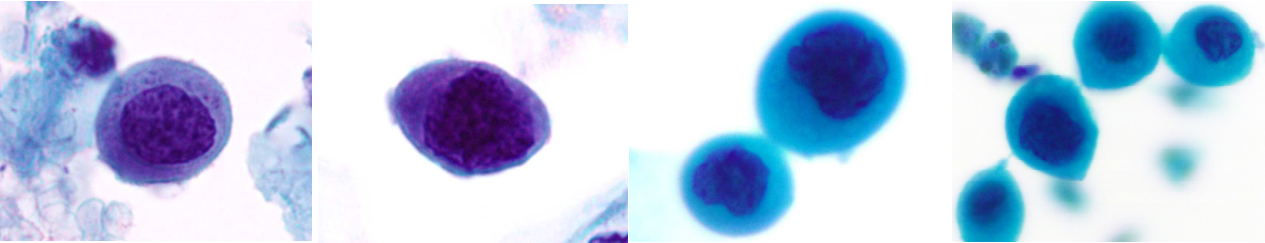
- Endometrial and/or endocervical cells with distinct cytomorphologic changes reflecting chronic irritation
- Two characteristic cell types:
 - Cells with vacuolated cytoplasm – ddx adenocarcinoma
 - Small, dark cells with scant cytoplasm, high N:C – ddx HSIL
- Found singly or in small clusters
- May show bi- or multinucleation
- Helpful distinguishing feature: nucleoli



Intrauterine Device Effect (small dark cells)



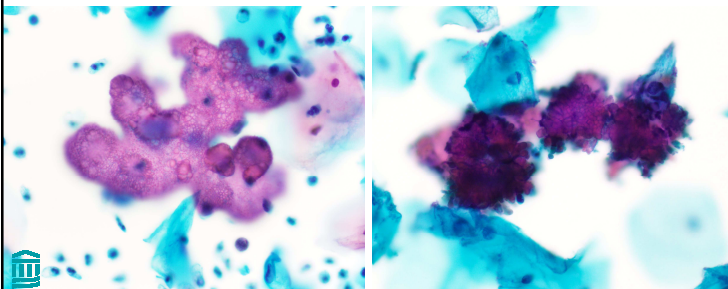
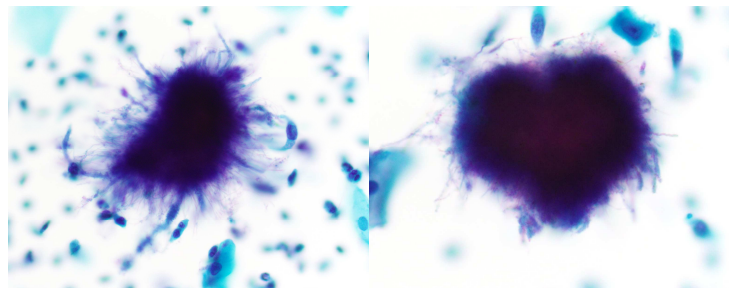
High Grade Squamous Intraepithelial Lesion



Background Findings Associated with IUD

Actinomyces

- Filamentous bacteria
- Appear as tangled clumps (“cotton ball”)
- Presence doesn’t dictate removal in absence of clinical features of infection
- Gram positive, GMS positive (aberrant)



Pseudoactinomycotic radiate granules

- Noninfectious
- Eosinophilic structures that appear spherical or in strips with club-like projections
- Gram stain with nonspecific staining, GMS negative



