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Diagnostic Dilemmas and Pitfalls in Pap Tests

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Objectives

- Highlight the challenges in cervical cytology given the overlap between benign and neoplastic processes
- Describe select benign processes with their corresponding neoplastic mimics
- Review illustrative examples of cytologic-histologic correlations in the context of glandular cells in cervical cytology
- Emphasize cytomorphologic features, diagnostic pitfalls, and approaches to handling these challenging cases

Introduction

- The Papanicolaou (Pap) test has been described as the most successful cancer reduction program ever devised which holds true
- Cervical cancer was the foremost cause of cancer deaths in the United States in the early 1900s
- After implementation of cervical cancer screening programs which began in the 1940s, it is not even in the top 10

Torous VF. Challenging lesions in cervical cytology: The elusive HSIL. Cytopathology. 2024 Jan;35(1):48-59

Introduction

- Large-scale studies show the impact of screening on mortality rates
- Mortality rates have significantly dropped after initiation of screening
- Globally, the regions and countries with the highest screening rates have been shown to have the greatest decrease in mortality

Torous VF. Challenging lesions in cervical cytology: The elusive HSIL. Cytopathology. 2024 Jan;35(1):48-59

Introduction

- Cervical cytology has been the cornerstone of cervical cancer detection since the development of the Papanicolaou test and implementation of screening programs
- Despite its long and widespread use, cervical cytology has remained a diagnostically challenging area
- Studies have shown that a false-positive diagnosis of cervical cancer will occur in ~10% to 15% of cases and interobserver reproducibility has only been moderate

Torous VF, Pitman MB. Interpretation pitfalls and malignant mimics in cervical cytology. J Am Soc Cytopathol. 2021 Mar-Apr;10(2):115-127

Introduction

- Pitfalls in Pap test can be defined as:
  - False positive (FP)
  - False negative (FN)
  - Underdiagnosed results
- Pitfalls lead to unnecessary diagnostic procedures or delayed and inadequate treatment
- Misdiagnosis can be a consequence of misinterpretation of certain morphological entities

Introduction

- Pitfalls can present diagnostic challenges for even the most experienced cytopathologists
- Focus on several benign and reactive processes that can mimic neoplastic processes in cervical cytology
- For each benign process, cytomorphologic features are highlighted as well as differential diagnosis with potential diagnostic pitfalls, and approach to interpretation and classification

Intrauterine device (IUD) effect

- A study by Fornari in 1974 described the atypical changes in glandular cells with:
  - Cytoplasmic vacuolization
  - Variation in nuclear size and shape
- Mimicking adenocarcinoma

M.L. Fornari. Cellular changes in the glandular epithelium of patients using IUCD—a source of cytologic error
Acta Cytol, 18 (1974), pp. 341-343

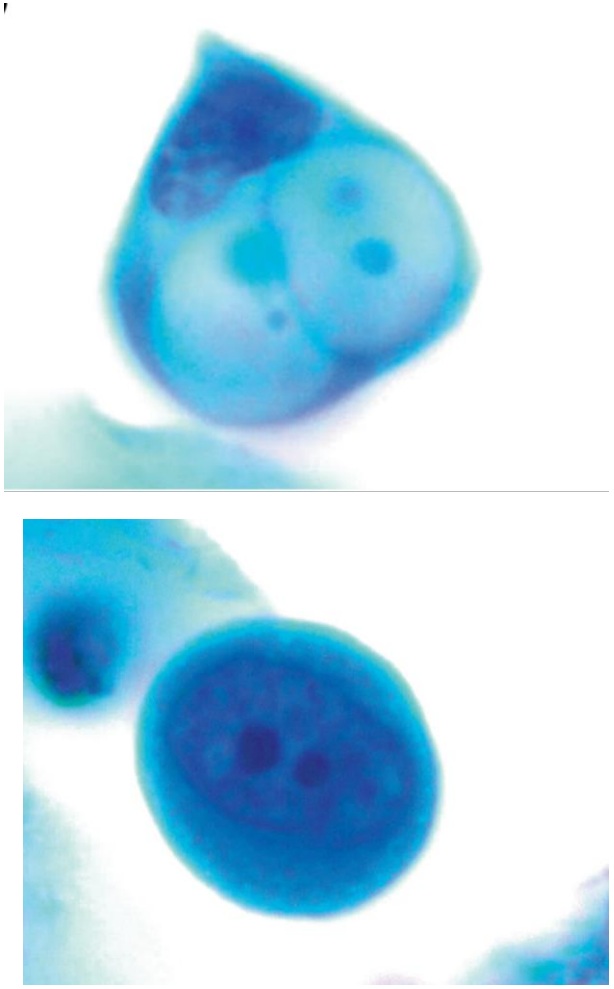
Intrauterine device (IUD) effect

- Later, a 1978 study by Gupta et al. described squamous atypia and indeterminate cells with:
  - High nuclear-cytoplasmic ratios
  - Hyperchromasia
  - Prominent nucleoli, occasional multinucleation
- Mimicking HSIL

P.K. Gupta, F. Burroughs, R.D. Luff, J.K. Frost, Y.S. Erozan. Epithelial atypias associated with intrauterine contraceptive devices (IUD) Acta Cytol, 22 (1978), pp. 286-29

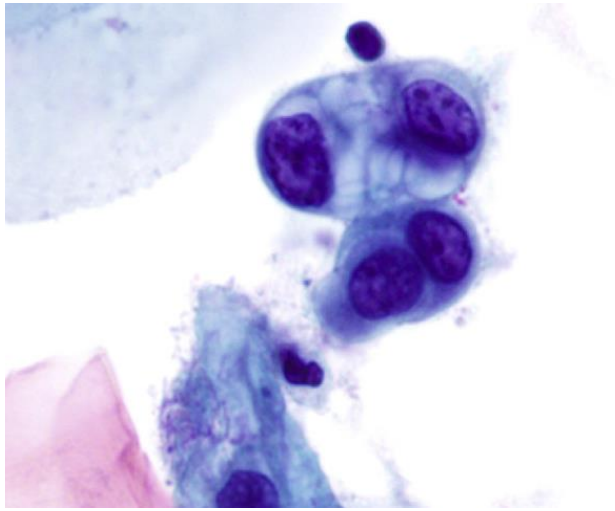
Intrauterine device (IUD) effect

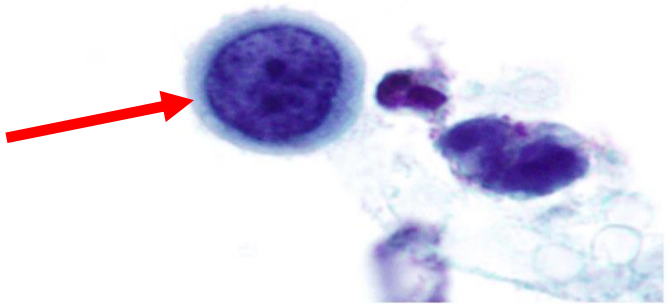
- 2 distinct cytomorphologic changes described with IUDs
- Caused by reactive changes in endometrial and/or endocervical cells due to chronic irritation



IUD effect - 2 distinct cells types

1. Glandular cells with vacuolated cytoplasm
2. Small dark cells mimicking HSIL, BUT…
   - SMOOTH NUCLEAR COUNTOURS
   - SMALL NUCLEOLI
   - MAY DEMONSTRATE MULTINUCLEATION
   - Significant nuclear enlargement and nuclear atypia with irregular nuclear contours will not be present
   - Background findings may include Actinomyces, calcification, debris, inflammation and reactive change





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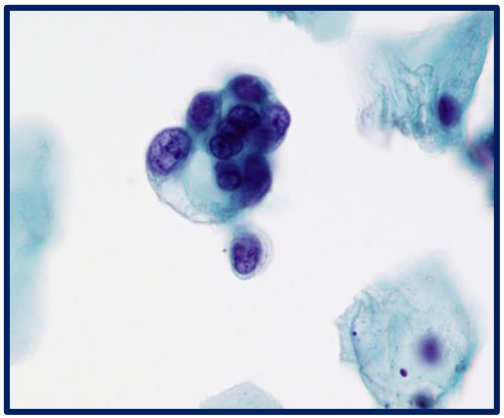
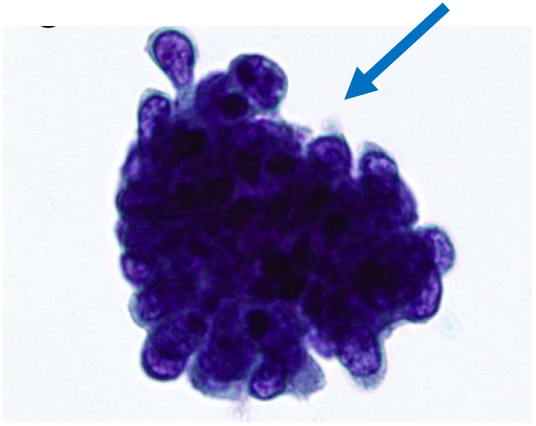
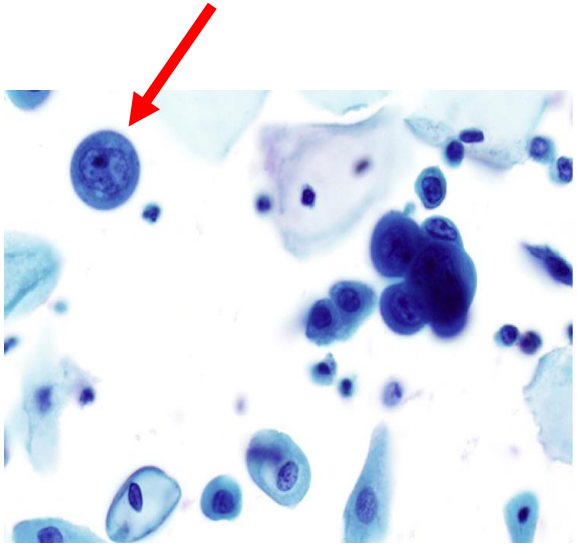
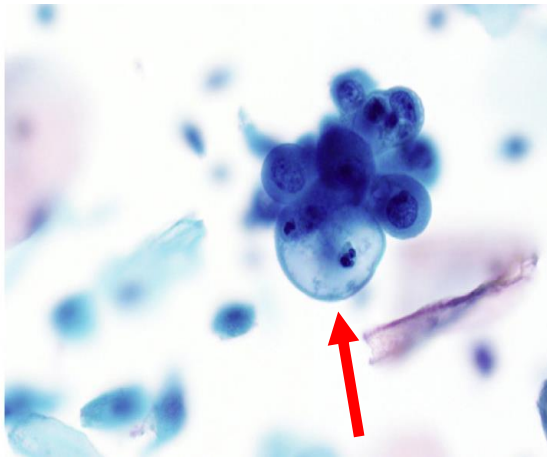
IUD effect vs. ….

