Mass General Brigham

MASSACHUSETTS GENERAL HOSPITAL

# HSIL and its Mimics

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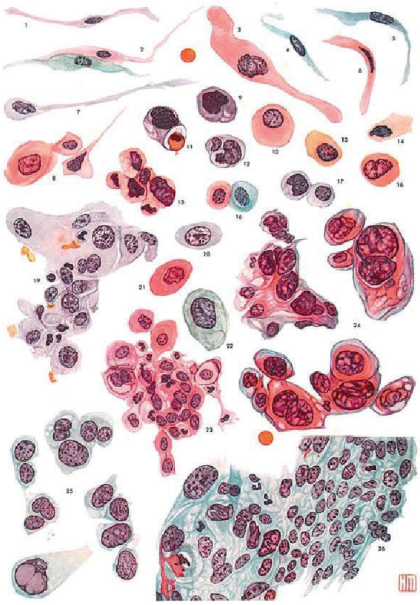
## History of the Pap Test


- 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



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Christopherson WM, Scott MA, Acta Cytol 1977
MacGregor JE, Teper S, Lancet 1978
Clarke EA, Anderson TW, Lancet, 1979
Laara E, Day NE, Hakam M, Lancet 1987
Anderson GH, et al Br Med J 1988

# The Pap smear is far from perfect…

- Sensitivity and specificity of preinvasive and invasive lesions vary
  - Preinvasive lesions: Mean sensitivity 47% (range 30-80%), mean specificity 95% (range 86-100%)
  - Invasive lesions: Sensitivity widely ranges from 16 to 82%
- Positive predictive value of an LSIL pap for CIN is 50-86% and an HSIL pap for CIN2+ is 60-80%
- False positive diagnoses of cervical cancer in 10-15% of cases
- Interobserver reproducibility of cytologic interpretations is moderate at best (K = 0.46) Stohler MH, Schiffman M, JAMA 2001

Nanda K et al, Ann Intern Med 2000
Levine PH et al, Diagn Cytopathol 2003
Mount S et al, Acta Cytol 2004

# Correlation with histology

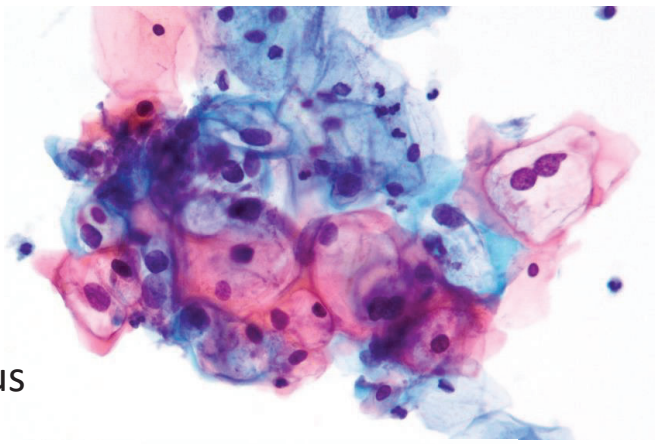
- Frequency of discrepancy ranges from 11 to 32%
  - Variables include interval between time of Pap test and biopsy, timing of review, individual performing the review, definition of what the discrepancy is
- Contribution from both sampling and laboratory error
  - Sampling error
    - Lesion sampling
      - ASCUS/LSIL Triage Study (ALTS) – 33-36% of CIN2 or worse lesions not identified by colposcopy
      - Patient age and menopausal status, visibility of squamocolumnar junction, lesion size and thickness, lesion distribution, endocervical extension, number of biopsies, training and experience of colposcopist, type of clinician
    - Cytology or histology prep issues including embedding / sectioning and technical difficulties
  - Cytologic or histologic interpretation errors – Less common
    - Cytologic overlap between benign and neoplastic processes
    - Cervical biopsy interpretation reproducibility also far from perfect

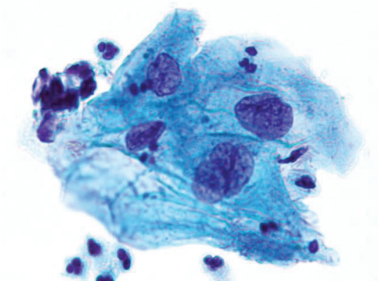
Abdul-Karim FW, Yang B. Adv Anat Pathol. 2017

***Key*** **to appropriate cytologic interpretation is to recognize *benign and reactive changes* that may mimic high grade lesions and to recognize *limitations in diagnosis* given the potential for morphologic overlap**

## Low Grade Squamous Intraepithelial Lesions

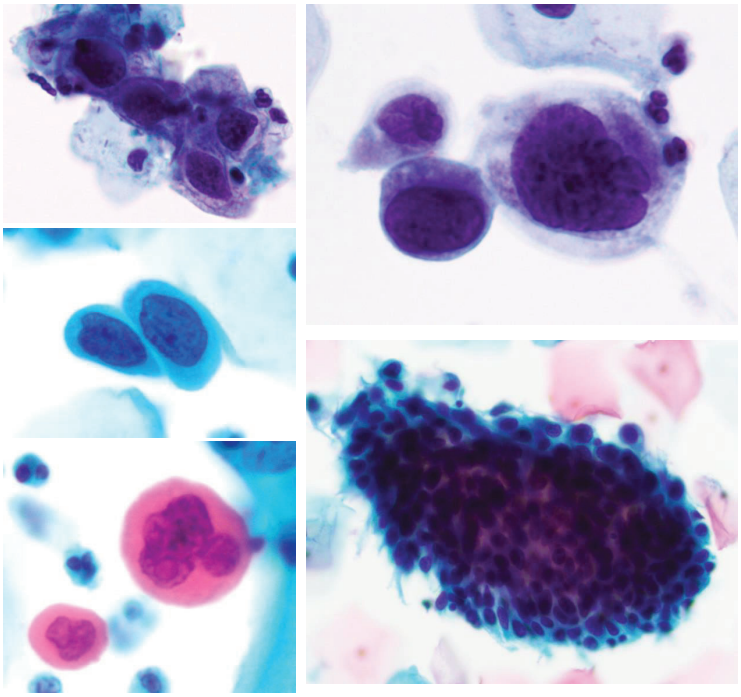
- Cytologic changes in 'mature' type cells
- Nuclear atypia:
  - Enlargement >3x that of intermediate cell
  - Irregular nuclear contours
  - Hyperchromatic
  - Slight chromatin coarseness
- Nucleoli generally absent or inconspicuous if present
- Bi- and multinucleation common
- Perinuclear cytoplasmic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