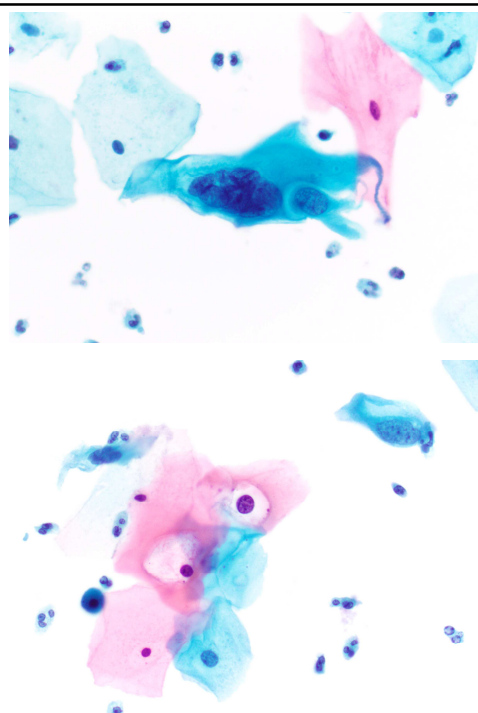
	Intrauterine Device Effect	High Grade Squamous Intraepithelial Lesions
Clinical	Premenopausal or perimenopausal women (contraceptive device); history of IUD may not be explicitly noted	Any age but usually younger women, peak in mid to late 30's; usually asymptomatic
Cellular pattern	Usually few in number; found singly or in small clusters	Usually cellular; found singly or in syncytia/ hyperchromatic crowded groups
Cytomorphology	Two distinct cell types: small dark cells with hyperchromatic nuclei and scant cytoplasm & vacuolated cells; nucleoli can be present; cells can be bi- or multinucleated	Parabasal-sized cells; nuclei will be enlarged, usually with notable contour irregularity, hyperchromasia, and chromatin coarseness; cytoplasm can be dense or squamoid or can be more delicate and might even have vacuoles; can be keratinizing
Distinguishing characteristics	Uniformity of cell population overall Prominent nucleolus can help distinguish small dark cells from HSIL Vacuolated cells might be indistinguishable from endometrial adenocarcinoma Background features can clue in that an IUD is present (actinomyces, pseudoactinomycotic granules, inflammation, reparative changes)	The atypical cell population will usually be cellular Cells will lack nucleoli The vast majority will be hrHPV positive



Torous VF and Pitman MP, JASC 2021

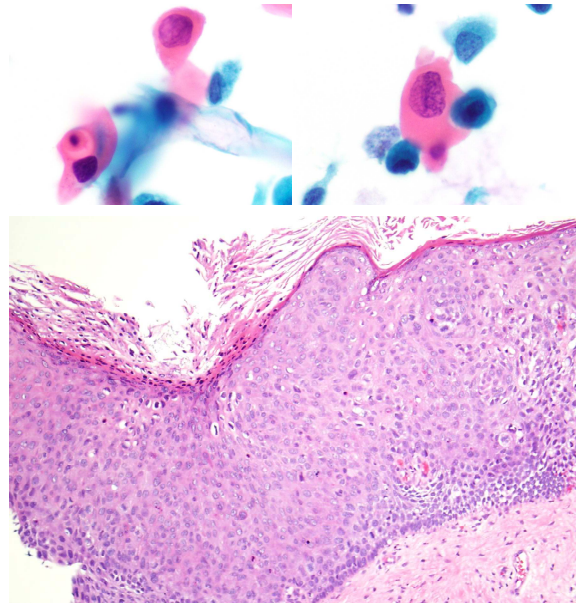
Low Grade vs High Grade SIL

- Cells of HSIL usually smaller and show less cytoplasmic maturity than cells of LSIL
- Degree of nuclear enlargement variable in HSIL but generally a marked increase in nuclear to cytoplasmic ratio
- Hyperchromasia, coarse chromatin, nuclear contour irregularity usually more severe in HSIL
- Some cases are more challenging
 - Pattern 1: Features that lie between low and high grade SILs
 - Pattern 2: Predominant LSIL pattern with few cells showing immature cytoplasmic features with higher N:C
- Cell quantity not a reliable indicator
 - If both LSIL and HSIL, report as HSIL
- In some cases, grading is challenging
 - "SIL, grade cannot be determined" or "LSIL, cannot exclude HSIL" with or without a comment



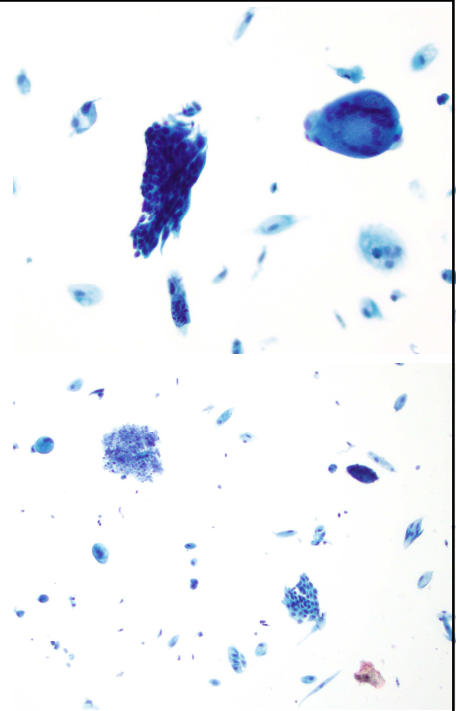
HSIL - Keratinizing Lesions

- Some HSILs have more abundant and abnormally keratinized cytoplasm
- Found singly or in clusters
- Enlarged, hyperchromatic nuclei
- Can be pleomorphic with variation in nuclear size and shape
 - DDX Invasive squamous cell carcinoma
 - Nucleoli and tumor diathesis absent
 - Challenging cases: *HSIL with features suspicious for invasion*