- Glandular-appearing IUD cells with <u>cytoplasmic vacuolization</u>
- Signet ring appearance
- Mimicking glandular entities
  1. Endometrioid endometrial adenocarcinoma (ACa) – low grade
  2. Exfoliated **endometrial cells**
- Features of **ACa** include:
  - prominent nucleoi
  - intracytoplasmic neutrophils









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Differential for IUD effect: HSIL

Characteristic Features of HSIL

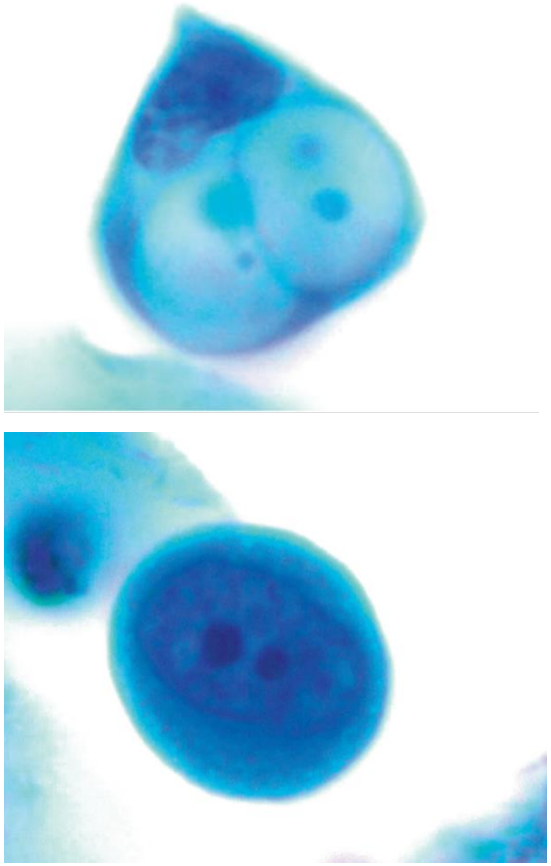
- Two main patterns:
  - syncytial-like aggregates (hyperchromatic crowded groups)
  - single cells
- **Enlarged nuclei** with hyperchromatic
- **Irregular nuclear contours**
- **Coarse chromatin**
- Cytoplasm often dense; can be delicate or vacuolated
- Nucleoli generally absent
- Majority hrHPV positive

Differential for IUD effect: Endometrial Adenocarcinoma

- Grade 1 tumors and endometrioid endometrial adenocarcinoma may have only mild atypia, few abnormal cells
- Tumor diathesis in 30% of cases; less on LB Paps
- **Nuclear enlargement** with variable hyperchromasia
- **Nucleoli**, small to prominent
- Cytoplasm, delicate and vacuolated; associated with cytoplasmic neutrophils, aka "**bag of polys**"
- Neoplastic cells, single or in small tight clusters
- Overlap with endometrial cell groups; menstrual specimens
- Associated with reactive/benign processes including IUDs and neoplastic processes

Diagnostic Approach to Differential of IUD effect

- Cellular features characteristic of an IUD include:
  - **Uniformity** of the cell type
  - **Low cellularity** of the atypical cells
  - **Prominent nucleolus**
  - Background **actinomyces** or pseudoactinomycotic granules, inflammation and/or reactive changes



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Diagnostic Approach to Differential of IUD effect

- Even with an IUD history, the differential may include:
  - Atypical squamous cells cannot exclude a high-grade squamous intraepithelial lesion (ASC-H)
  - Atypical glandular cells (AGC, NOS)
- Patient age:
  - Older age - higher suspicion for a glandular lesion
  - Women of childbearing age with atypical endometrial hyperplasia or endometrioid adenocarcinoma grade 1 may be treated with Mirena IUD to preserve fertility
- Add a note:
  - Suggest repeat sampling after removal of the device
  - Cytologic features will lessen within a few months after removal

Tubal Metaplasia

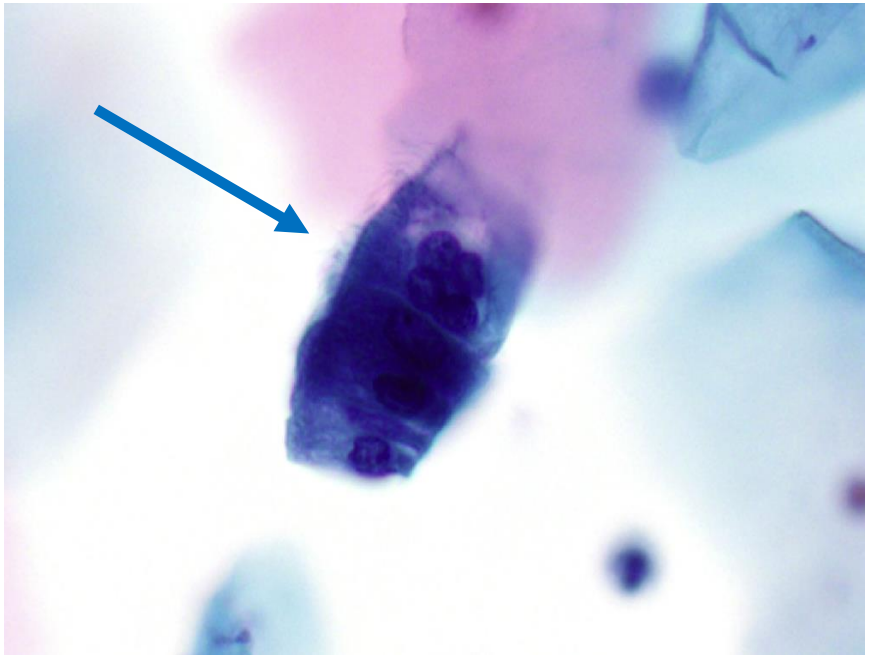
- In TM the endocervical epithelium is replaced by an epithelium that recapitulates fallopian tube epithelium
- TM commonly seen in the upper endocervix
- Frequency of up to:
  - 10% in cervical cytology specimens
  - 30% in cone excision/hysterectomy

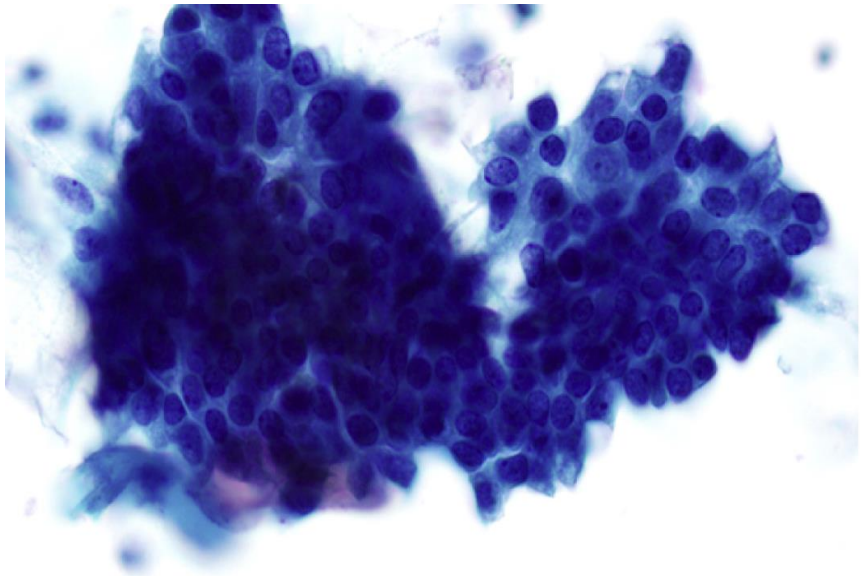
Tubal Metaplasia

- Tubal metaplasia is an important diagnostic pitfall**
- Morphologic overlap with other glandular processes:
  - Endocervical adenocarcinoma in situ (AIS)
  - Squamous intraepithelial lesions (SIL) involving endocervical glands →HSIL, syncytial type

Tubal Metaplasia

- Tubal metaplastic cells can be single, in pseudostratified strips, flat sheets, or in crowded clusters
- Cells will be columnar in shape and tend not to lose their cytoplasm
- Presence of apical **terminal bars and cilia**





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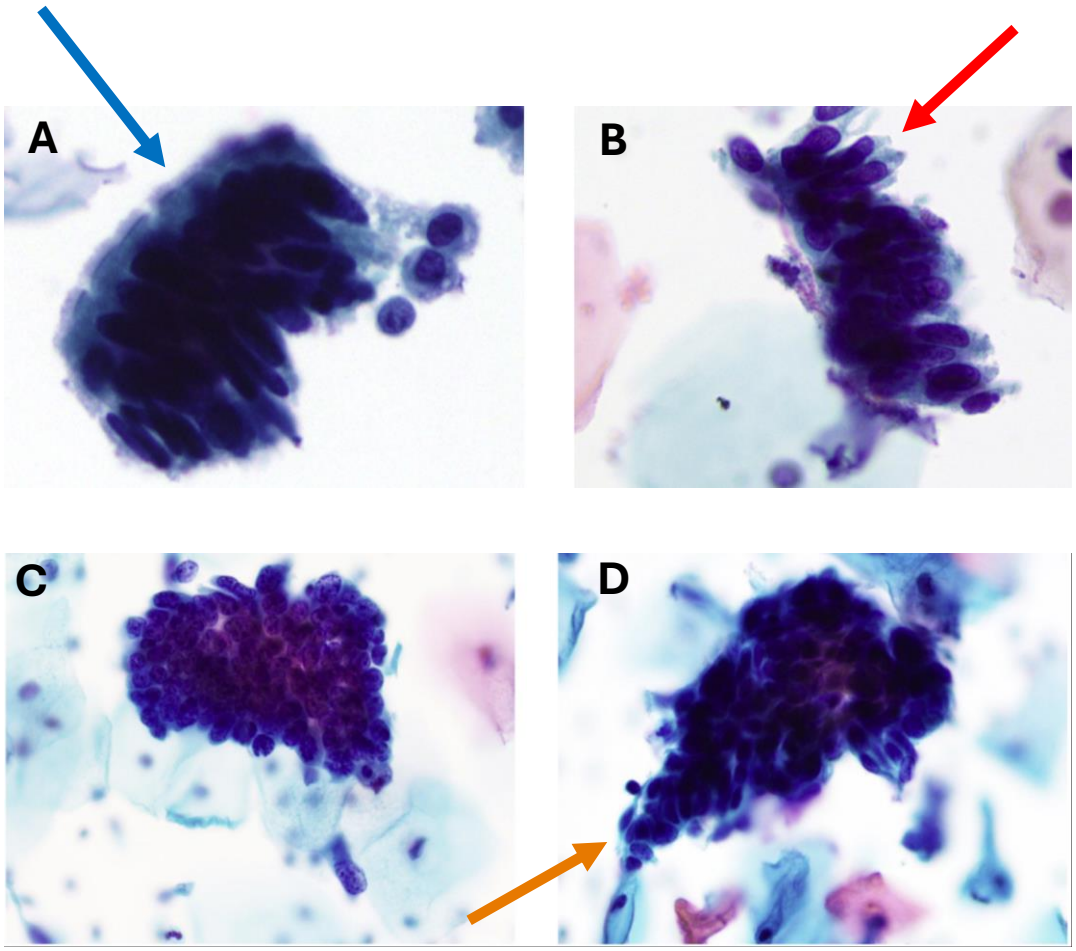
Tubal Met vs. …