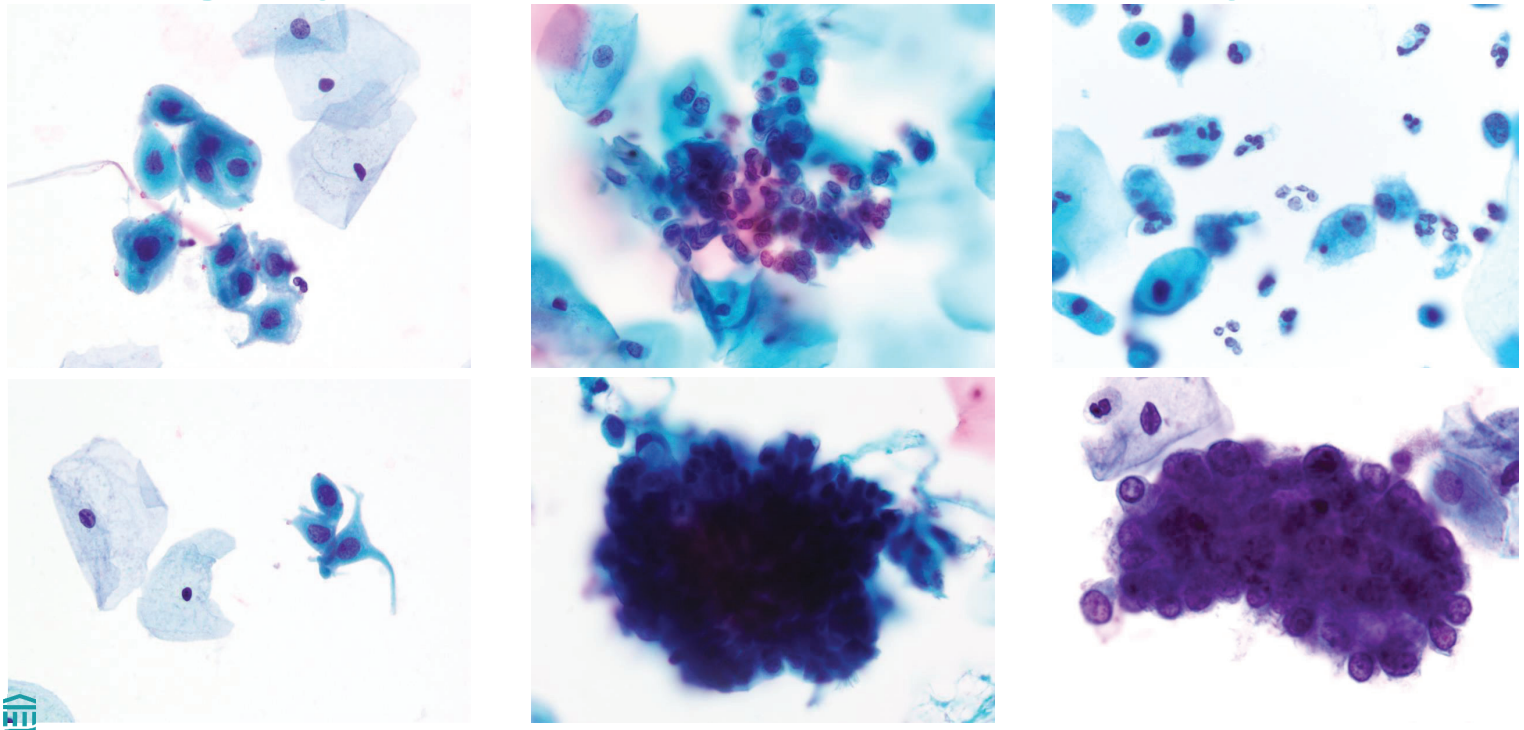


# High Grade Squamous Intraepithelial Lesions

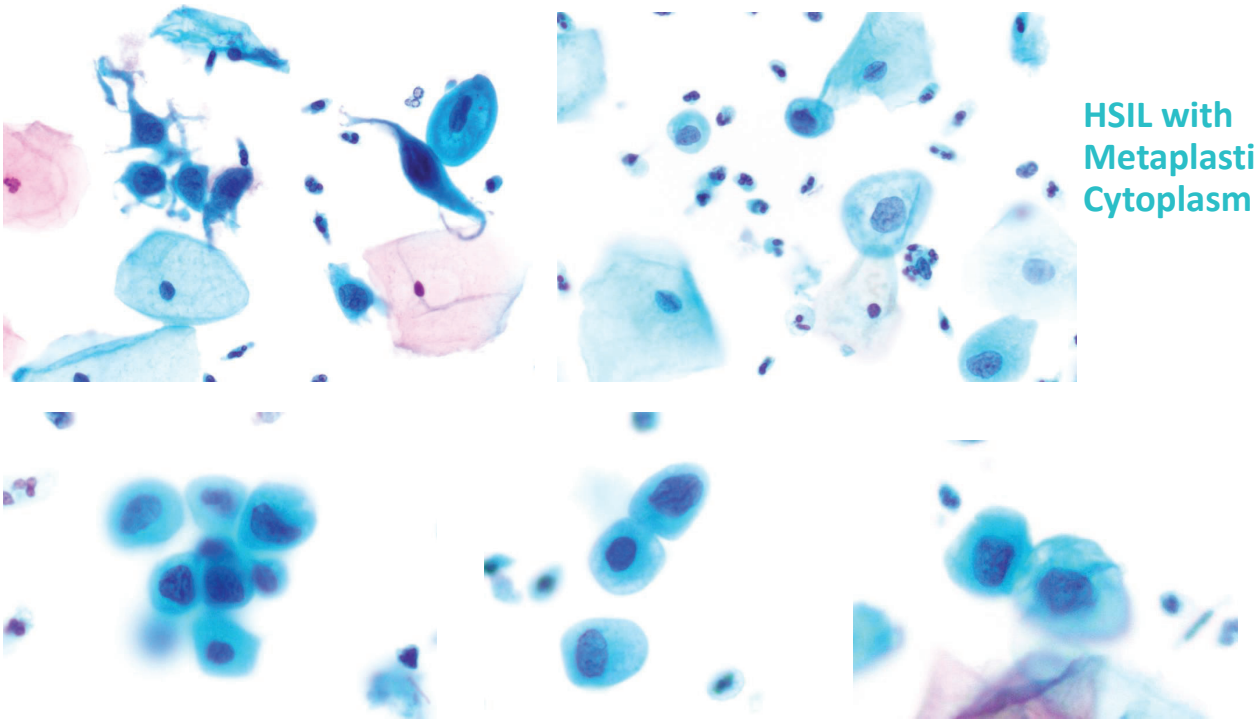
- Benign mimics
  - Benign epithelial cells (squamous / tubal metaplastic cells, endometrial cells)
  - Inflammatory cells (histiocytes, lymphocytes)
  - Immature squamous metaplasia
  - Herpes
  - IUD effect
  - Transitional cell metaplasia
  - Endocervical polyp
- Neoplastic mimics
  - LSIL, SCC, AIS

- Problematic patterns:
  - Syncytial aggregates
  - SIL with endocervical gland involvement
  - HSIL resembling endometrial cells
  - Single and rare small HSIL cells
  - Abnormal stripped nuclei
  - HSIL in atrophy
  - Keratinizing lesions
  - LSIL with some features suggestive of concurrent HSIL

# Benign Epithelial Cells and Inflammatory Cells

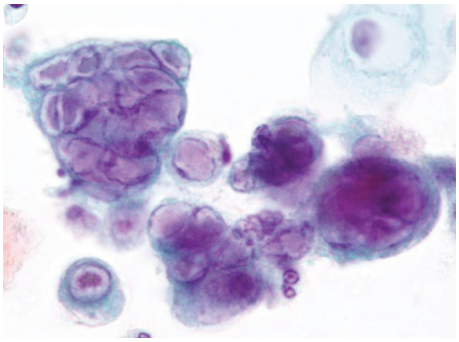
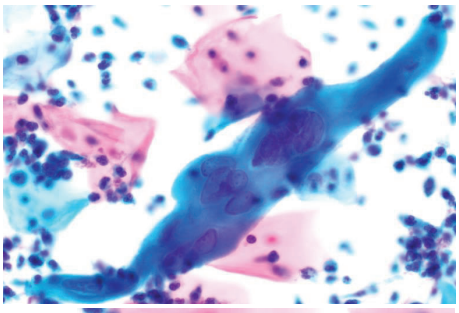
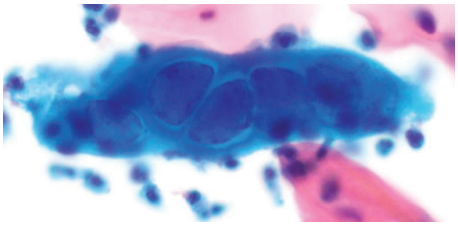
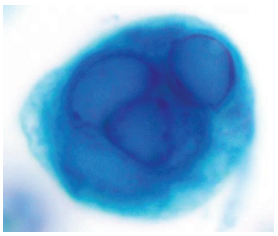
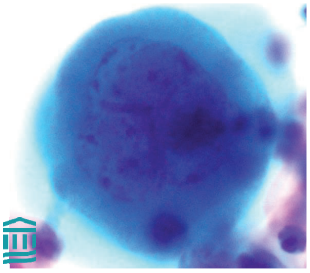
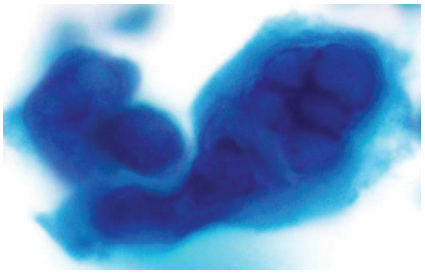
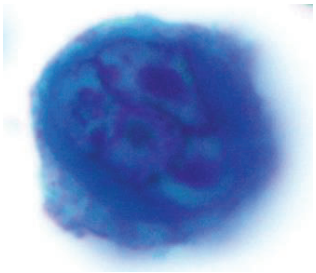


## HSIL with Metaplastic-Type Cytoplasm

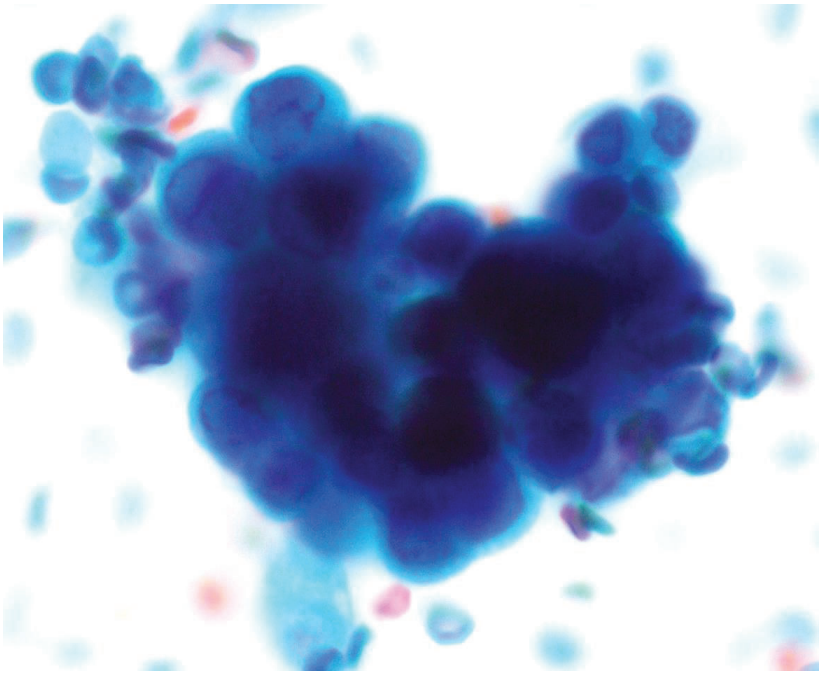
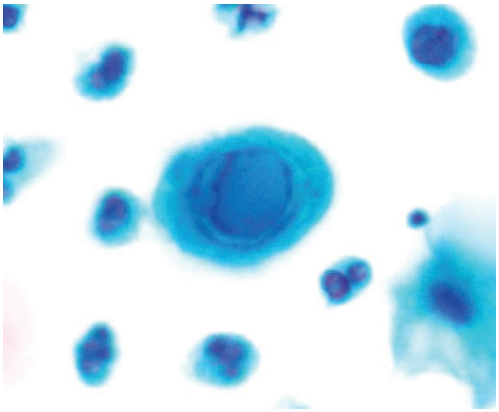
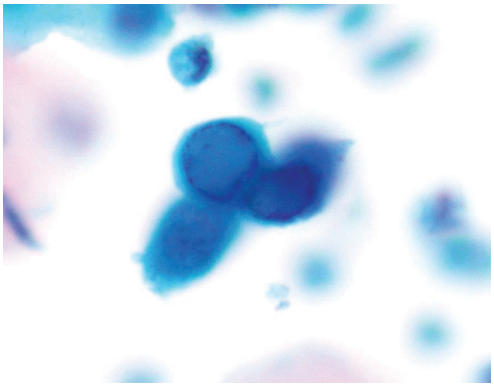


# Herpes Simplex Virus

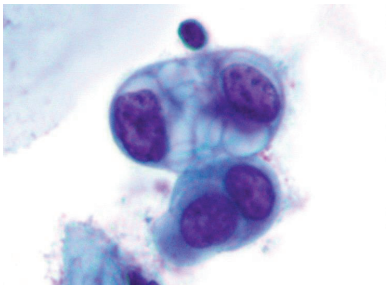
- Cytopathic effect
  - 3 M's: multinucleation, molding, margination
  - Ground-glass nuclei
  - Dense eosinophilic intranuclear (Cowdry) inclusions
- Relatively reproducible
- DDX LSIL and HSIL
  - Homogenous (ground-glass) appearance versus hyperchromatic nuclei of SILs

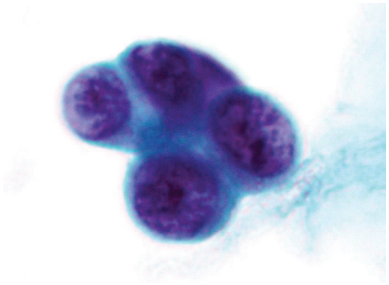
# Herpes Viral Cytopathic Effect mimicking HSIL

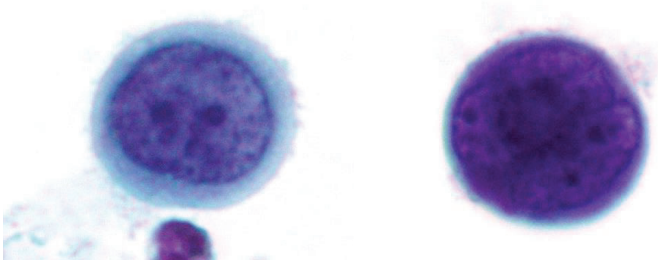




# Intrauterine Device Effect



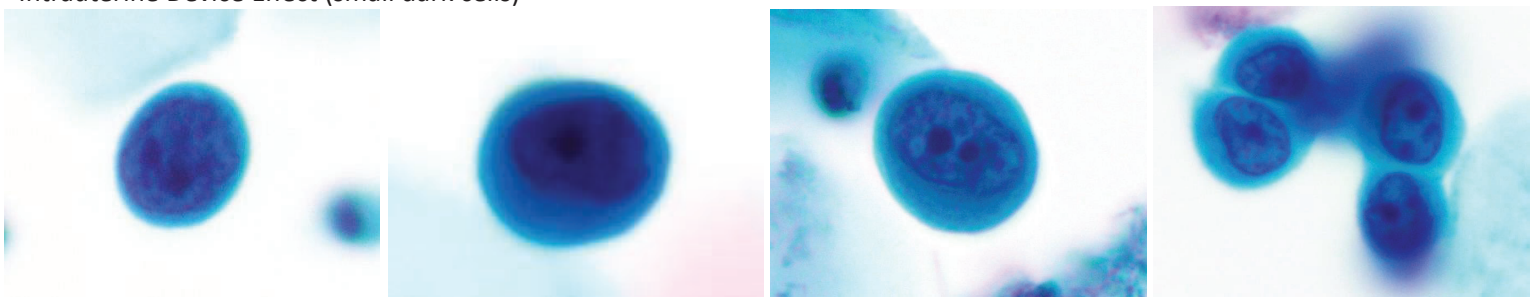
- Endometrial and/or endocervical cells with distinct cytomorphologic changes reflecting chronic irritation
- Two characteristic cell types:
  - Cells with vacuolated cytoplasm – ddx adenocarcinoma
  - Small, dark cells with scant cytoplasm, high N:C – ddx HSIL
- Found singly or in small clusters
- May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