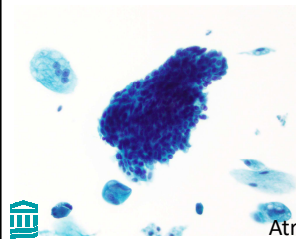
Atrophy

- Decreased estrogen stimulation leading to thinned immature squamous epithelium
 - Menopause, postpartum, s/p bilat oophorectomy, use of exogenous hormones or drugs
- Variable looks, patterns, degree of atrophy, background changes
- Flat monolayered sheets with preserved nuclear polarity and little nuclear overlap
 - Patterns with dispersed parabasal-type cells may predominate
- Uniform chromatin distribution, regular nuclear contours
- Variably sized histiocytes
- Inflammatory exudate, clumped basophilic granular material ≠ tumor diathesis

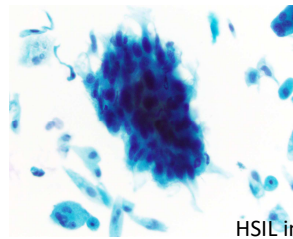


HSIL in Atrophy

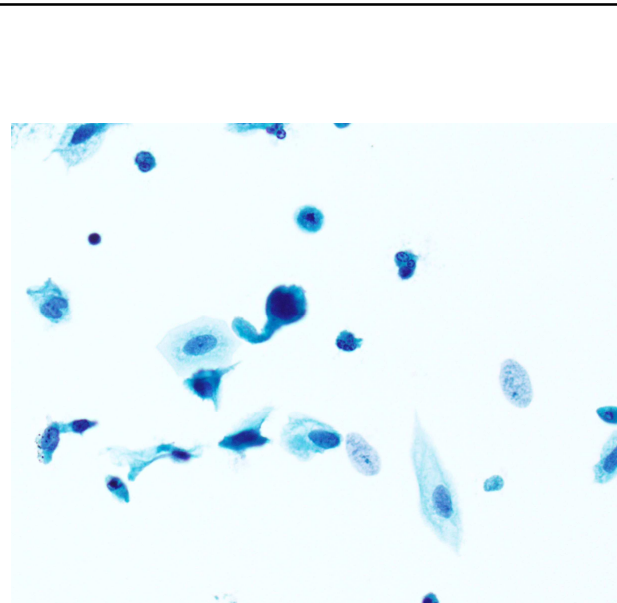
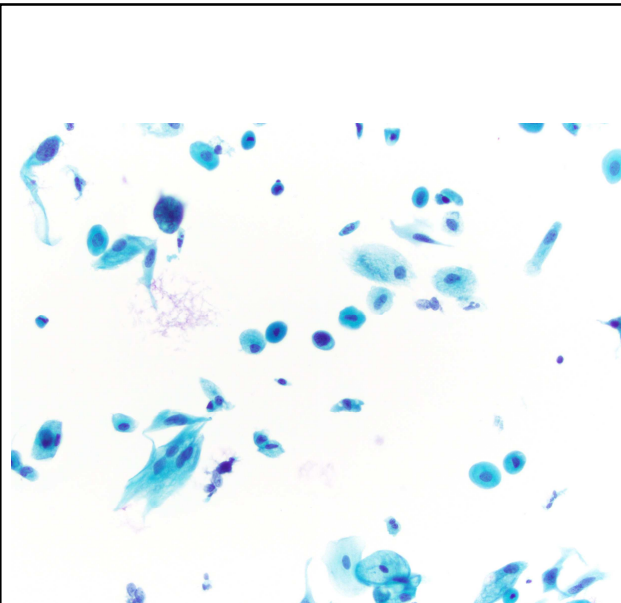
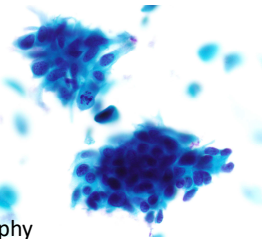
- Challenging due to immaturity of background squamous cells and similarity between immature squamous cells and high grade dysplastic cells
- Parabasal cells may have slight nuclear enlargement and higher N:C but lack nuclear membrane irregularity
- Nuclei may appear hyperchromatic but chromatin tends to be smudgy rather than coarse
- Mitotic activity usually not seen
- Hyperchromatic groups without crowding / nuclear overlapping