A. **Tubal metaplastic** cells pseudostratified with cytologic atypia, nuclear enlargement

B/C. Endocervical AIS with pseudostratified strips and nuclear enlargement, crowding, overlapping, hyperchromasia, and coarse chromatin as well as peripheral feathering

D. HSIL, syncytial type, flat edge



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Differential for Tubal Metaplasia: Endocervical AIS

Characteristic features of Endocervical AIS

- Cellular, hyperchromatic crowded groups or strips
- Glandular differentiation with columnar cells; rosettes
- Nuclear “feathering”, nuclear enlargement and hyperchromasia
- Nuclear crowding, overlap
- Nuclear stratification and palisading
- Chromatin with moderate to coarse granularity
- Nucleoli inconspicuous
- Few to numerous mitoses; apoptosis
- Background usually clean

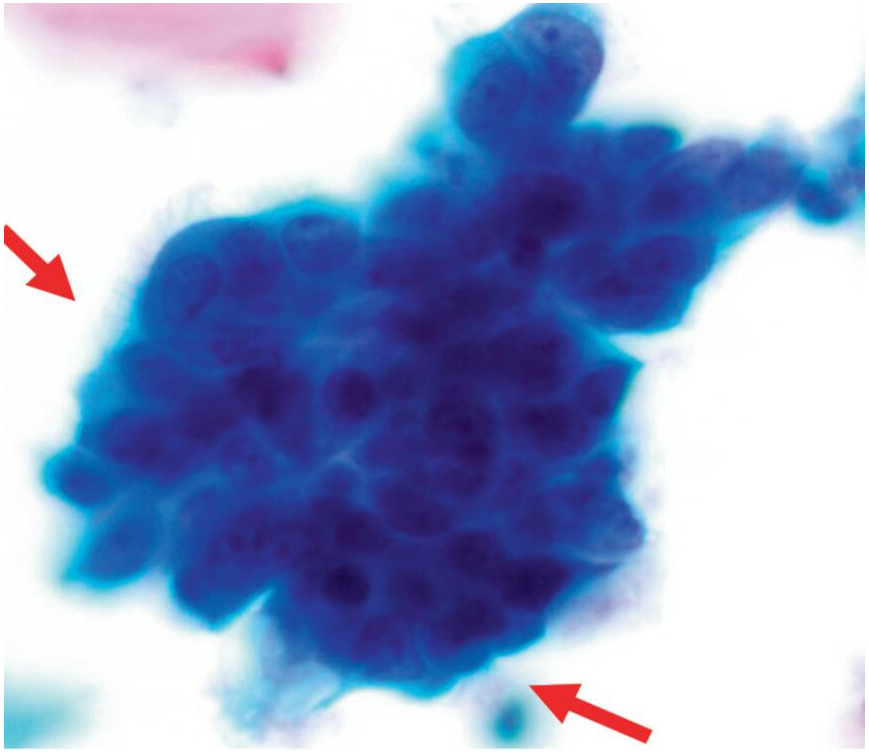
Differential for Tubal Metaplasia: HSIL involving glands

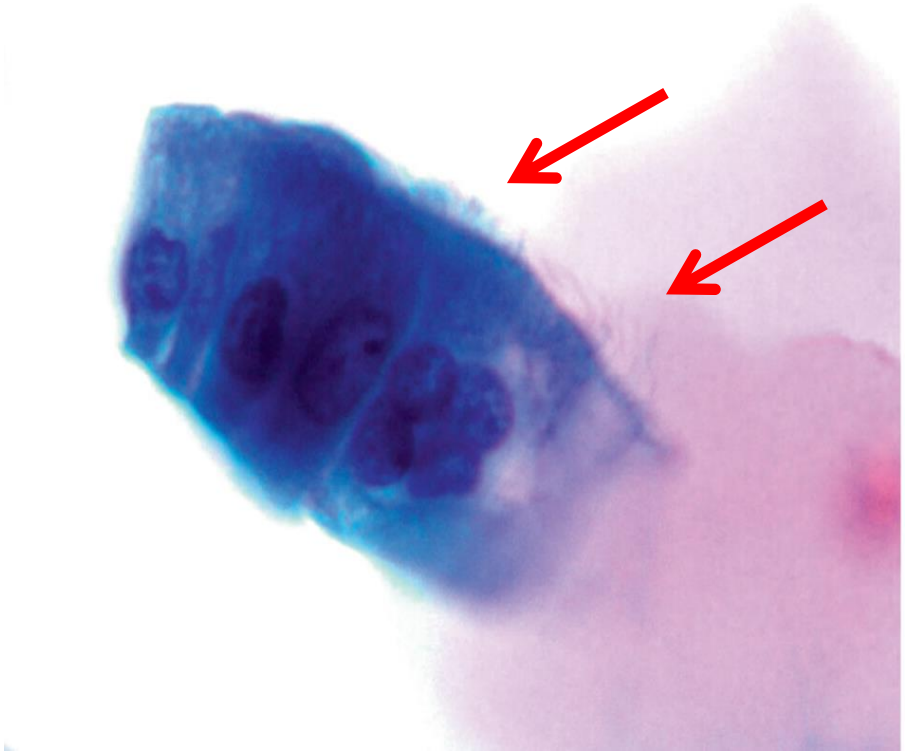
- Characteristic features of **HSIL** involving glands
  - **Cellular**
  - **Hyperchromatic crowded** groups
  - Look for cell cluster **edges with flattening of the nuclei** with a smooth/rounded cell group borders
  - **Spindling** of the cells in cell clusters
  - **Nuclear atypia** best seen at cluster edges
  - **No rosettes or pseudostratified strips**
  - Feathering uncommon

Diagnostic Approach to Differential of Tubal Metaplasia (TM)

- Careful examination with active movement through planes of focus to search for **terminal bars and/or cilia**
  - **Favors benign**
  - Presence of cilia does not exclude a lesion
- Chromatin granularity:
  - **Fine chromatin favors TM**
  - Coarse chromatin favors neoplastic processes
- Compared to AIS:
  - **TM lacks feathered edges and rosettes**
  - TM shows rare mitotic figures; apoptosis is typically absent

Tubal Metaplasia





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Diagnostic Approach to Differential of Tubal Metaplasia

- Awareness of the **differential diagnosis** and attention to the **cytologic criteria** discussed can lead to the correct diagnosis
- In challenging cases an atypical interpretation of **AGC, NOS or ASC-H** helps triage for further evaluation
- **High-risk HPV** status and cell block preparation can be considered with **p16** showing block-like positivity in HSIL and endocervical AIS

Atrophy

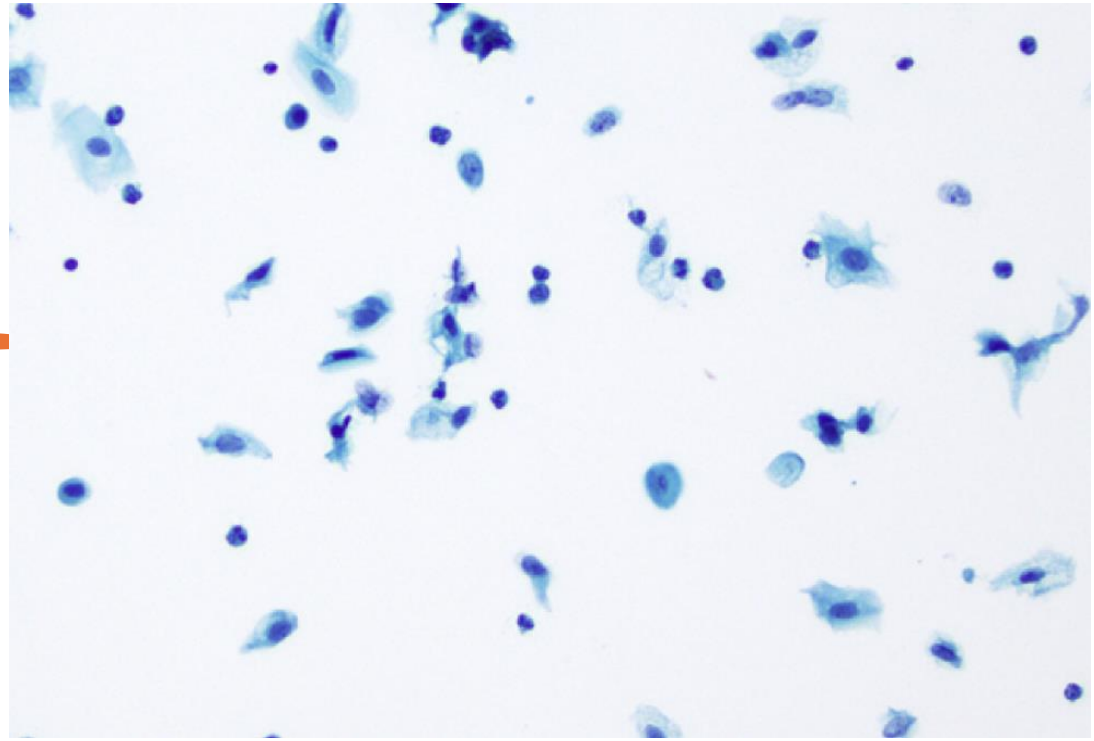
- Decreased estrogen stimulation
  - Menopause
  - Postpartum
  - Bilateral ophorectomy
  - OCPs, IUD with Progestin
  - Exogenous hormones; gender-affirming testosterone hormonal therapy in female-to-male transgender patients