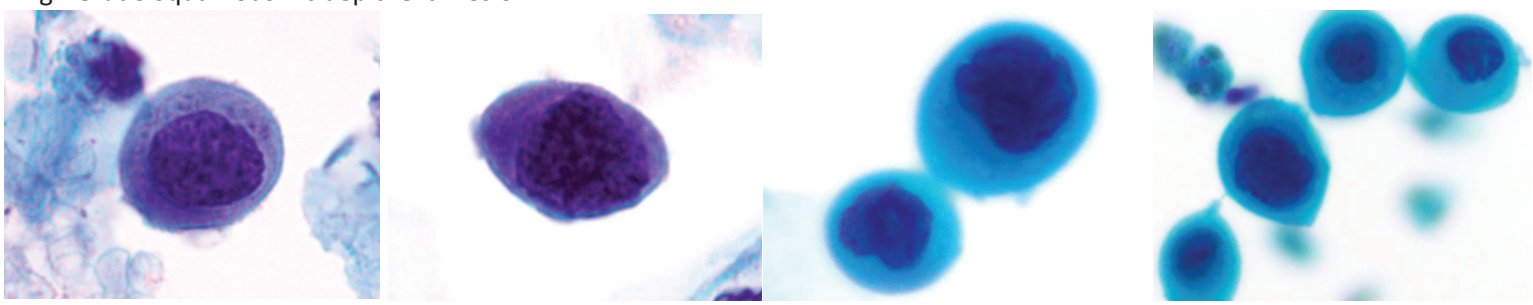


Torous VF and Pitman MP, JASC 2021

Intrauterine Device Effect (small dark cells)



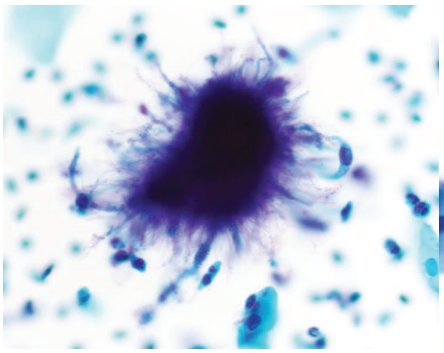
High Grade Squamous Intraepithelial Lesion

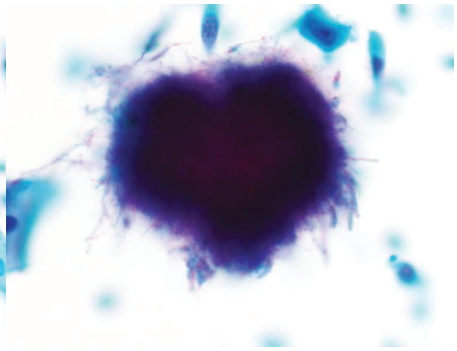


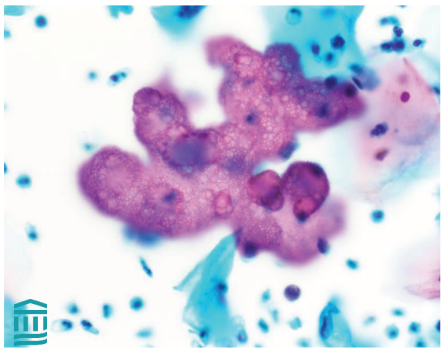
# Background Findings Associated with IUD

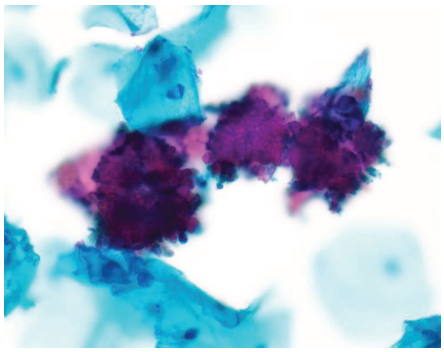
Actinomyces

- 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)