Atrophy

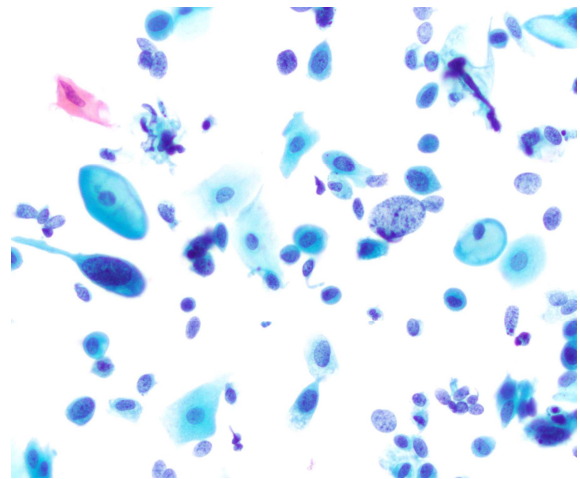
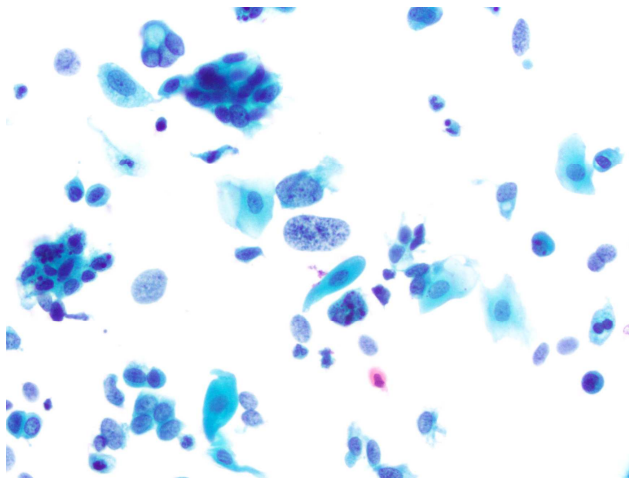