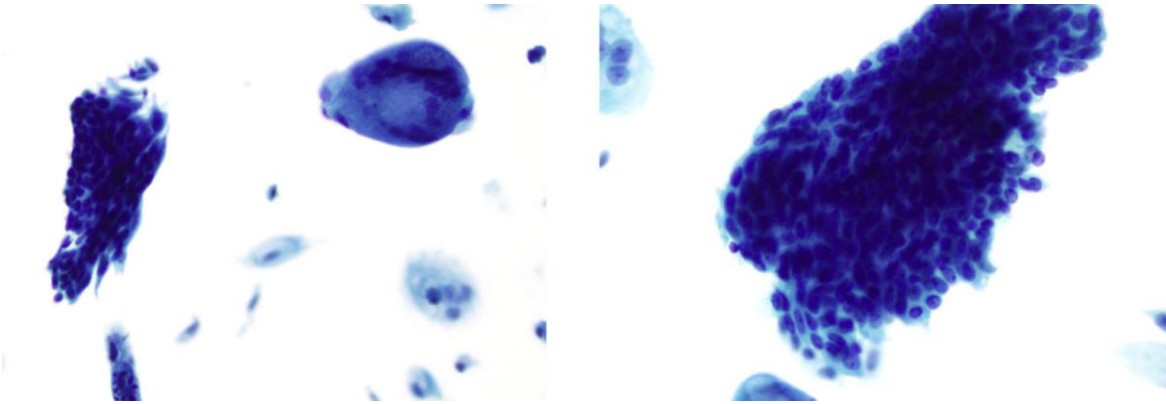
Atrophy

- Leads to thinned immature squamous epithelium
- Challenging due to the associated background metaplastic and inflammatory changes
- Cytologic features exist to avoid false-positive interpretations

Atrophy

- Single parabasal and intermediate cells
- Multinucleated histiocytes
- Hyperchromatic, crowded groups (HCG)





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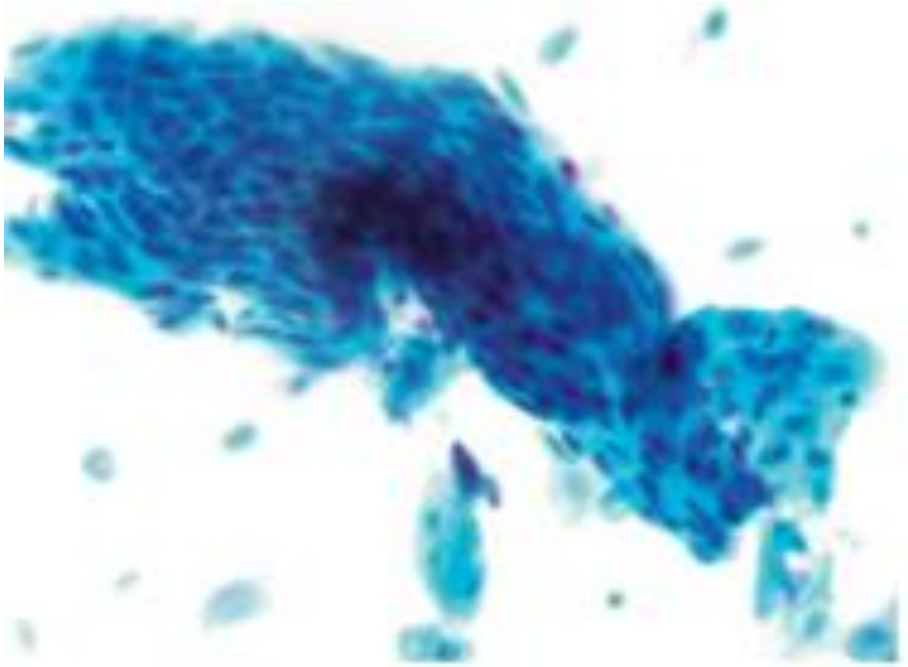
Atrophy

- Parabasal cells in flat sheets
  - **“school of fish”**
- No significant nuclear overlap
- Slight nuclear enlargement with increased N:C and mild hyperchromasia
- Smooth nuclear contours and uniform chromatin
- Mitotic activity not usual



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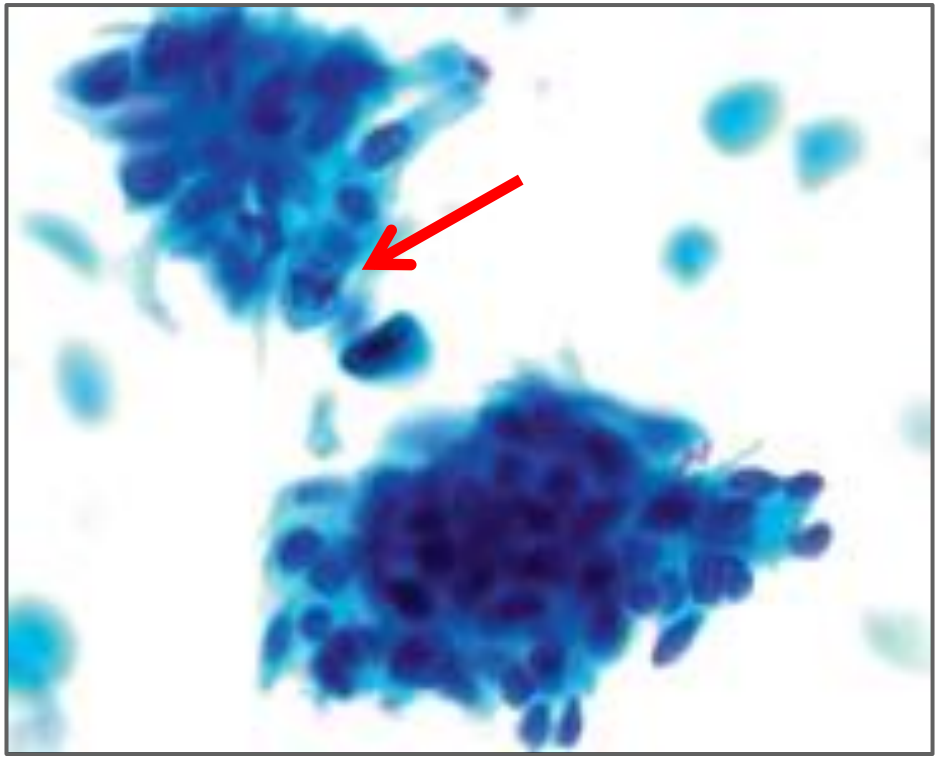
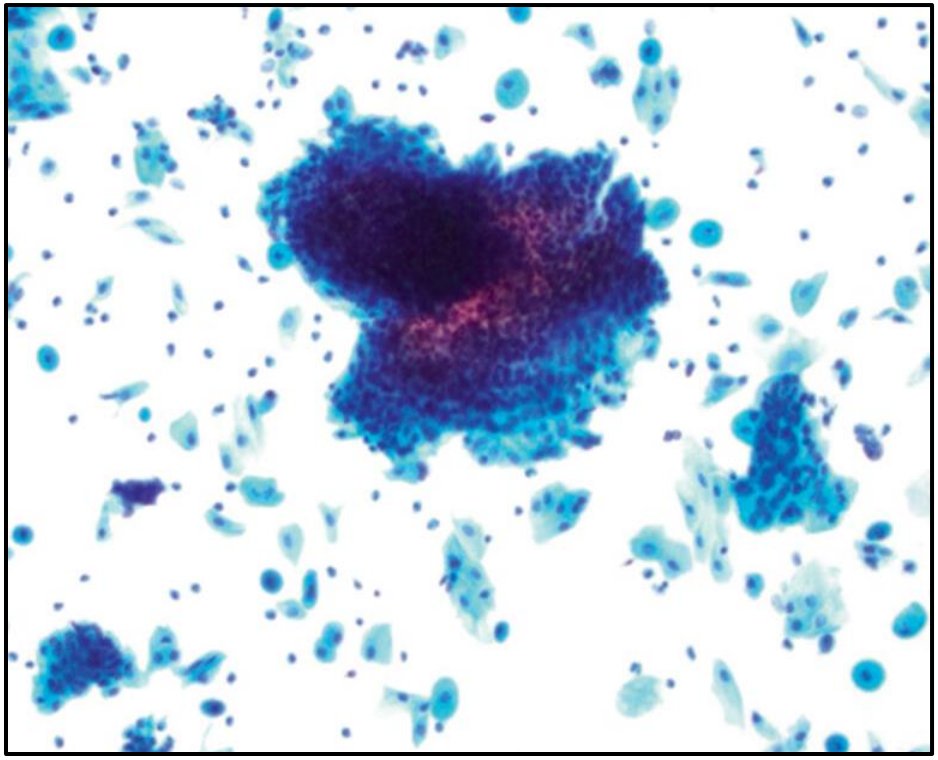
Differential for Atrophy: HSIL

Characteristic features of **HSIL**

- HSIL can occur as single cells and sheets
- Both HSIL patterns overlap with atrophy
  - Cell clusters of HSIL show a **loss of polarity**
  - Single cells show nuclear atypia
- Prominent **nuclear overlapping** and crowding
- At high power nuclear atypia will be present:
  - Nuclear enlargement, increased N:C
  - Nuclear **contour irregularity**
  - Hyperchromasia, **coarse chromatin** (not smudgy)
- Mitotic activity varies; look for abnormal cells