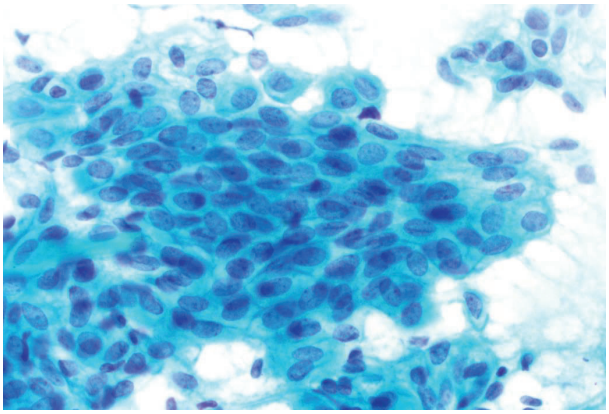


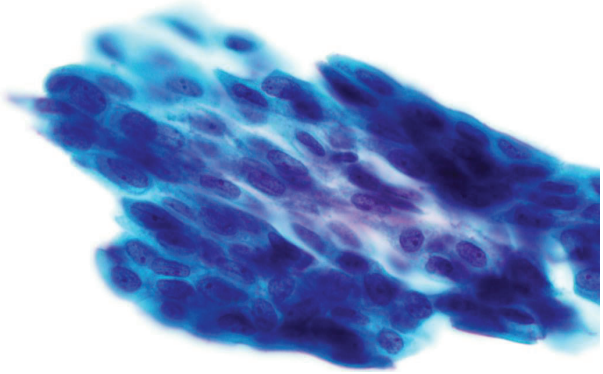
Pseudoactinomycotic radiate granules

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

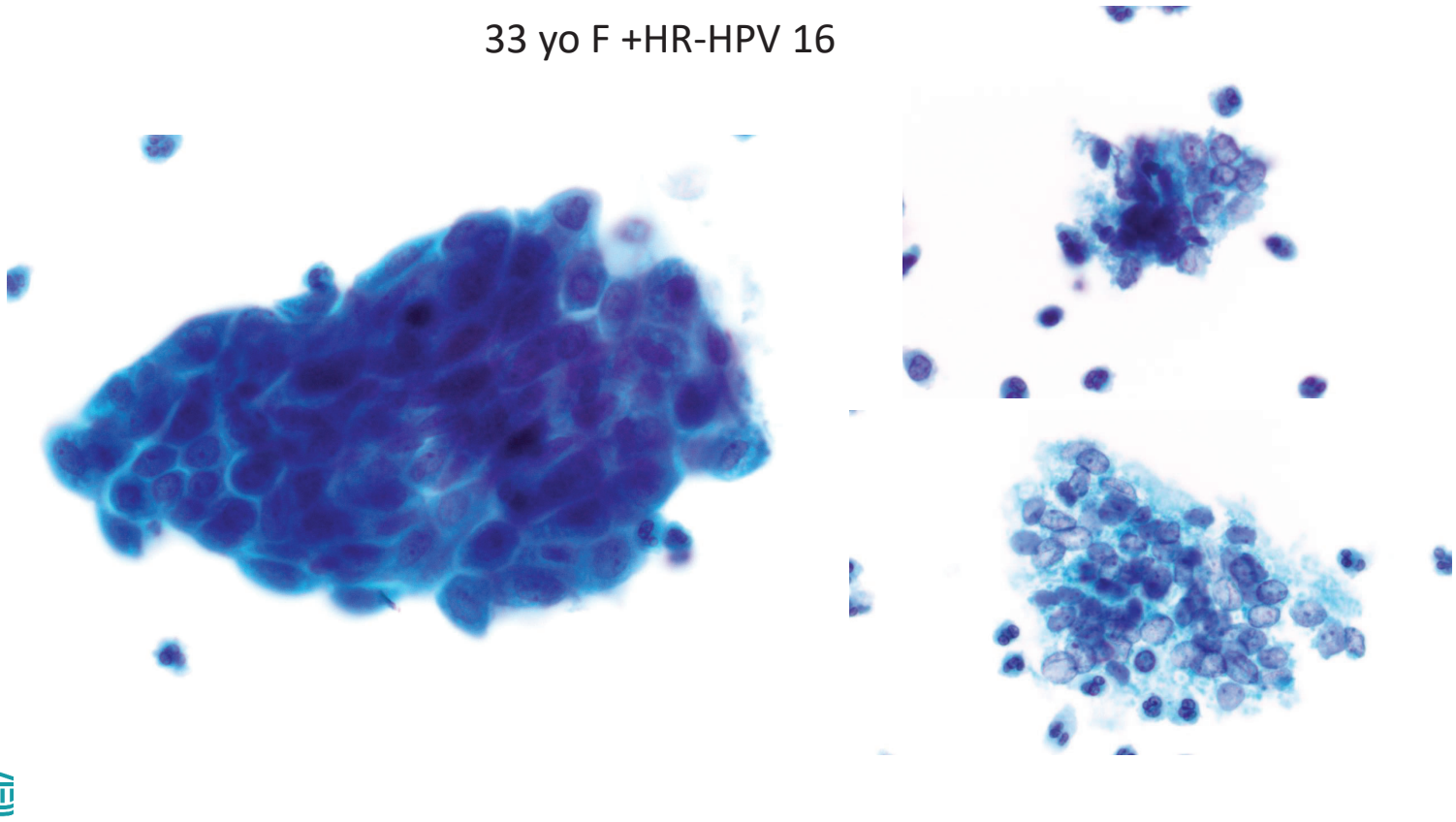
# Transitional Cell Metaplasia

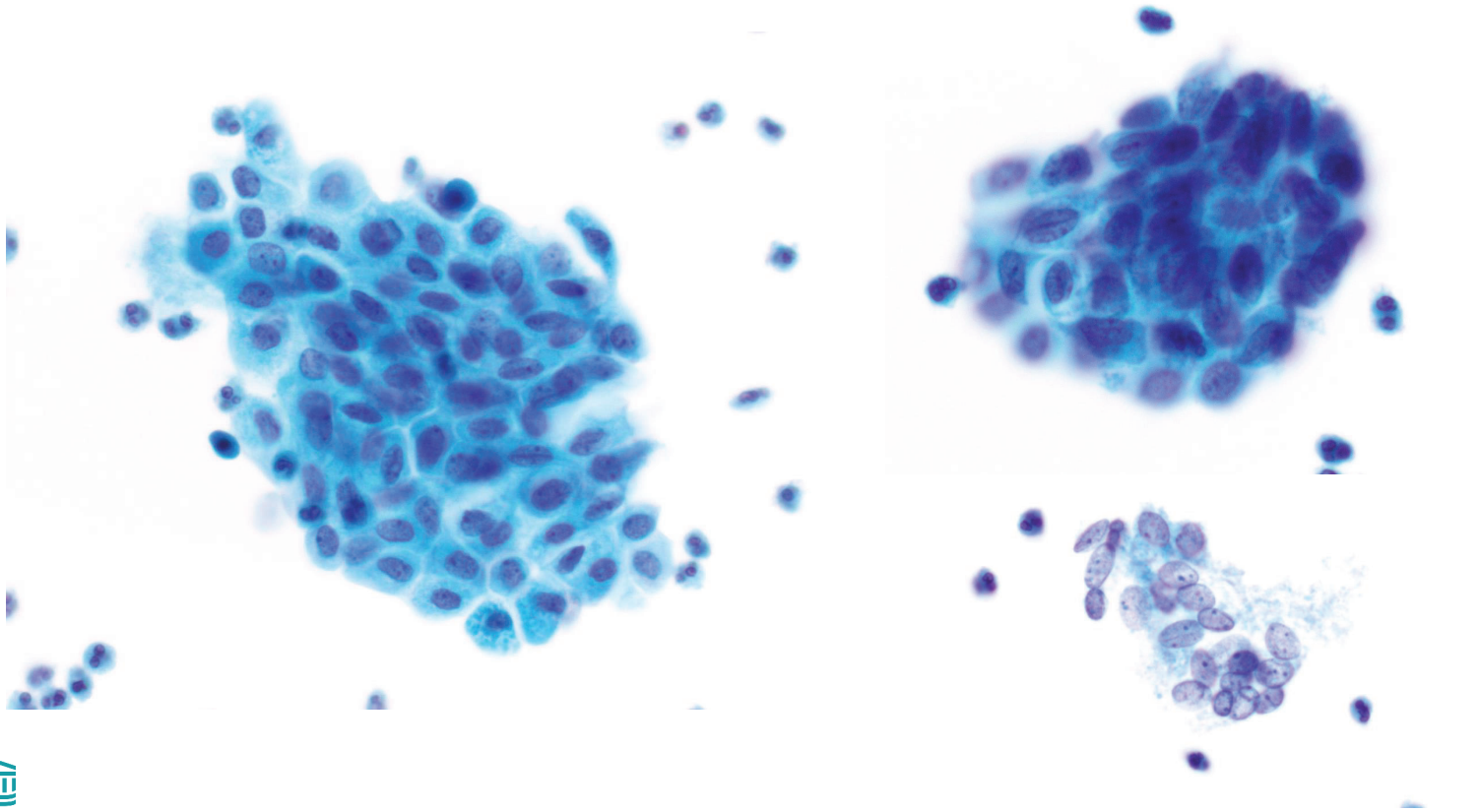
- Atrophic epithelium resembling transitional cell epithelium
- Flat sheets of cells
- Wrinkled, oval to spindle-shaped nuclei, powdery chromatin, small nucleoli, small perinuclear halos
- Prominent longitudinal nuclear grooves (“coffee bean nuclei”)





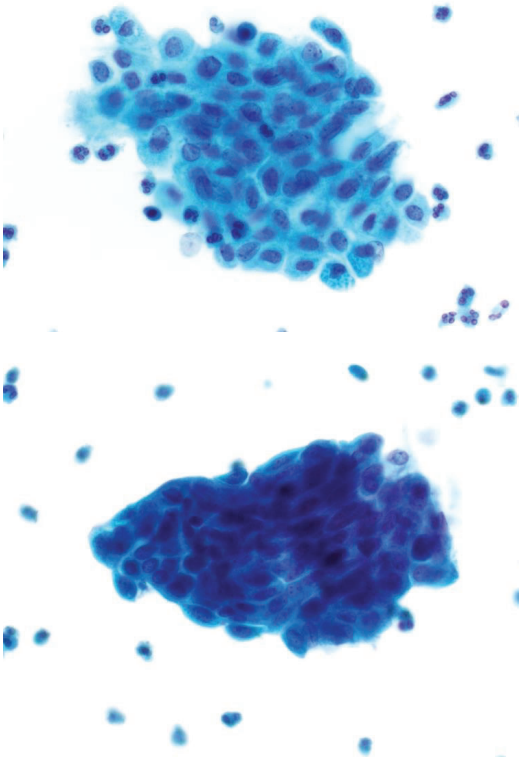
33 yo F +HR-HPV 16





## Female-to-male transgender patient on testosterone hormone therapy

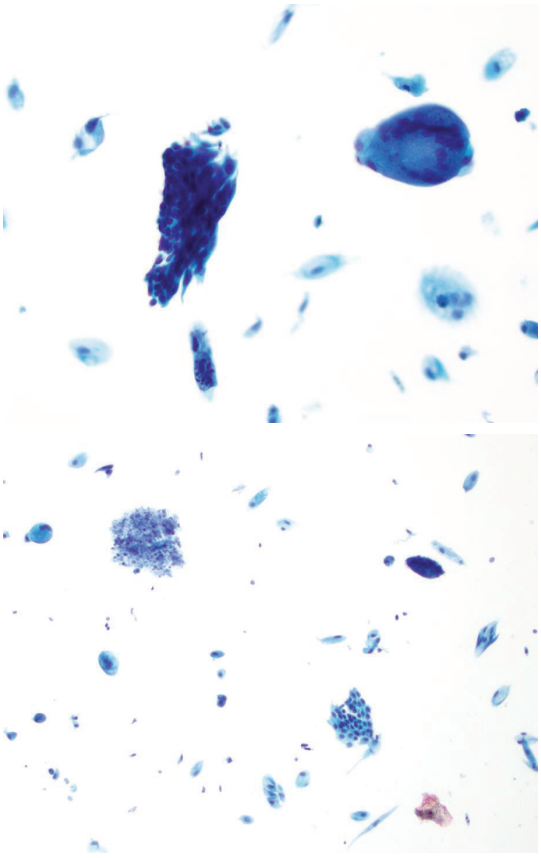
- TCM traditionally, described in peri or postmenopausal women, now, known to occur frequently in those on androgen / testosterone therapy
  - Found in up to 88% of Pap tests from transgender men; MGH 43%
  - Atrophy NOS found in 62% to 93% of transgender Pap tests; 92% MGH)
  - Abnormality rates 5.7% to 29.2%; HR-HPV+ rates 8% to 33%
    - Abnormality rates correspond to the HR-HPV+ Rate



Torous VF, Cancer Cytopathol, *2023*

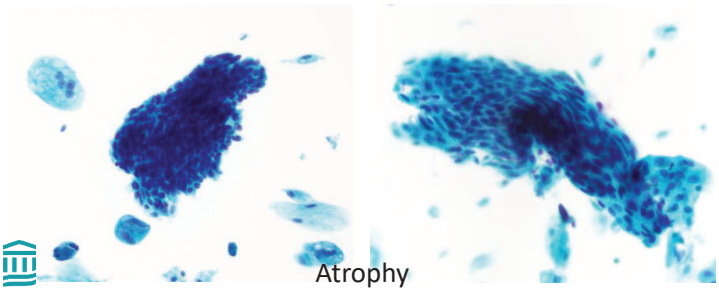
## Atrophy

- 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

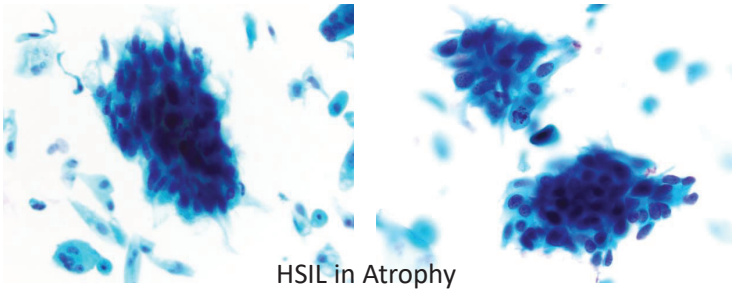


# HSIL in Atrophy

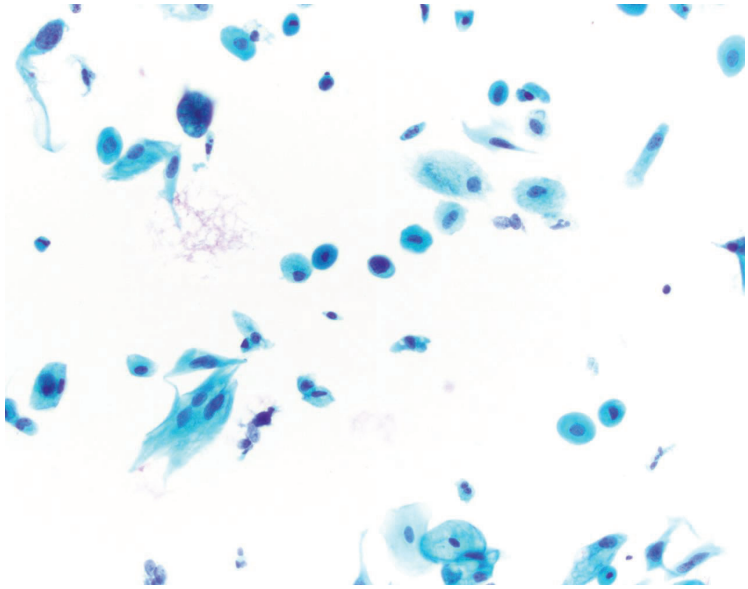
- Challenging due to immaturity of background squamous cells and similarity between immature squamous cells and high grade dysplastic cells
- Parabasal cells may have slight nuclear enlargement and higher N:C but lack nuclear membrane irregularity
- Nuclei may appear hyperchromatic but chromatin tends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