HSIL in Atrophy

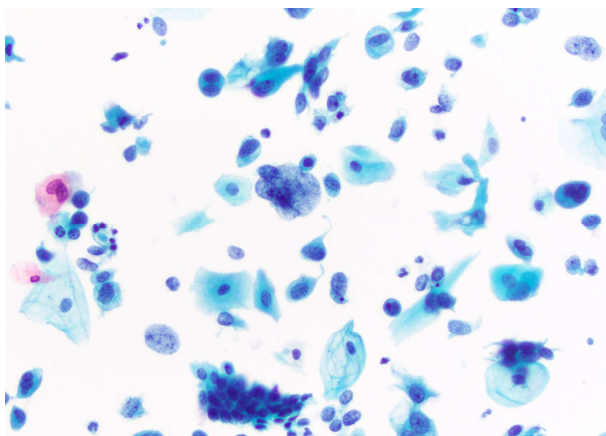


HSIL in Atrophy Single Cell Pattern

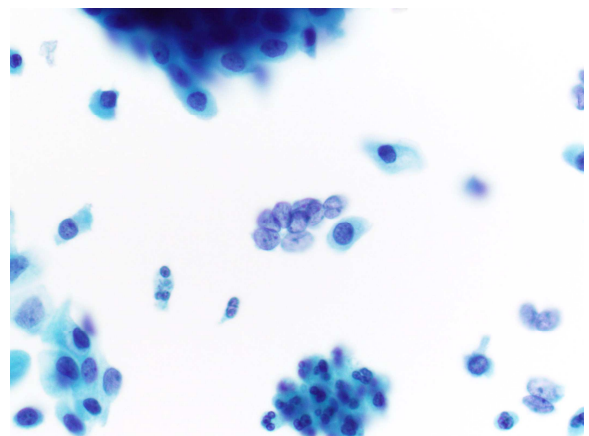




HSIL atypical stripped cells

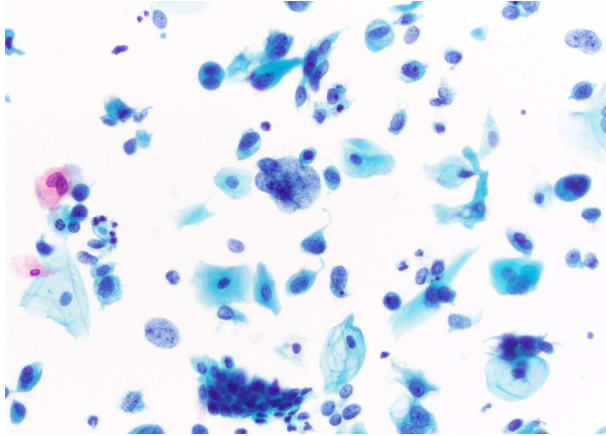


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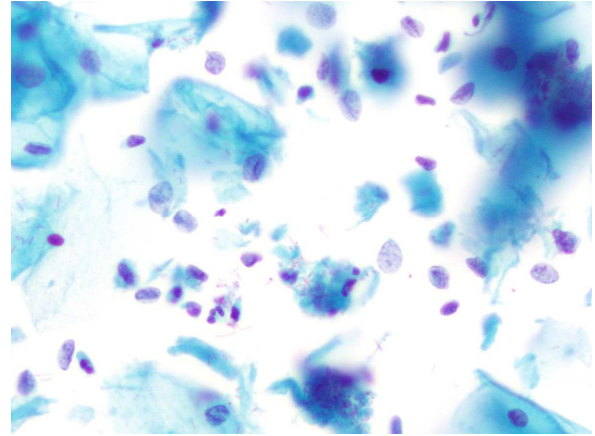
Atypical stripped cells in HSIL

Benign "small blue cells" in atrophy



Atypical stripped cells in HSIL

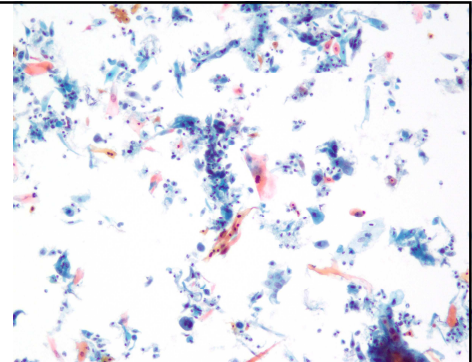
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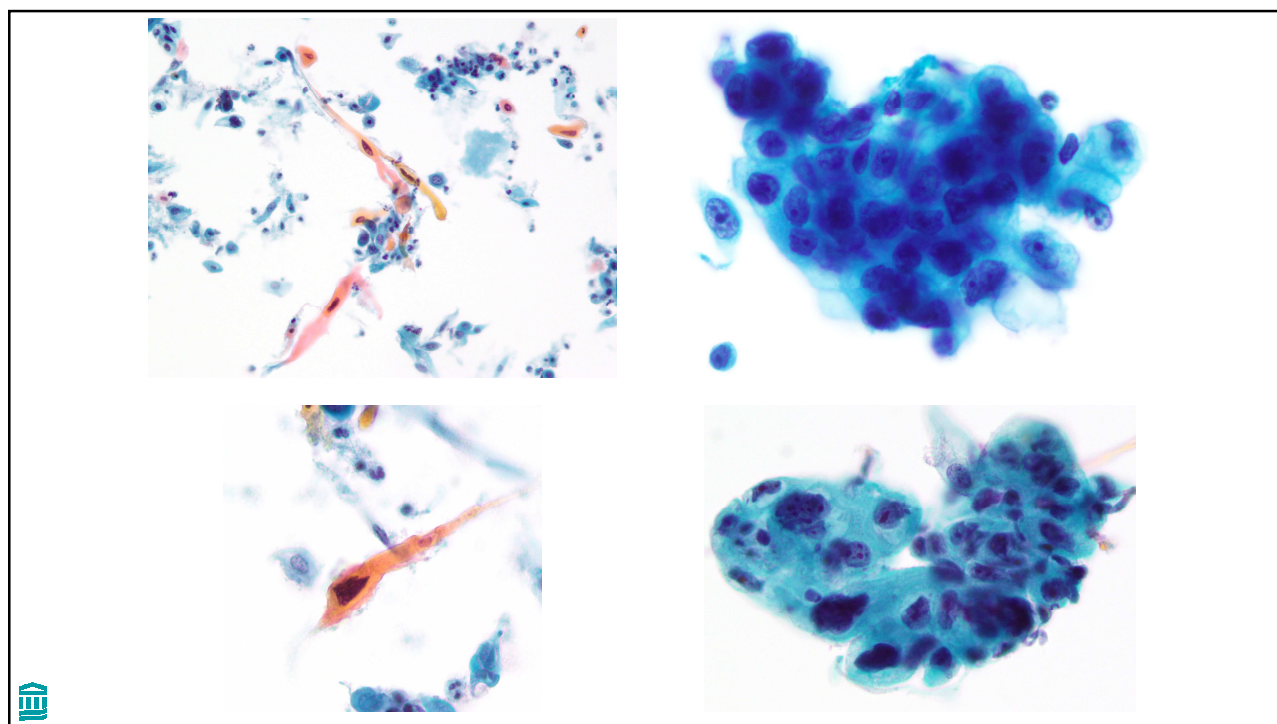