Atrophy vs.HSIL





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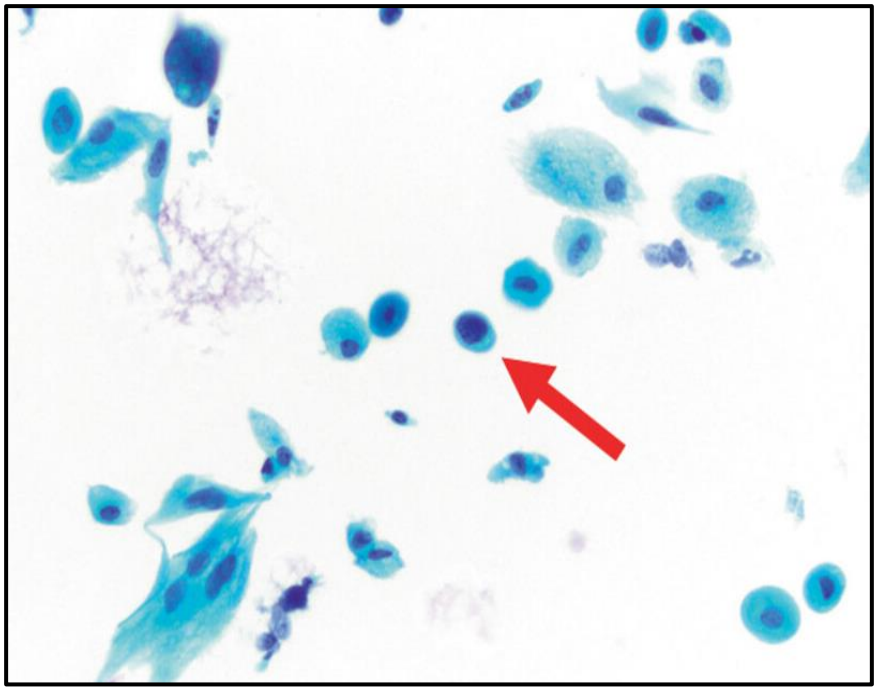
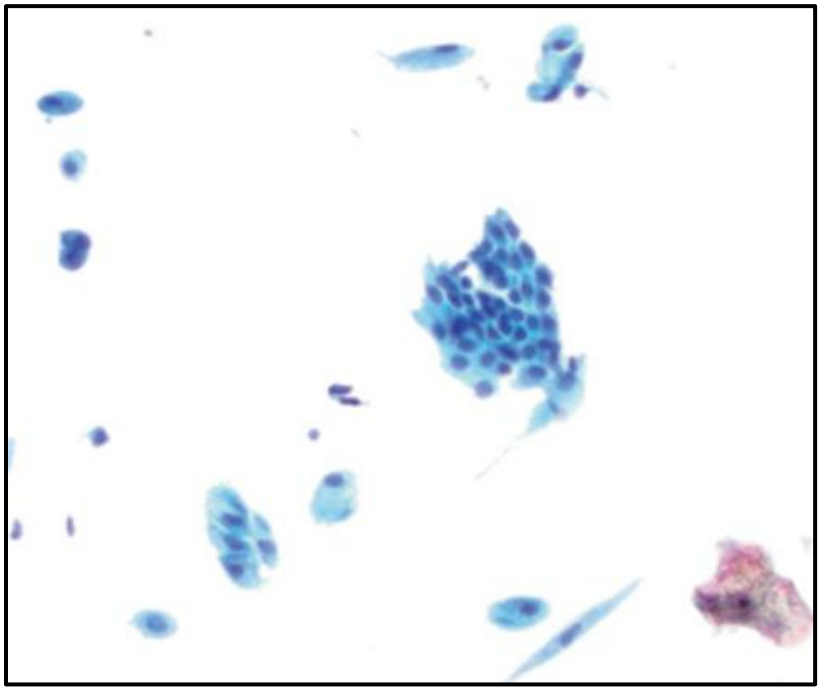
Atrophy vs.
HSIL + atrophy





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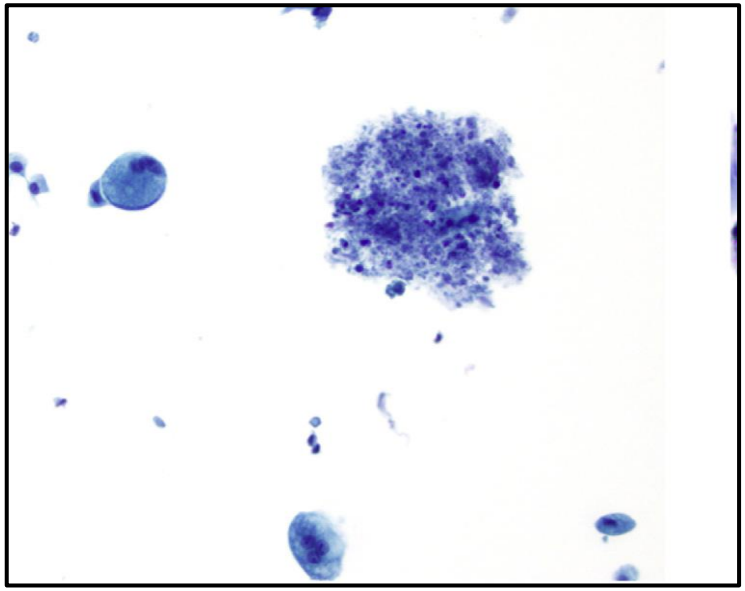
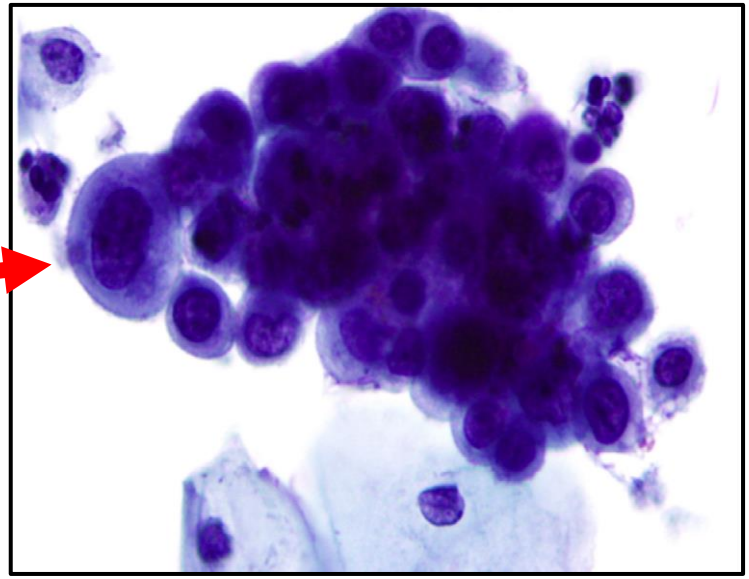
Atrophy vs. HSIL

- Granular debris and histiocytes; not tumor diathesis
- **HSIL** in hyperchromatic group with nuclear atypia and overlap





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Diagnostic Approach to Differential: Atrophy vs. HSIL

Atrophy and its lesional mimicker HSIL

	Atrophy	**HSIL**
Clinical	Low estrogen, postmenopausal, postpartum, exogenous hormones	Any age, usually younger women, with peak in mid- to late 30s
Cellular pattern	Can be a diffuse pattern change, found singly or in syncytium-like sheets	Usually cellular; found singly or in syncytia, hyperchromatic crowded groups

Diagnostic Approach to Differential: Atrophy vs. HSIL

Atrophy and its lesional mimicker HSIL

	Atrophy	**HSIL**
Distinguishing cytologic characteristics	Cells within **sheets** can be crowded, no significant nuclear overlap; **smooth nuclear borders**, **even chromatin**; N:C may be increased; generally, no mitotses	**Crowded groups**, cellular **overlap, loss of polarity**; **atypical nuclear features**, exceptions possible; can be **mitotically active;** hrHPV positivity may be helpful

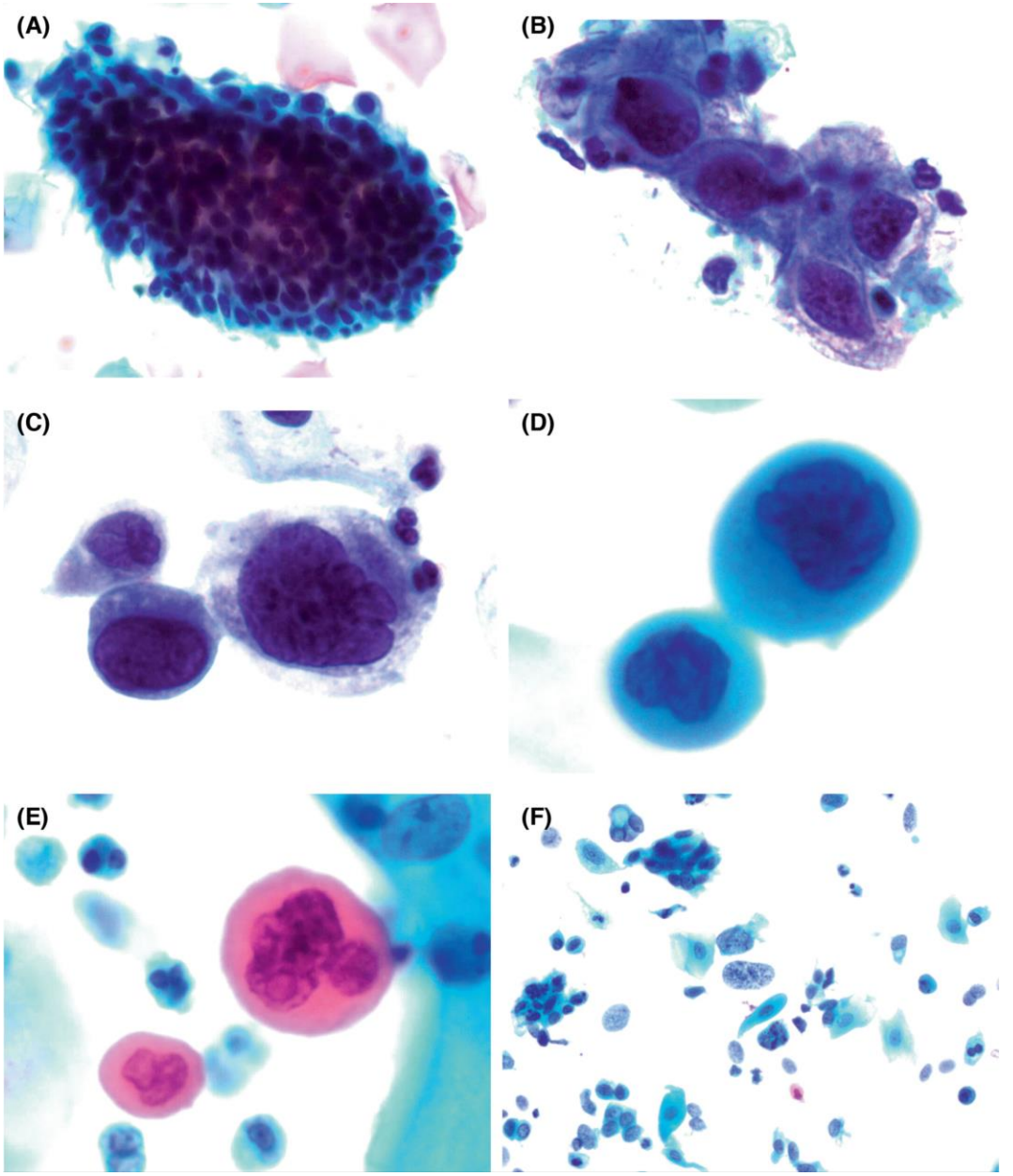
Patterns of HSIL

(A-C) HSIL in different patterns varying from large syncytial aggregates/HCG, to smaller groups and single cells