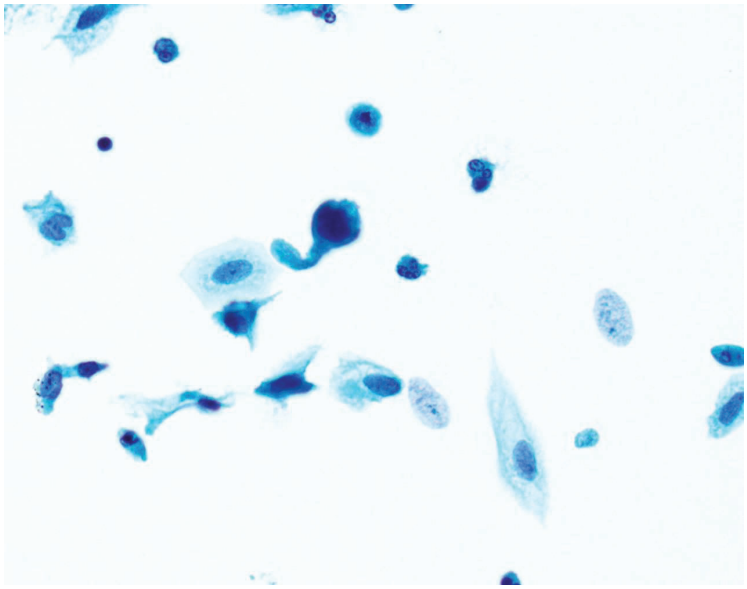


Atrophy

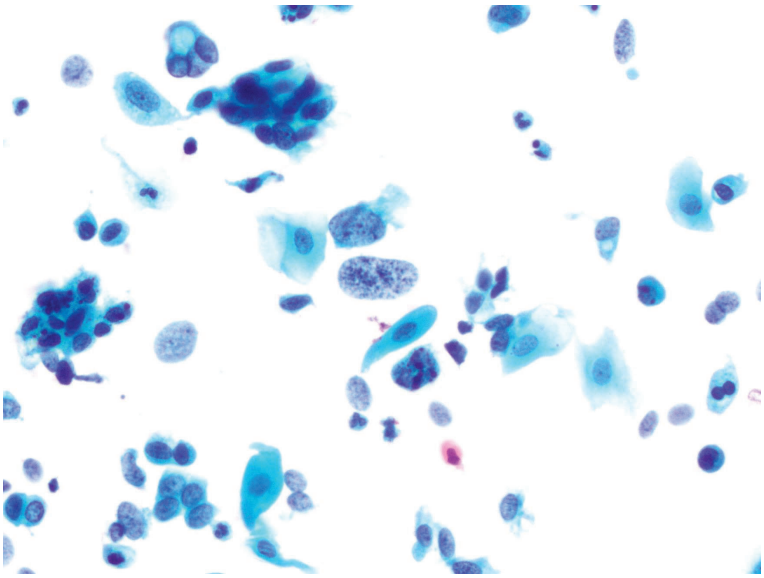


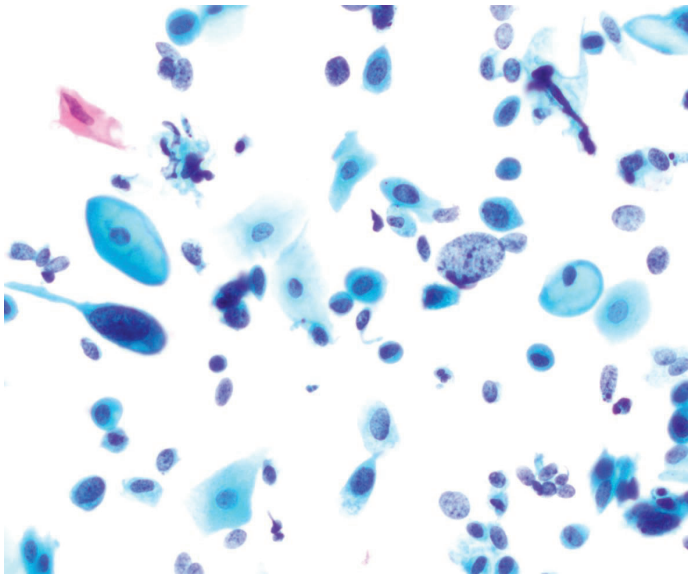
HSIL in Atrophy



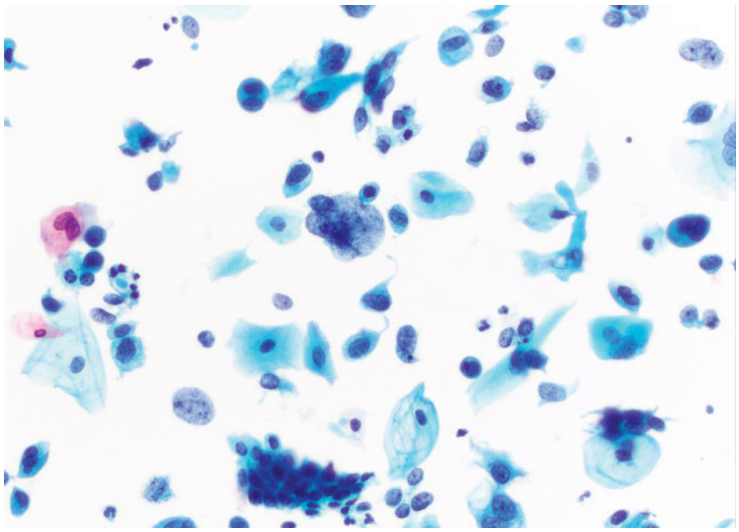


HSIL in Atrophy Single Cell Pattern





HSIL atypical stripped cells



Atypical stripped cells in HSIL

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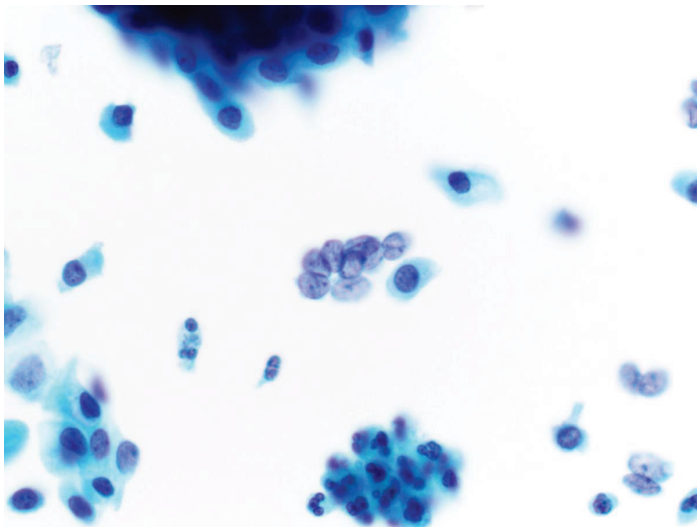
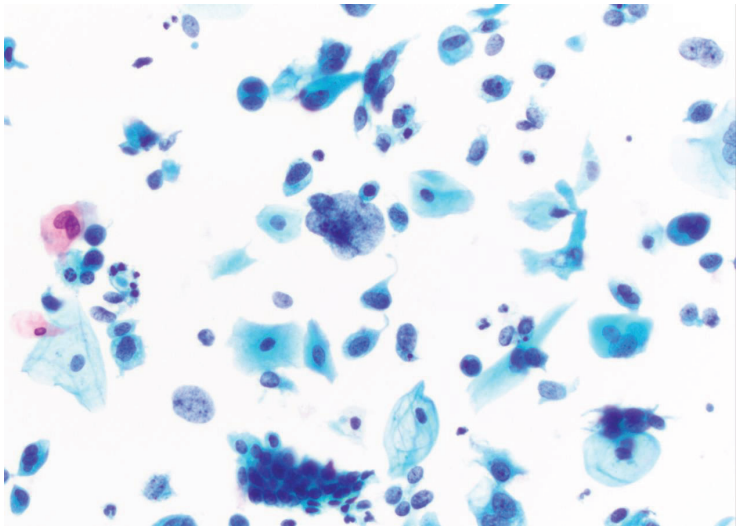