Benign stripped nuclei in cytolysis

Squamous Cell Carcinoma

- Cytomorphologic features of HSIL plus:
 - Macronucleolus
 - Irregular chromatin distribution
 - Tumor diathesis
- Keratinized cells with irregular/bizarre shape (tadpoles, fiber cells) in keratinizing type
- Often with population of HSIL cells



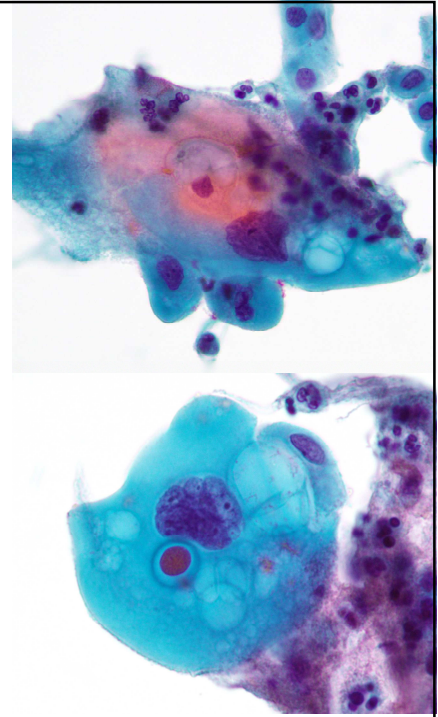


Squamous Cell Carcinoma

- Benign mimics
 - Benign cells (endometrial cells)
 - Radiation change
 - Repair
 - Atrophy
 - Behcet disease
 - Pemphigus vulgaris
- Neoplastic mimics
 - HSIL

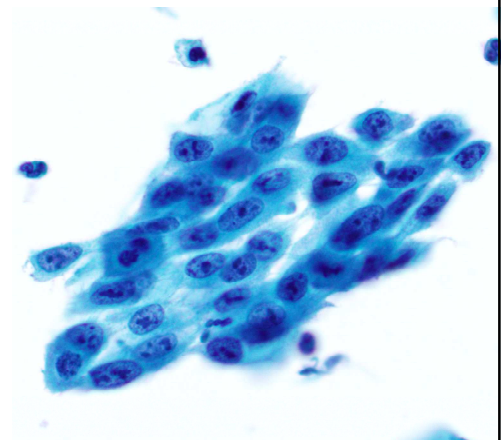
Radiation Change

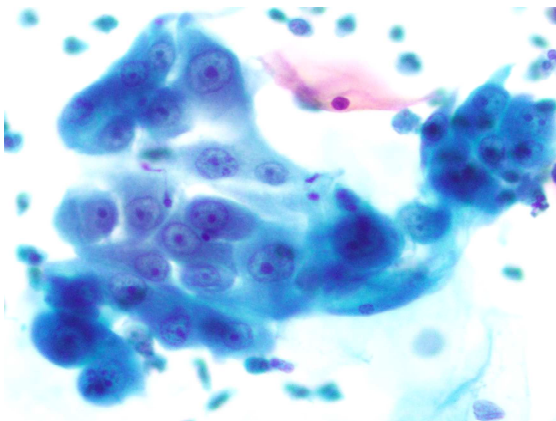
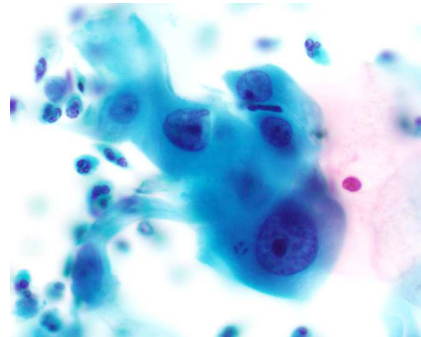
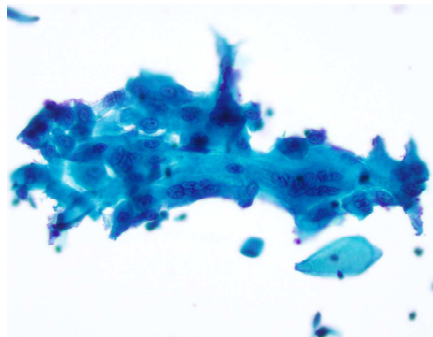
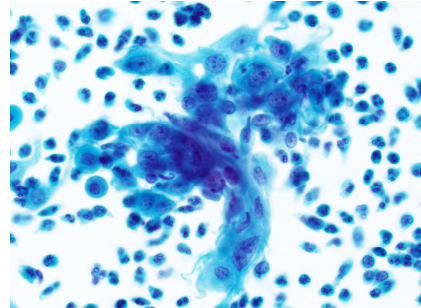
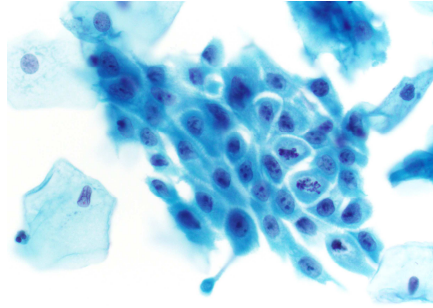
- Cytologic sampling is a simple, accurate, and cost-effective method for detecting residual CA in women treated for cervical CA
- High false negative rate (50 to >75%) due to sampling and interpretation issues
- Cytologic changes can be striking:
 - Cytoplasmic vacuolization (earliest effect)
 - Polychromasia
 - Cytomegaly (macrocytes)
 - Bizarre cells
 - Nuclear changes – multinucleation (common), membrane irregularity; chromatin clumping and hyperchromasia possible but usually smudgy
 - Background necrosis, inflammatory exudate