(D) Metaplastic appearance with dense blue-green cytoplasm

(E) Keratinized forms

(F) Atypical stripped cells; clue to look for additional diagnostic HSIL cells



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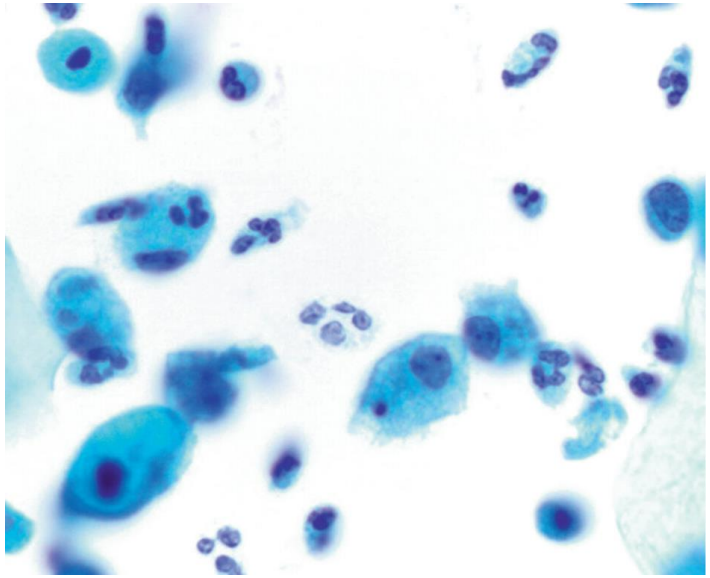
Benign Inflammatory Cells:
Histiocytes or lymphocytes can mimic HSIL

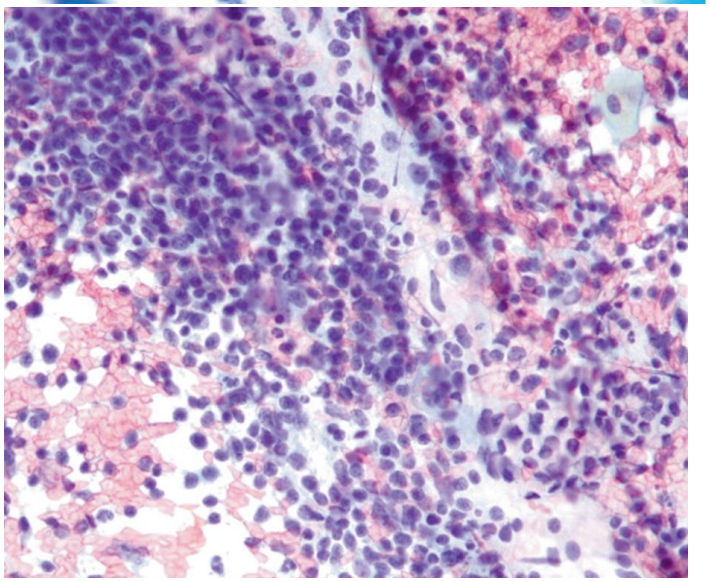
- Histocyte cytologic features
  - Similar in size to HSIL/ASC-H cells
  - Irregular nuclear contours
  - Finely textured chromatin
  - More abundant and delicate cytoplasm
- Lymphocyte cytologic features
  - High N:C ratio
  - Coarse chromatin potentially
  - Lack irregular nuclear contours
  - Usually smaller than HSIL cells
  - Enlarged in reactive processes; look for other inflammatory cells; tingible body macrophages, and plasma cells

Benign Inflammatory Cells

Top: Typical histiocytes with low N:C ratio and abundant delicate cytoplasm (LBP)

Bottom: Follicular cervicitis with abundant lymphocytes; high N:C ratios; small in size; lack nuclear irregularity; look for plasma cells or dendritic cells can be helpful (conventional smear)





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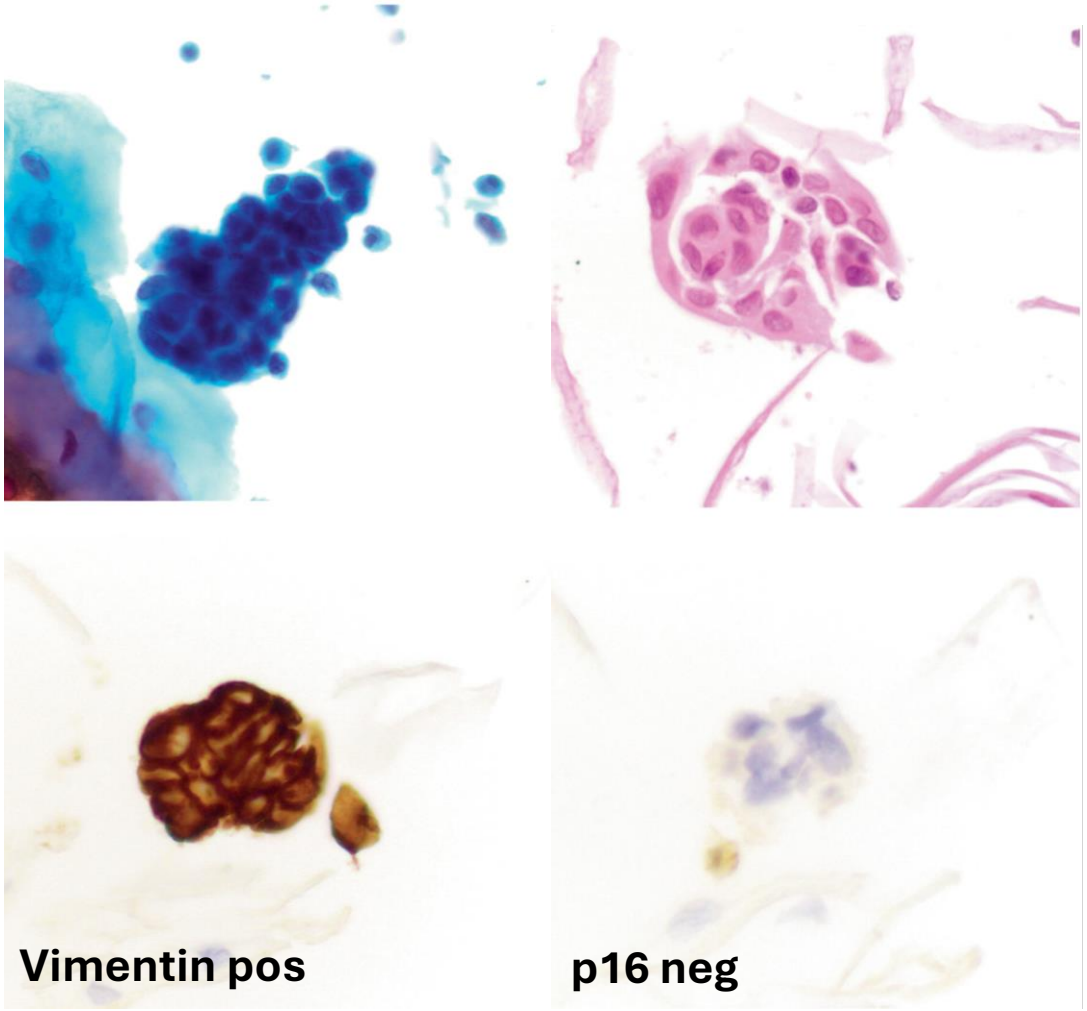
Benign Endometrial Cells:
Endometrial cells can mimic HSIL/ASC-H

- Exfoliated endometrial cell cytologic features
  - Single cells or dense aggregates (HCG)
  - Small cells with dark nucleus
  - Scant cytoplasm with high N:C ratio
  - Smaller than HSIL cells
  - Classic 3-D groups of endometrial cells
  - Smooth to scalloped cell group borders
  - Nuclear wrapping
  - Clusters of endometrial cells mimic the syncytial HSIL
  - Lack anisonucleosis
  - Apoptotic bodies present
  - In challenging cases, cell blocks can be helpful

Benign Endometrial Cells vs. HSIL

Endometrial cells and it's lesional mimicker syncytial HSIL

- Young woman with recent history of loop electrosurgical excisional procedure (LEEP) for HSIL
- Hyperchromatic cellular group on Pap
- H&E Cell block prepared
- IHC supported endometrial cell group



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Squamous Metaplasia:
Squamous metaplastic can mimic HSIL/ASC-H

- Squamous metaplasia may cause FPs in cervical cytology
- Review of specific features to distinguish LSILs from HSILs showed the only predictors of misclassified cases were:
  - Number of atypical squamous metaplastic cells and HSIL cells
- Squamous metaplasia involves the proliferation of endocervical reserve cells and differentiation into squamous cells
  - Reserve cells differentiate into immature squamous metaplasia

Staats PN, Davey DD, Witt BL, et al. Performance of specific morphologic features in distinguishing low-grade squamous intraepithelial lesions from high-grade squamous intraepithelial lesions in borderline cases: a College of American Pathologists Cytopathology Committee multiobserver study. J Am Soc Cytopathol. 2022;11(2):102-113

Squamous Metaplasia:
Squamous metaplasia can mimic HSIL/ASC-H

Cytologic features of squamous metaplasia

- Dense aqua-colored cytoplasm, round sharp cell borders
- Cytoplasmic vacuolization
- Size and shape varies with the degree of maturation
- Nuclei round to oval
- Smooth nuclear membranes and fine chromatin
- immature cells have high N:C ratio
- Nuclear atypia may be seen with reactive change
- Nucleoli support squamous metaplasia

Squamous Metaplasia
Squamous metaplasia can mimic HSIL/ASC-H

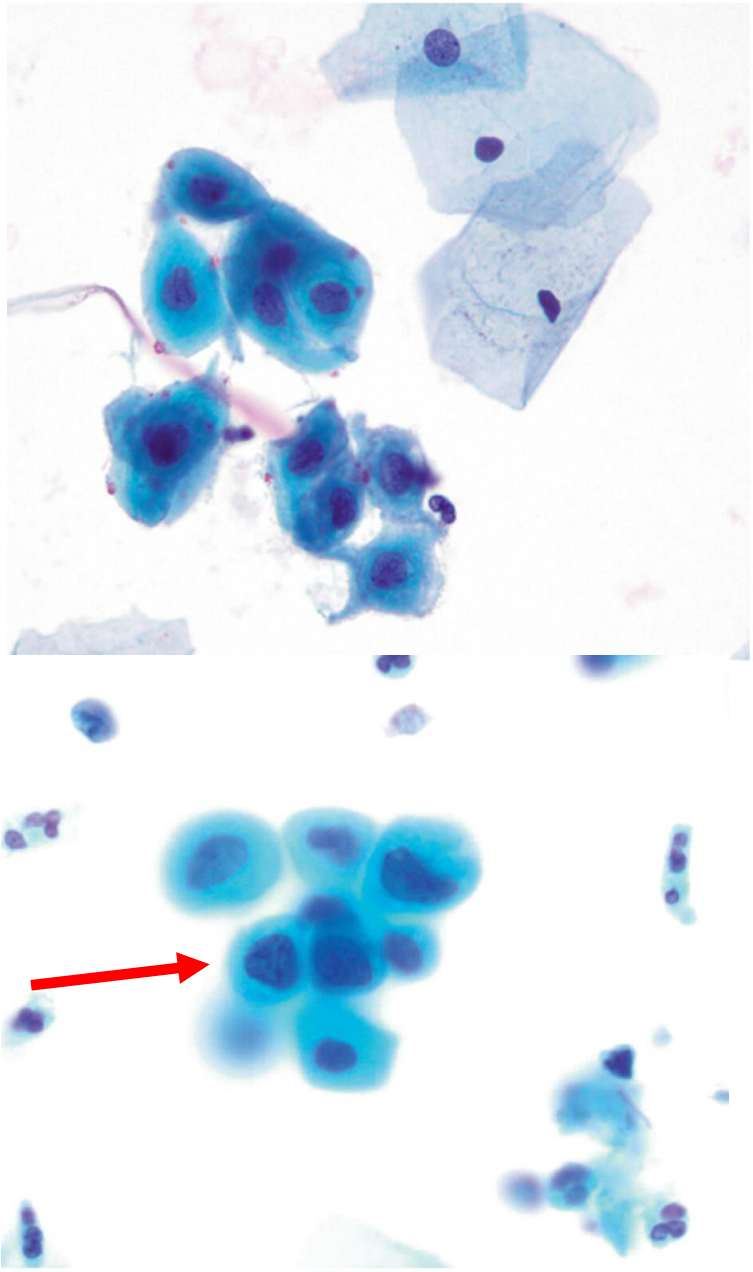
- Cytologic features of HSIL
  - Dense metaplastic-type cytoplasm
  - high N:C ratio
  - Nuclear enlargement
  - Hyperchromasia
  - Membrane irregularity
  - Coarse chromatin
- In challenging cases, ASC-H interpretation may be considered