Benign “small blue cells” in atrophy



Atypical stripped cells in HSIL

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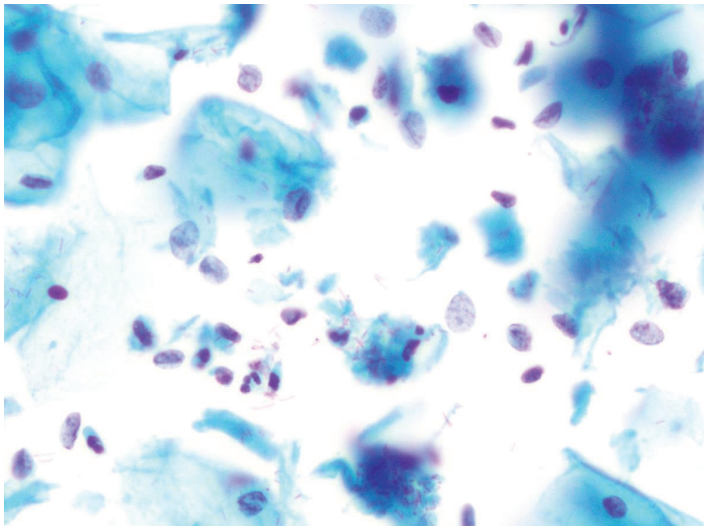
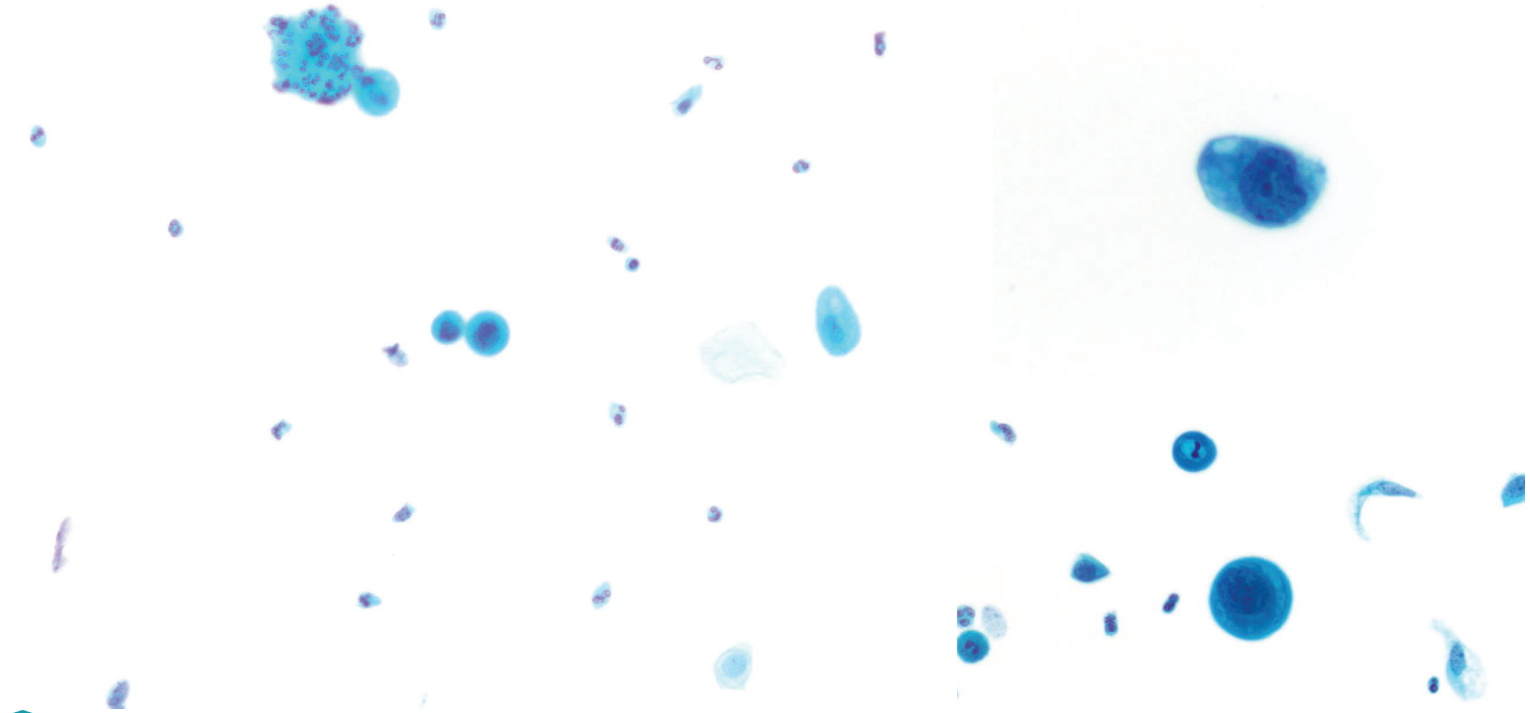
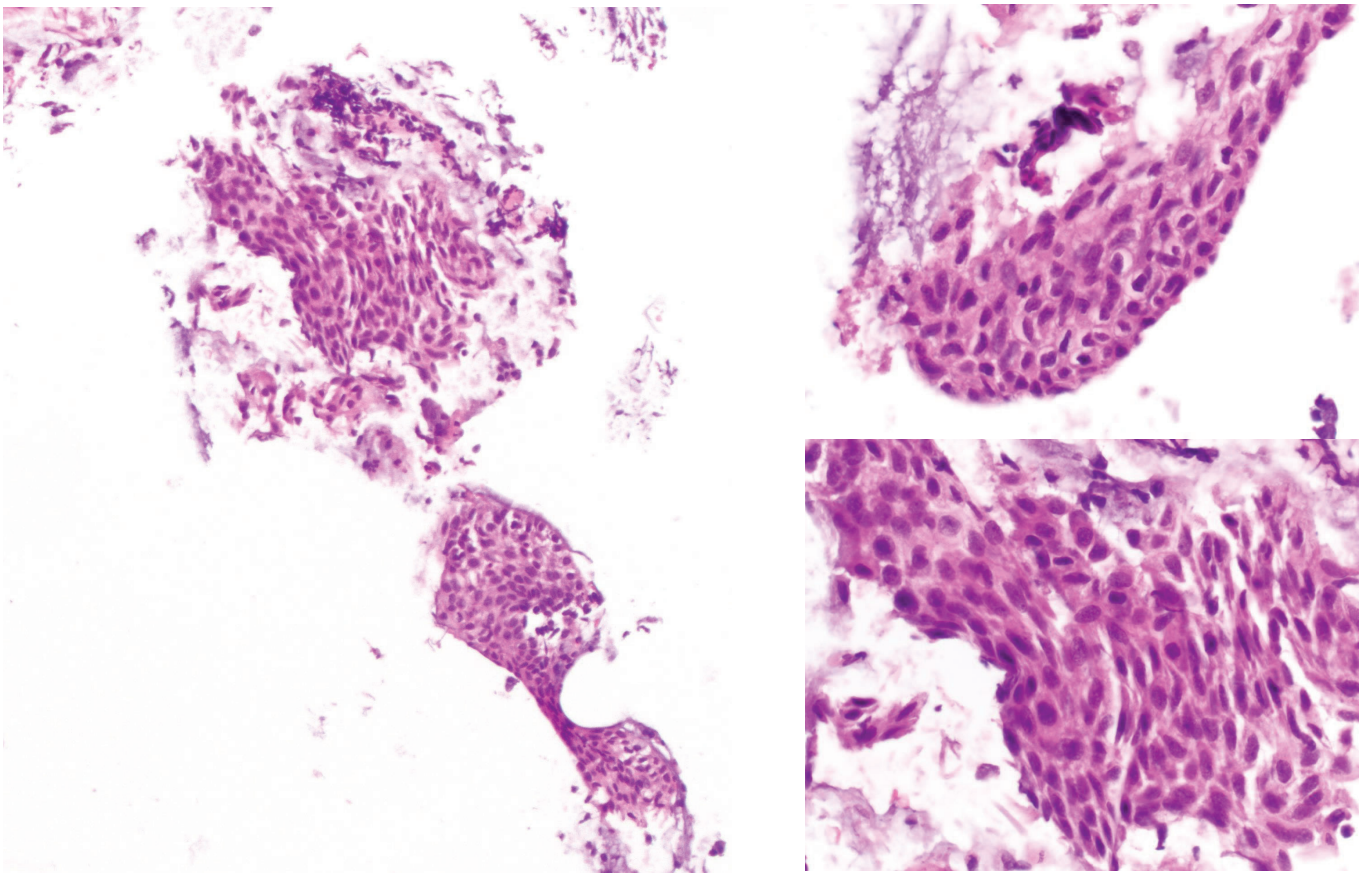
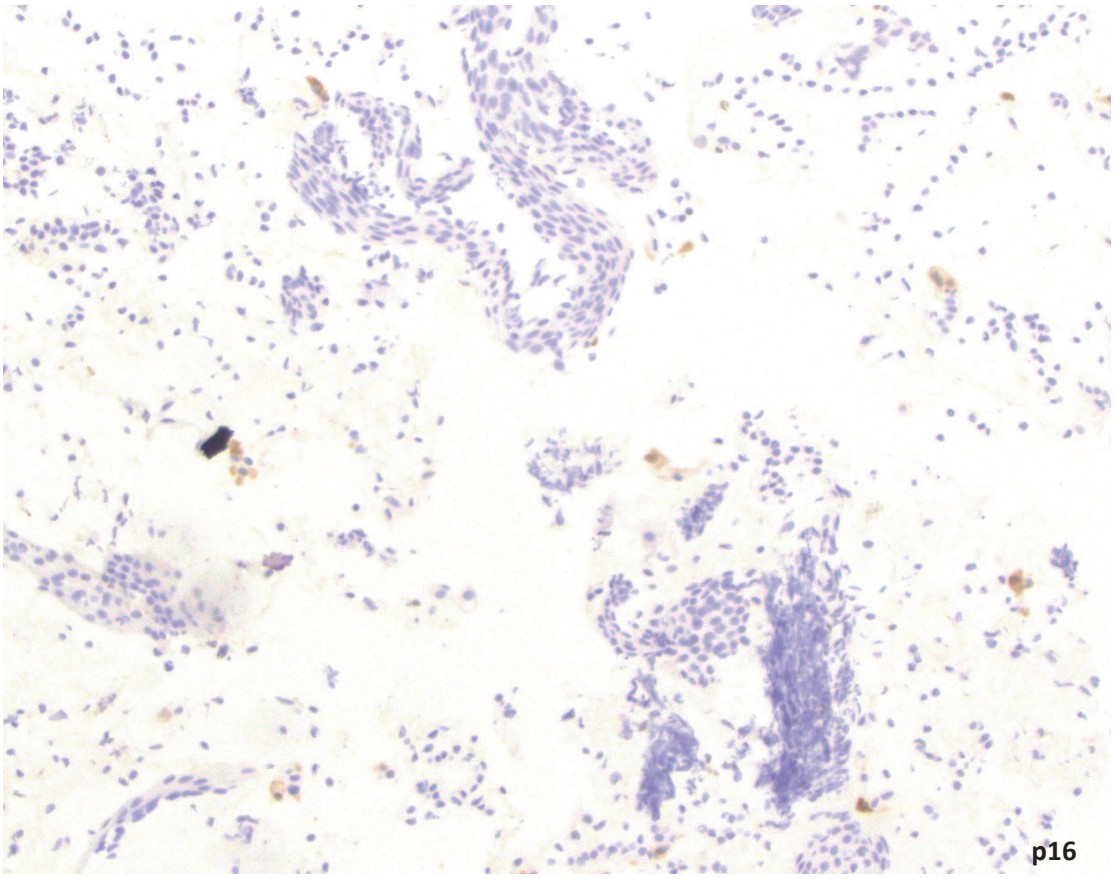


Benign stripped nuclei in cytolysis

58 yo F +HR-HPV “other”



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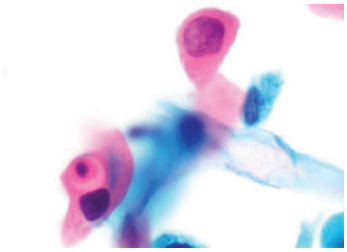
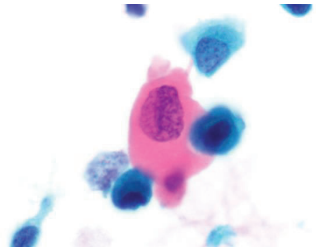
# Atypia of Atrophy

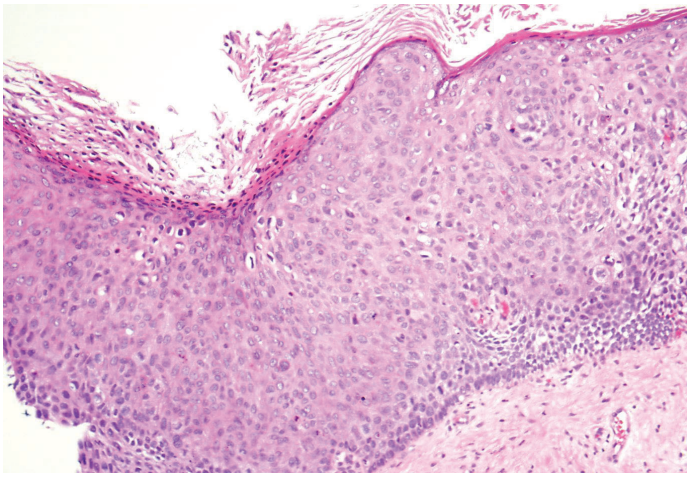
- Some atrophy cases may show more marked nuclear enlargement and hyperchromasia
  - These cases are usually best placed within an ASC-US category, although some may be challenging to distinguish from a SIL or invasive carcinoma
- Historically, a course of intravaginal estrogen cream was recommended followed by repeat Pap test, but this has now been excluded from current management guidelines

# HSIL - Keratinizing Lesions

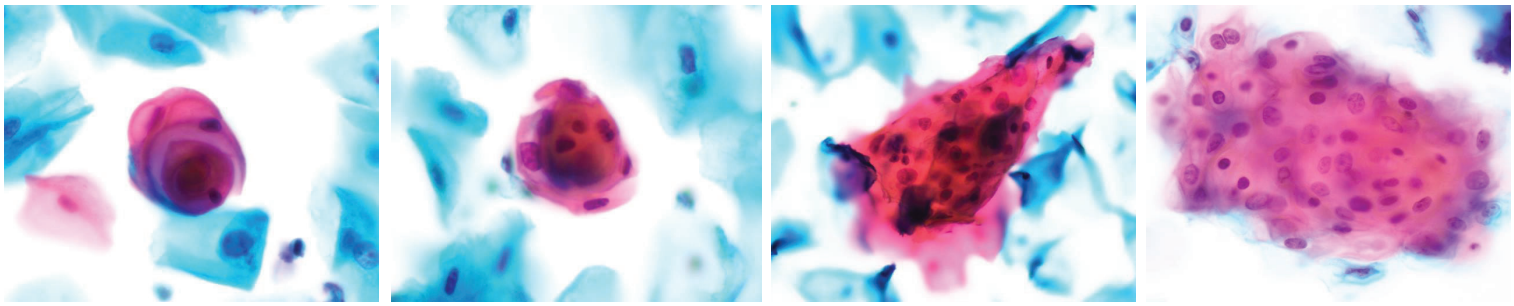
- Some HSILs have more abundant and abnormally keratinized cytoplasm
- Found singly or in clusters
- Enlarged, hyperchromatic nuclei
- Can be pleomorphic with variation in nuclear size and shape
  - DDX Invasive squamous cell carcinoma
    - Nucleoli and tumor diathesis absent
    - Challenging cases: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