Repair

- Result from injury to cervical epithelium with proliferation of reserve cells
- Typical repair:
 - Cohesive, flat sheets (no isolated atypical cells)
 - Streaming (school of fish) or pulled out (taffy) appearance
 - Large nucleus, size variation
 - Large nucleolus
 - Pale, finely granular chromatin
 - Mitoses
 - Inflammation; no necrosis
- Atypical repair:
 - Cellular crowding and overlap, marked anisonucleosis, prominent and irregular nucleoli, irregular chromatin distribution



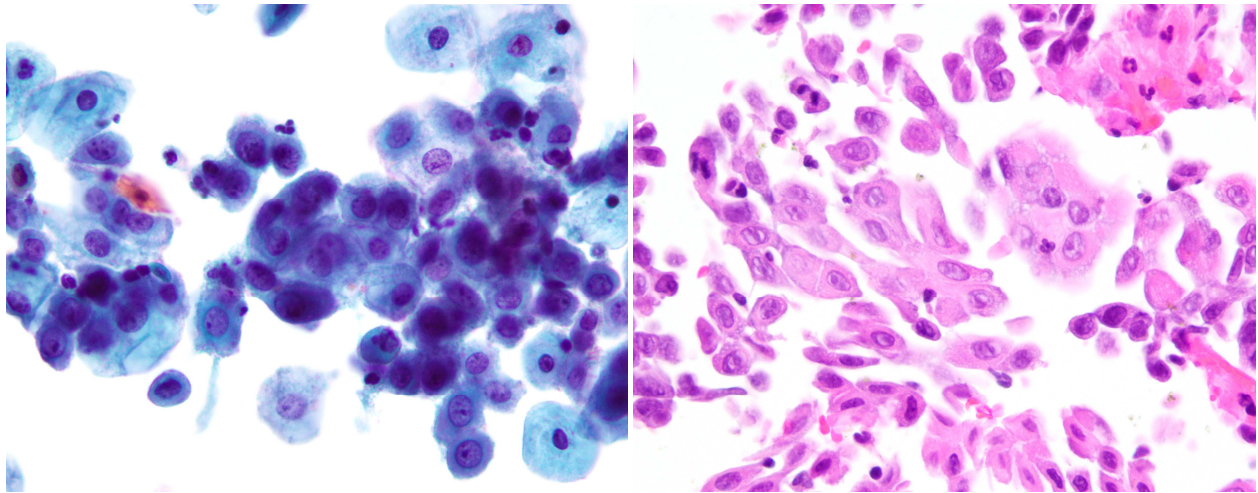


Repair



Squamous cell carcinoma





Pemphigus Vulgaris



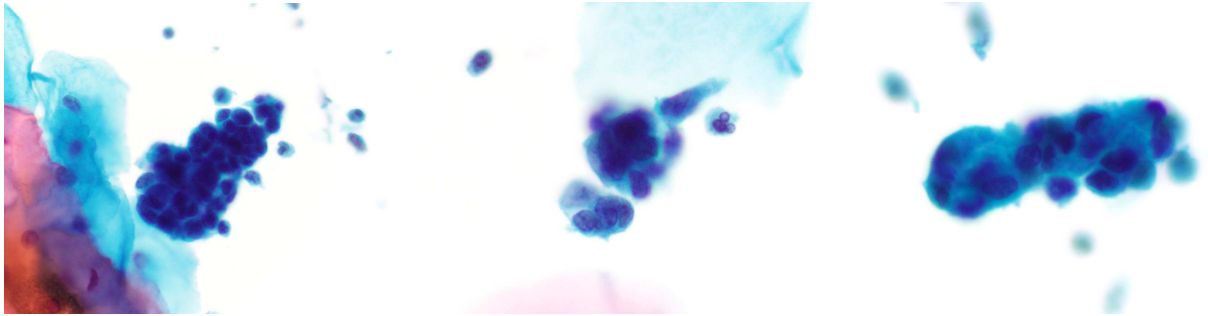
A Word on Atypical Squamous Cells

- ASC does not represent a single biologic entity
- ASC is essential: bridges the gap between clearly normal and clearly abnormal
 - ASCUS is the most common nonnegative interpretation (4.3%)
- Should be kept to a minimum
 - Less than 5% of all Pap cases or ASC/SIL <3:1 (opportunity for feedback)
- Factors: “Quantity and quality,” preservation, clinical setting
- Patterns: Mature/intermediate-type cytoplasm or incomplete koilocytosis (ASCUS), atypical squamous metaplasia (ASCH), atypical cells in atrophy, atypical parakeratosis, atypical repair



A Word on Cell Blocks