Squamous Metaplasia vs. HSIL

Squamous metaplasia and it's lesional mimicker **HSIL**

Top: Squamous metaplastic cells - variable N:C ratio depending on the level of maturation; classic blue-green dense cytoplasm, regular nuclear features, even chromatin

Bottom: HSIL with metaplastic type cytoplasm - nuclear atypia, hyperchromasia, irregular nuclear contours and increased N:C ratio



Torous VF. Challenging lesions in cervical cytology: The elusive HSIL. Cytopathology. 2024 Jan;35(1):48-59

Reparative/Reactive Changes

- Reparative changes involve both squamous and endocervical cells
- Source of both FP and FN diagnoses shown in various College of American Pathologist slide survey studies
- High variability in the degree of atypia
- Overlap with squamous and glandular neoplastic processes
- Studies show in this atypical category there may be:
  - No identifiable pathologic findings
  - Benign to neoplastic processes, including infection, a previous surgical procedure, IUD changes, radiotherapy, atrophy, uterine prolapse, polyp, and low- or high-grade intraepithelial squamous lesions (LSIL, HSIL)

Reparative changes and the false-positive/false-negative Papanicolaou test: a study from the College of American Pathologists interlaboratory comparison program in cervicovaginal cytology. Arch Pathol Lab Med, 125 (2001), pp. 134-140

P.H. Levine, P.A. Elgert, P. Sun, A. Simsir. Atypical repair on Pap smears: clinicopathologic correlates in 647 cases. Diagn Cytopathol, 33 (2005), pp. 214-217

Reparative/Reactive Changes:
2 categories: typical vs. atypical

- Typical repair with mild change identified as benign
  - Nuclear binucleation or multinucleation
  - cytoplasmic changes can include vacuolization, perinuclear cytoplasmic halos, and polychromasia
- Atypical repair can have striking nuclear changes including:
  - Nuclear enlargement, anisonucleosis, irregular nuclear contours, irregularities in chromatin distribution, prominent and irregular nucleoli
  - Mitoses can be present
  - Arranged in cohesive groups and flat sheets
  - Pulled out cytoplasm with “pulled taffy” appearance
  - Crowded with more prominent nuclear overlap

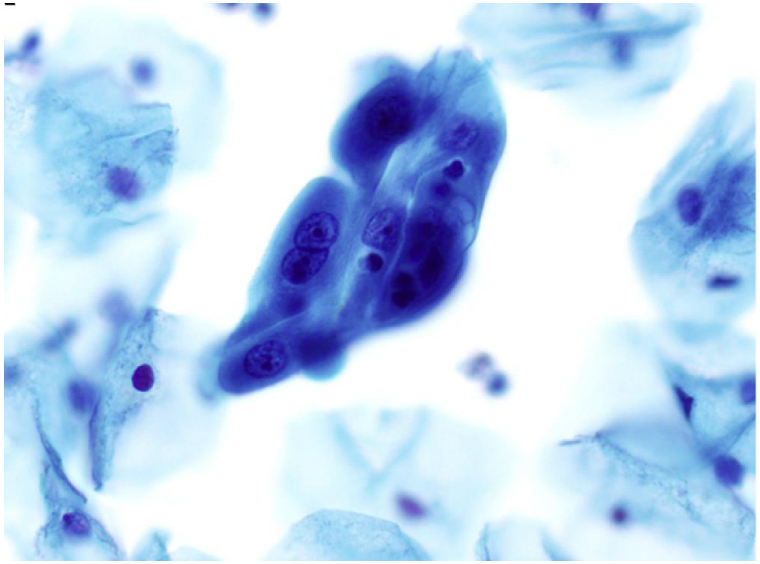
Typical Reparative Changes

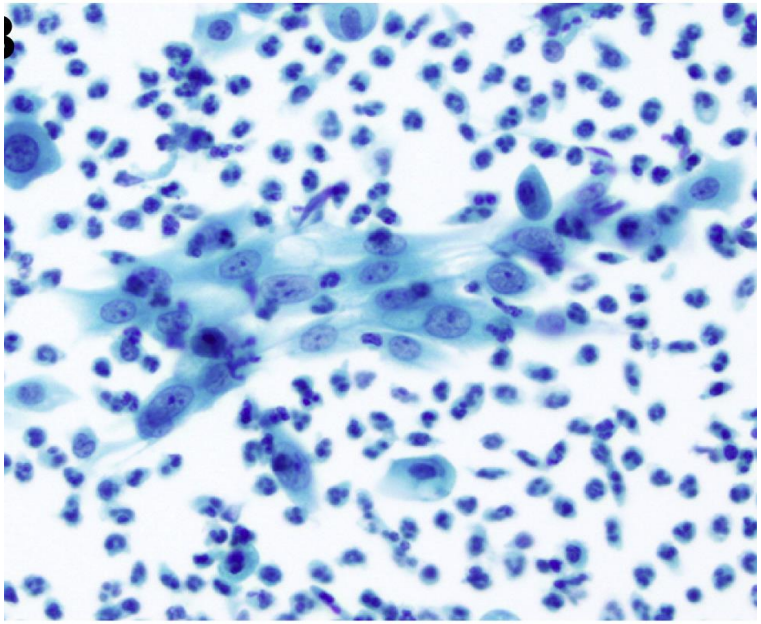
Top

- Bi- and multinucleation,
- Prominent nucleoli, not typical of intraepithelial lesions.

Bottom

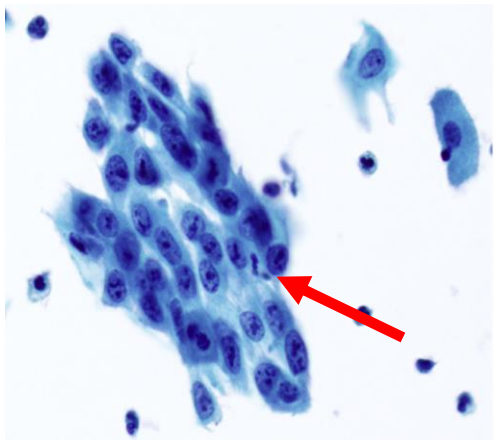
- Mild nuclear enlargement.
- Nuclear contours smooth
- Prominent nucleoli
- Streaming or pulled taffy arrangement of the cells

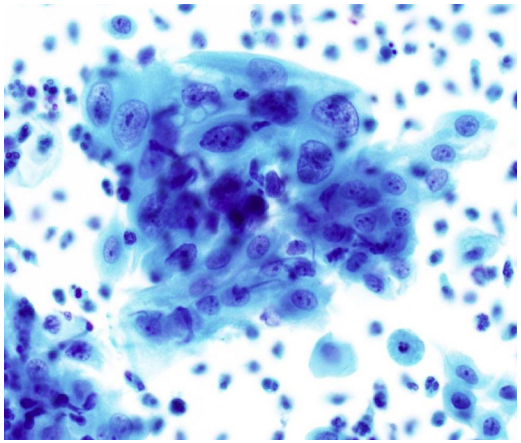




Torous VF, Pitman MB. Interpretation pitfalls and malignant mimics in cervical cytology. J Am Soc Cytopathol. 2021 Mar-Apr;10(2):115-127

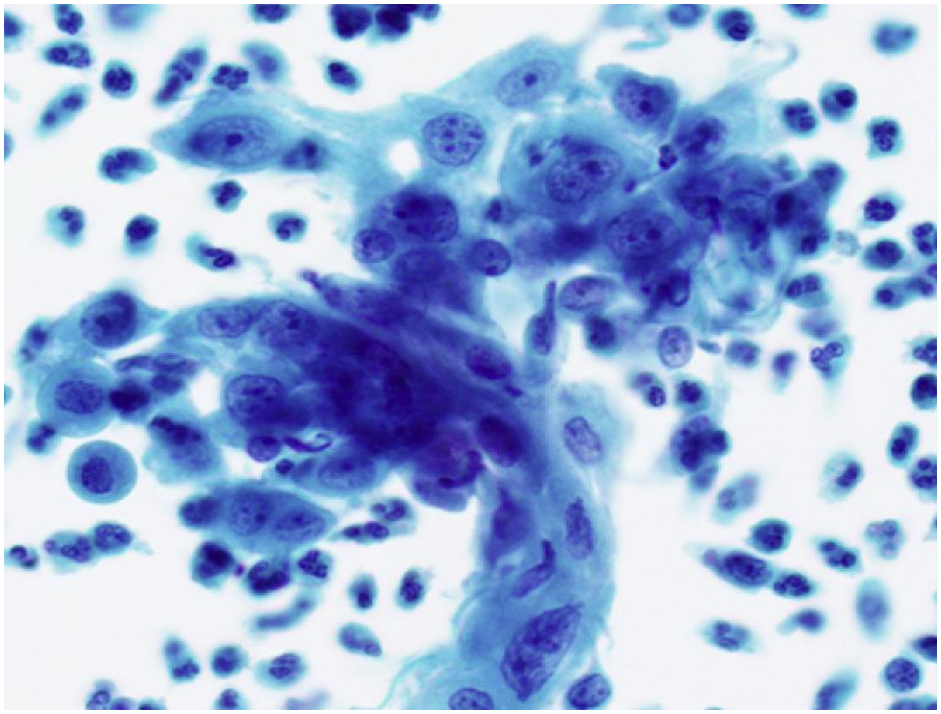
Atypical Reparative Changes





Nuclear atypia more marked

- Pronounced nuclear enlargement
- Anisonucleosis
- Nuclear contours more irregular
- Irregularity to the chromatin distribution
- No significant crowding
- Some streaming
- Mitotic figures can be seen (red arrow)