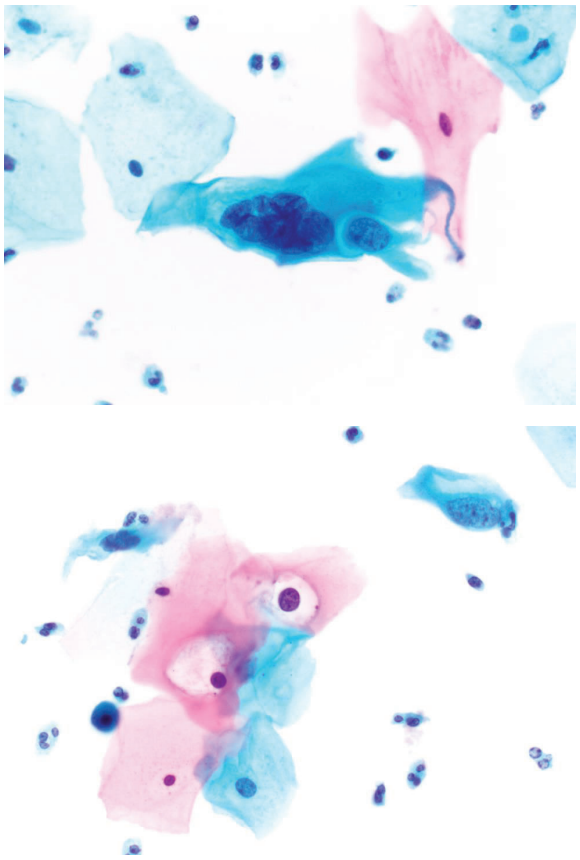
# Keratinized Squamous Cells



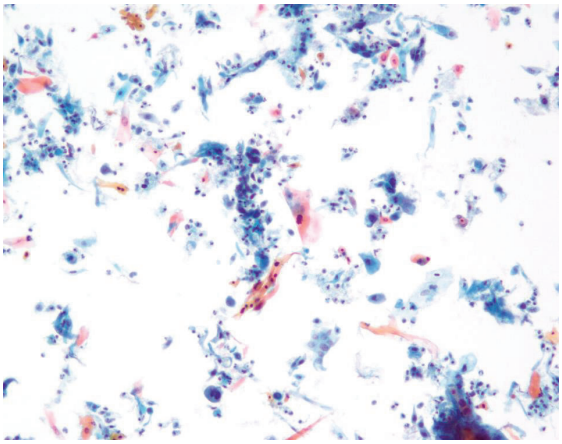
Atypia

# Low Grade vs High Grade SIL

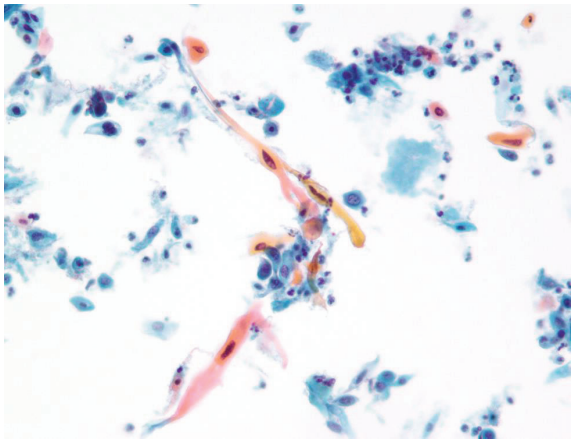
- Cells of HSIL usually smaller and show less cytoplasmic maturity than cells of LSIL
- Degree of nuclear enlargement variable in HSIL but generally a marked increase in nuclear to cytoplasmic ratio
- Hyperchromasia, coarse chromatin, nuclear contour irregularity usually more severe in HSIL
- Some cases 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

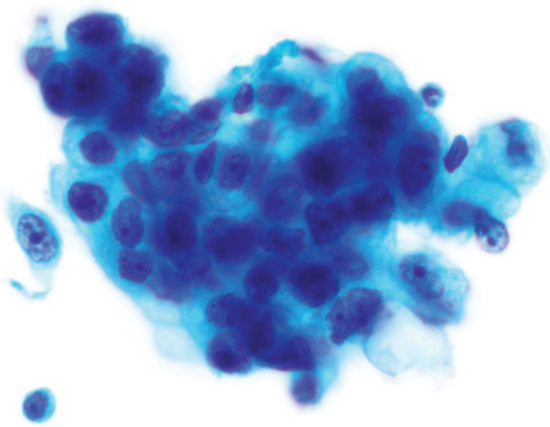


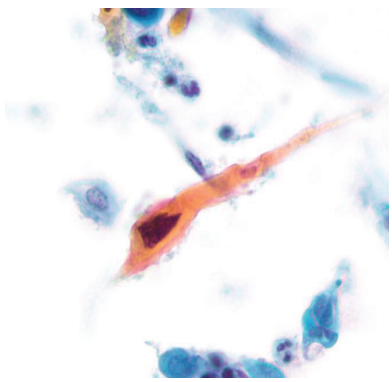
# Squamous Cell Carcinoma

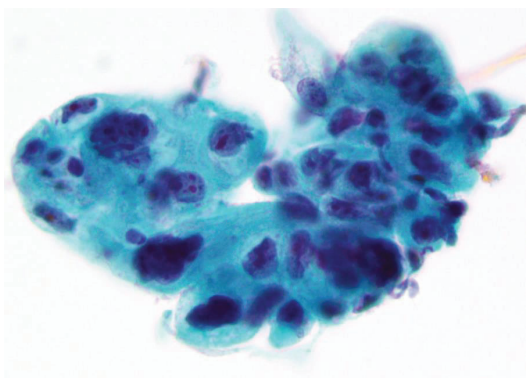


- Cytomorphologic features of HSIL plus:
  - Macronucleolus
  - Irregular chromatin distribution
  - Tumor diathesis
- Keratinized cells with irregular/bizarre shape (tadpoles, fiber cells) in keratinizing type
- Often with population of HSIL cells







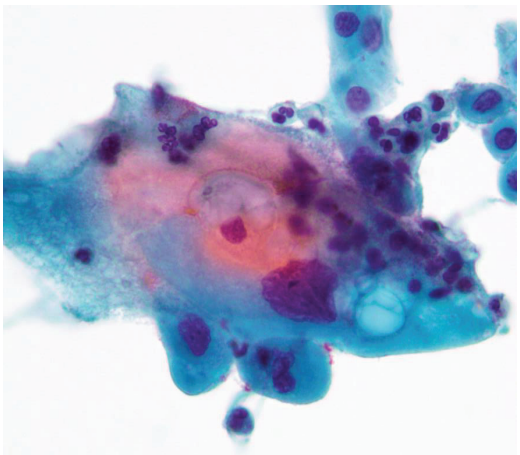


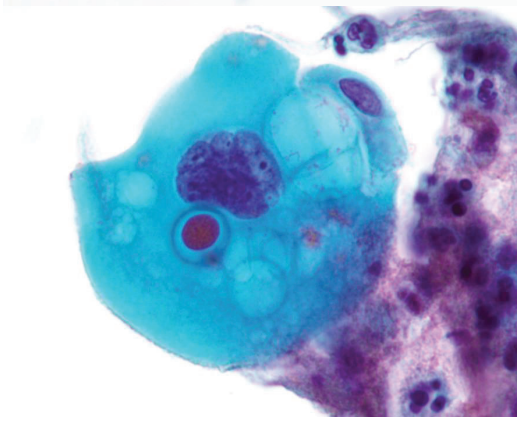
# Squamous Cell Carcinoma

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

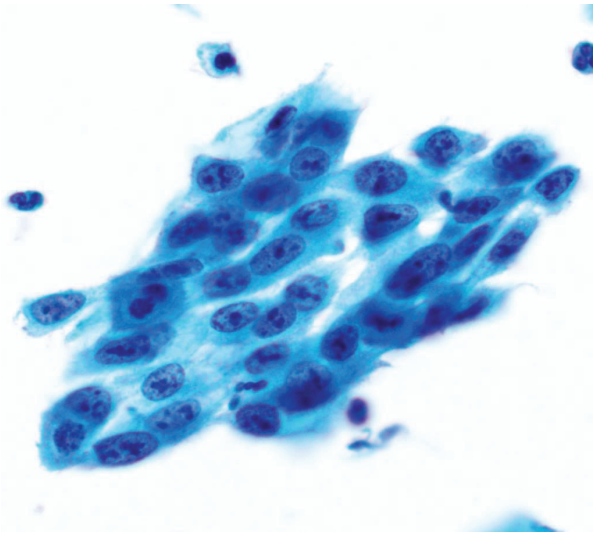
# Radiation Change

- 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

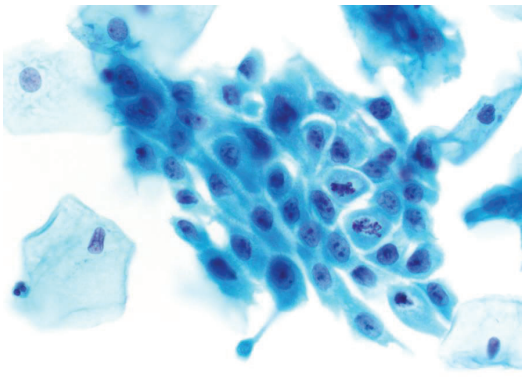


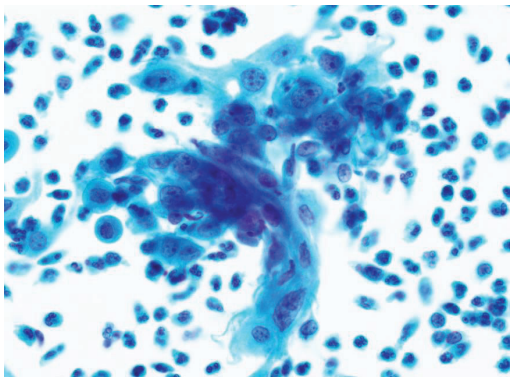


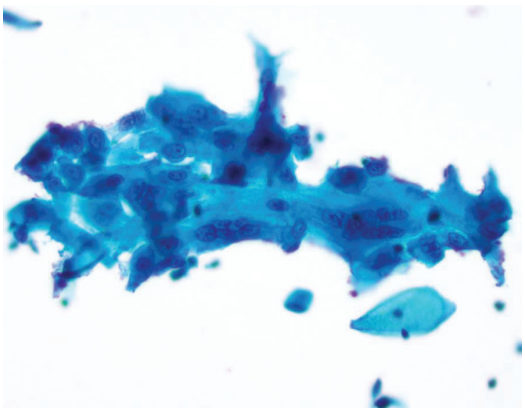
# Repair

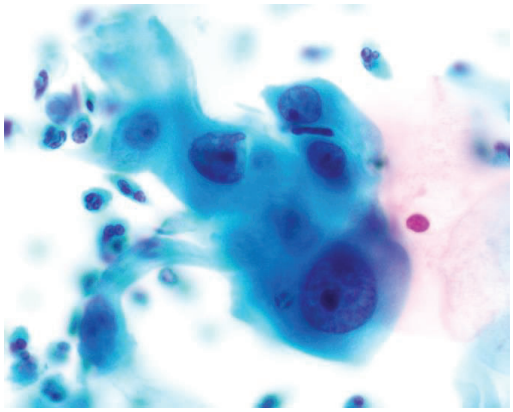


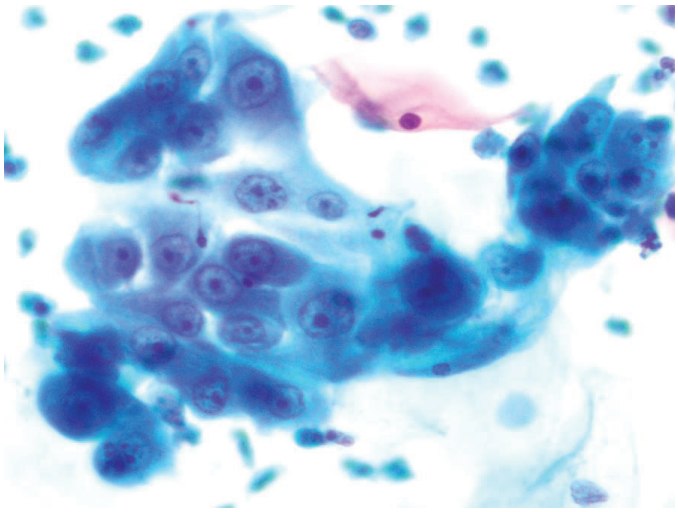
- Result from injury to cervical epithelium with proliferation of reserve cells
- Typical repair:
  - Cohesive, flat sheets (no isolated atypical cells)
  - Streaming (school of fish) or pulled out (taffy) appearance
  - Large nucleus, size variation
  - Large nucleolus
  - Pale, finely granular chromatin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