Cell blocks can be helpful in some cases

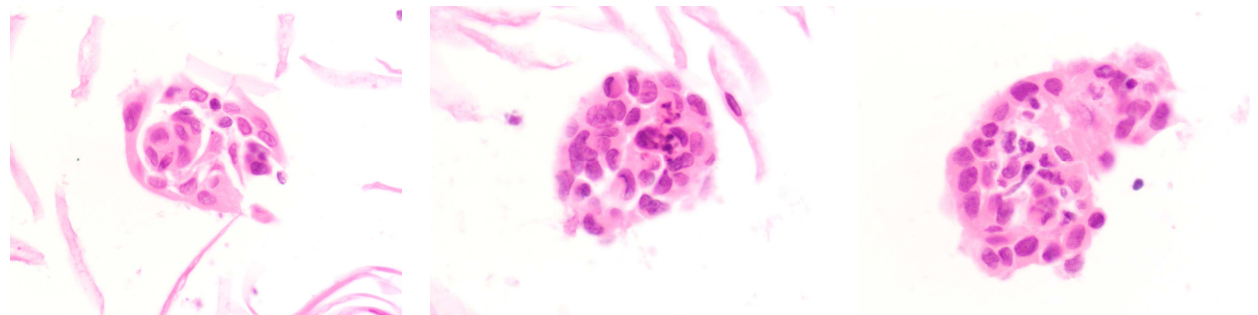


Cervical Pap from a 27-year-old woman with recent history of LEEP for CIN3 which was involving glands



A Word on Cell Blocks

Cell blocks can be helpful in some cases

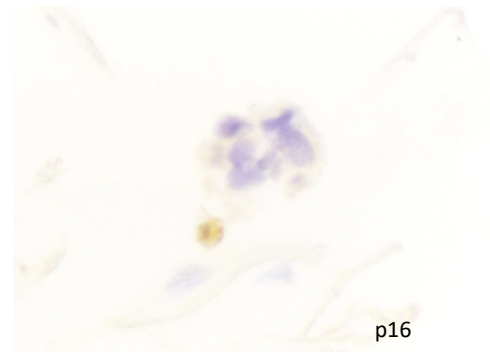
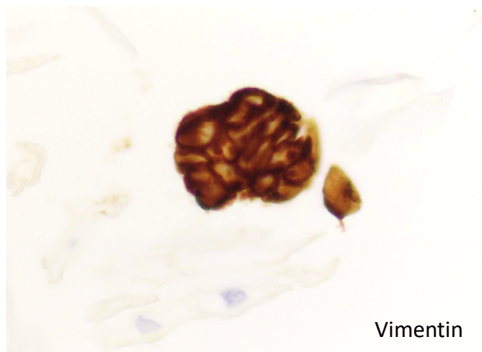


Cervical Pap from a 27-year-old woman with recent history of LEEP for CIN3 which was involving glands



A Word on Cell Blocks

Cell blocks can be helpful in some cases



Cervical Pap from a 27-year-old woman with recent history of LEEP for CIN3 which was involving glands

➤ **Menstrual sample with benign endometrial cells**



Summary

- The Pap test is considered the most successful cancer reduction program ever devised
- Identification of high grade SILs is of particular importance given the implications for management
- Although many cases can be definitively and accurately diagnosed as HSIL, knowledge of overlapping benign entities is needed to help avoid misidentification
- Cell blocks can be helpful in some cases
- An atypical category can be used for challenging cases which is integral to preserve the sensitivity of this screening test and to ensure that the other categories remain pure