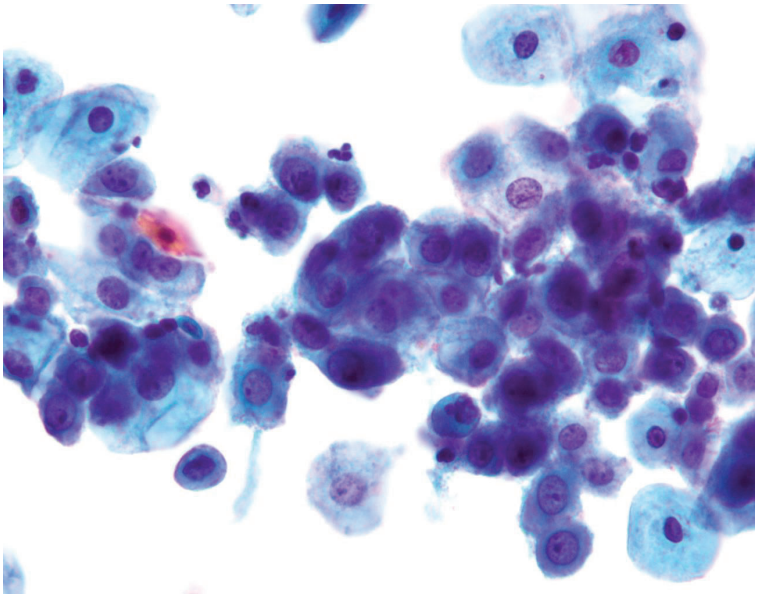


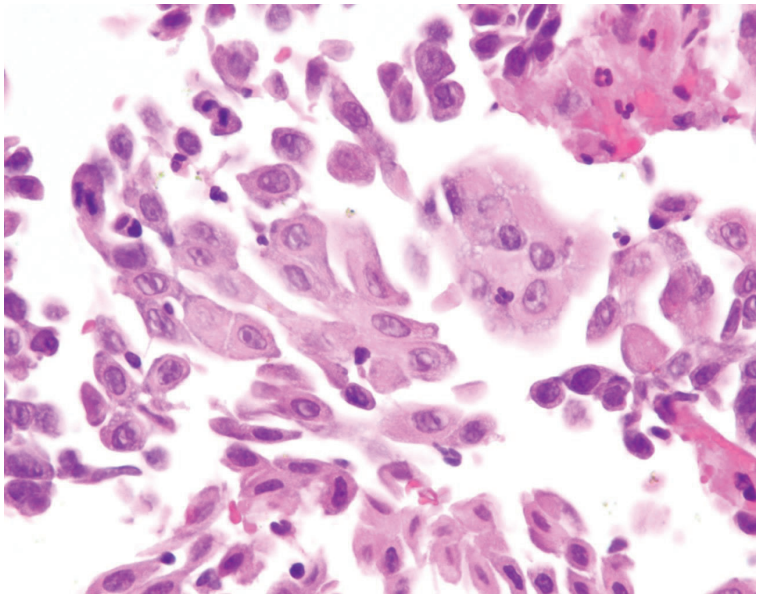




Repair

Squamous cell carcinoma





Pemphigus Vulgaris

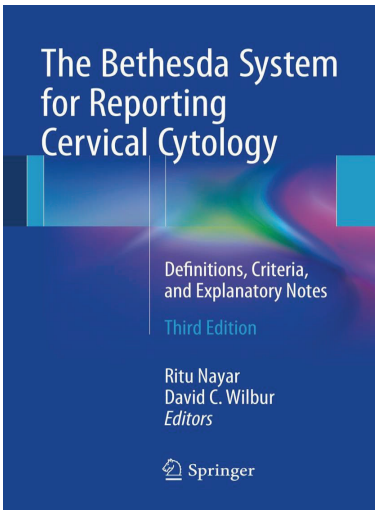
# Reporting Squamous Lesions using The Bethesda System

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




# A Word on Atypical Squamous Cells

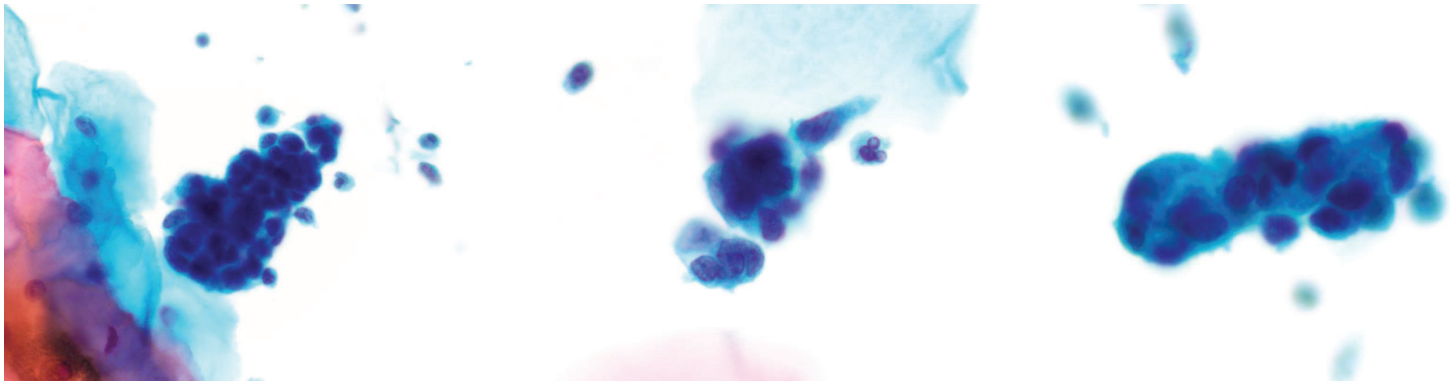
- ASC does not represent a single biologic entity
- ASC is essential: bridges the gap between clearly normal and clearly abnormal
  - ASCUS is the most common nonnegative interpretation (4.3%)
- Should be kept to a minimum
  - Less than 5% of all Pap cases or ASC/SIL <3:1 (opportunity for feedback)
- 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 Quality Assurance

- ASC is essentially a diagnosis of uncertainty
  - Specific reference values based on specific cytologic prep used with general guideline ASC rate <5% (~90% ASCUS 10% ASCH) (CAP Checklist, Bethesda System)
    - Low risk population? High risk population?
- Other measures: ASC/SIL ratio and hrHPV+ ASCUS
  - Quantitative, physician specific performance data
    - Current recommendation ASC:SIL 2-3:1 (Bethesda System)
    - Approx "expected" ASCUS hrHPV rate ~50% (Stoler, AJCPI 2007; ALTS trial)
  - ASC:SIL is a fraction (ie depends on two variables) so added value to correlating hrHPV positivity with ASCUS cases
    - Multilayered approach to assessment

# A Word on Cell Blocks

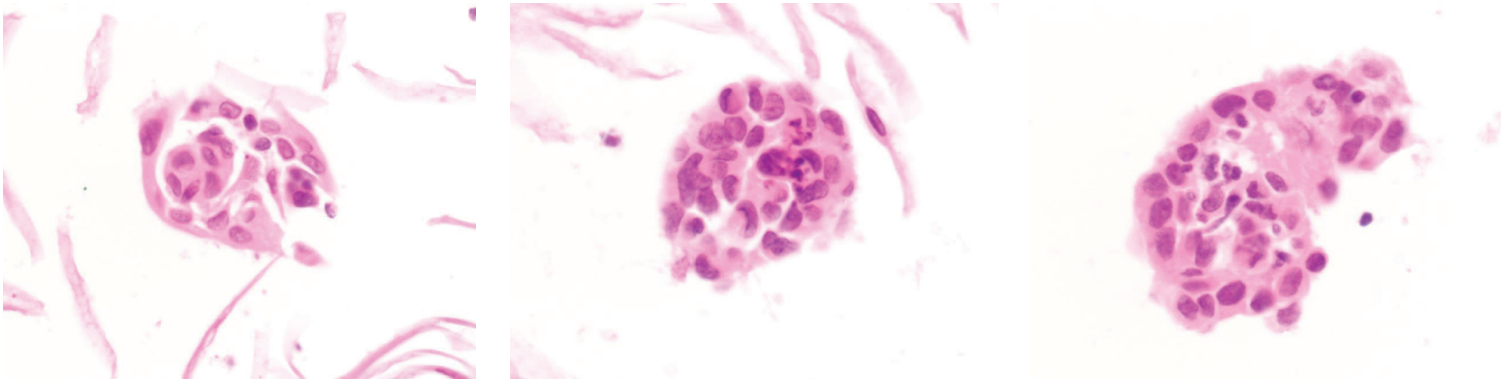
Cell blocks can be helpful in some cases



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



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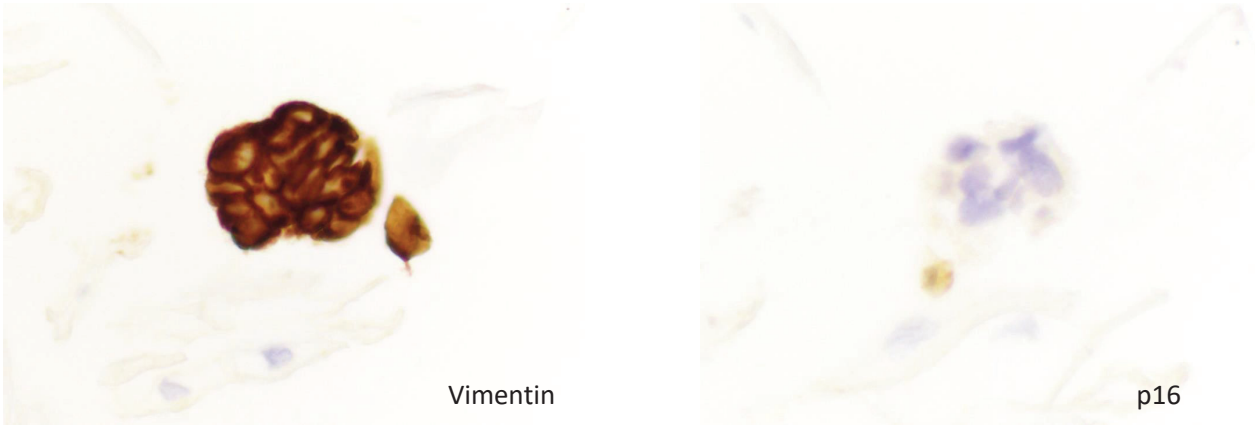

Vimentin p16

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, knowledge of overlapping benign entities is needed to help avoid misidentification
- 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, and also serving as a key performance indicator