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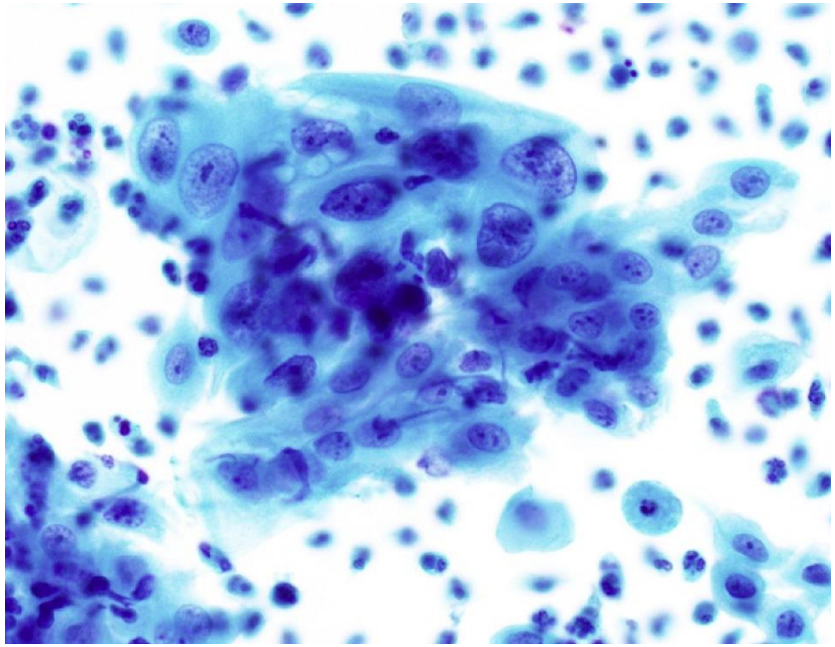
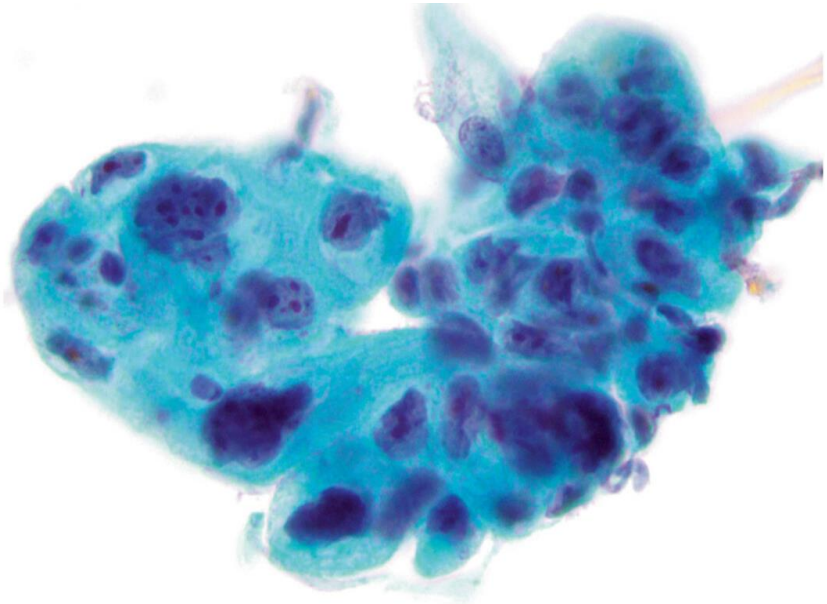
Differential for Repair:
Squamous Lesions

- Reparative changes can mimic both LSIL and HSIL
  - LSIL and HSIL lack nucleoli
- Given the prominent nucleoli, SqCC can be a major diagnostic consideration, however:
  - SqCC more discohesive
  - Background isolated malignant cells
  - More pronounced nuclear atypia
    - Dyskeratotic cells in keratinizing type, tadpole cells
    - Angulated nuclei
  - Necrotic background (tumor diathesis) rather than an inflammatory background

Repair vs. Poorly Differentiated Squamous Cell Carcinoma (SqCC)





Repair and it's lesional mimicker, poorly differentiated SqCC

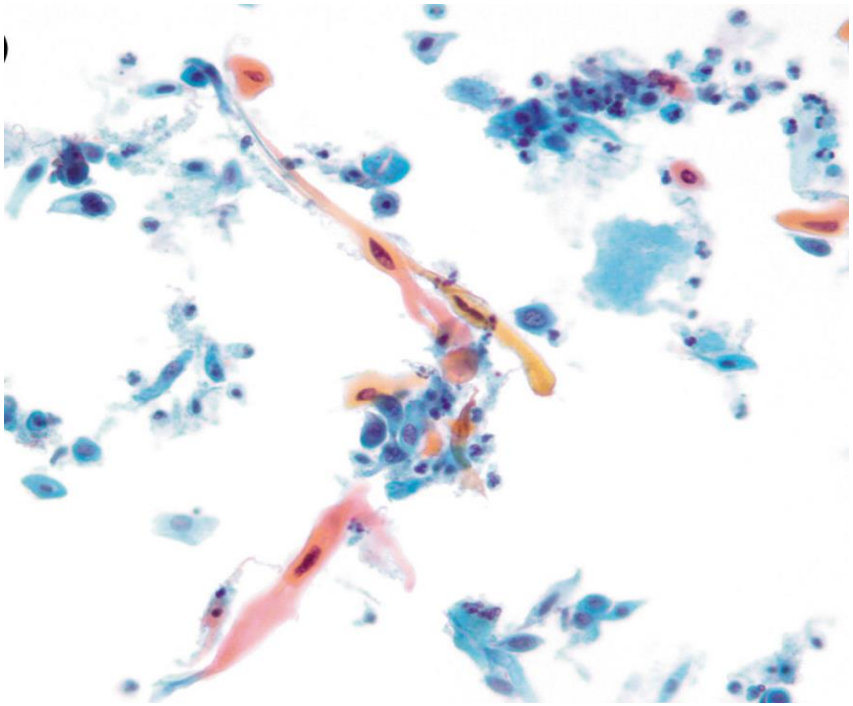
Top: Repair - prominent nucleoli but fine chromatin and smooth nuclear contours; acute inflammation; no tumor diathesis

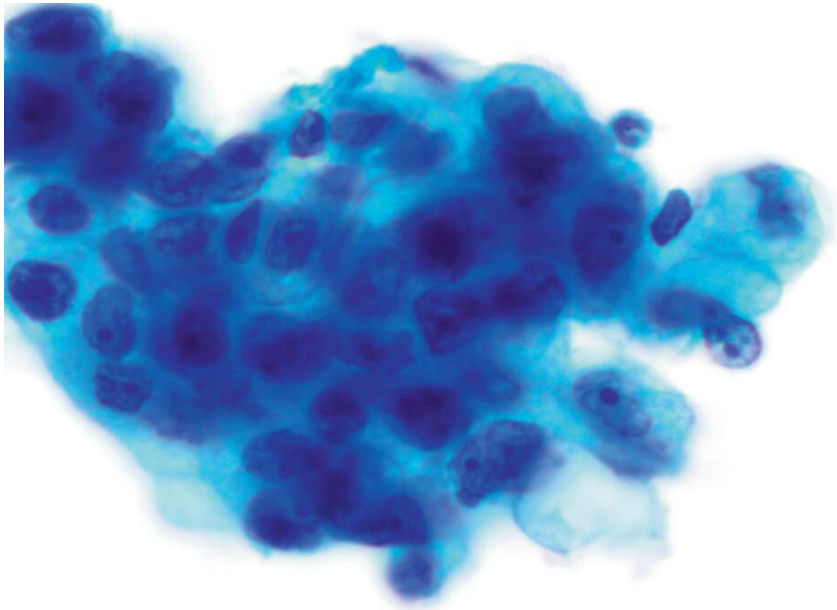
Bottom: Nonkeratinizing SqCC - marked nuclear atypia, enlargement, coarse chromatin and irregular nuclear contours; background necrosis is helpful if present

Torous VF. Challenging lesions in cervical cytology: The elusive HSIL. Cytopathology. 2024 Jan;35(1):48-59

Squamous Cell Carcinoma (SqCC)

- Single cells
- Marked atypia
- Atypical keratinization
- Angulated nuclei
- Tadpole cells
- Tumor diathesis





Torous VF. Challenging lesions in cervical cytology: The elusive HSIL. Cytopathology. 2024 Jan;35(1):48-59

Differential for Repair:
Glandular Lesions/AIS

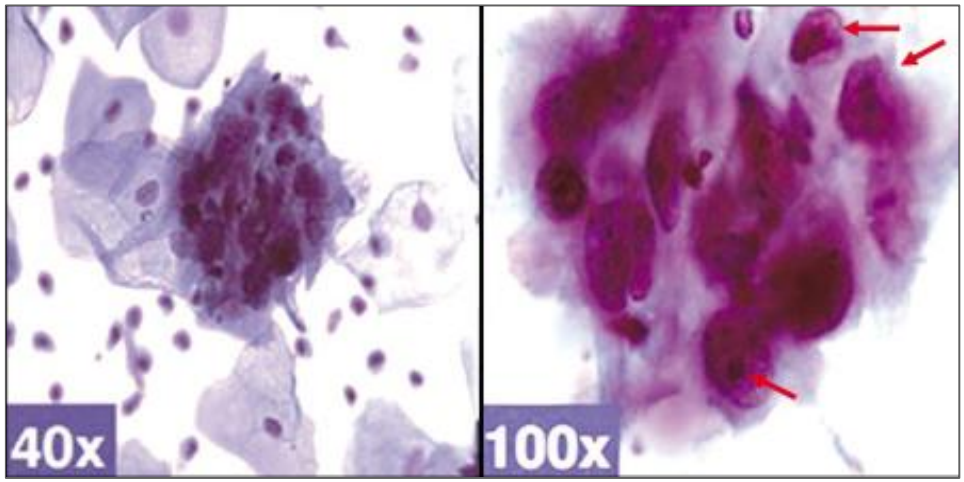
- Reparative changes in glandular cells can be nonspecific for the glandular lesion they mimic
- Endocervical AIS:
  - **Endocervical AIS** has notable **hyperchromasia**
  - **Coarser chromatin** texture
  - **Mitoses and apoptosis** more readily seen
- Repair:
  - **No prominent nucleoli**
  - **No rosettes, pseudostratification, or feathering**
  - **No prominent anisonucleosis**

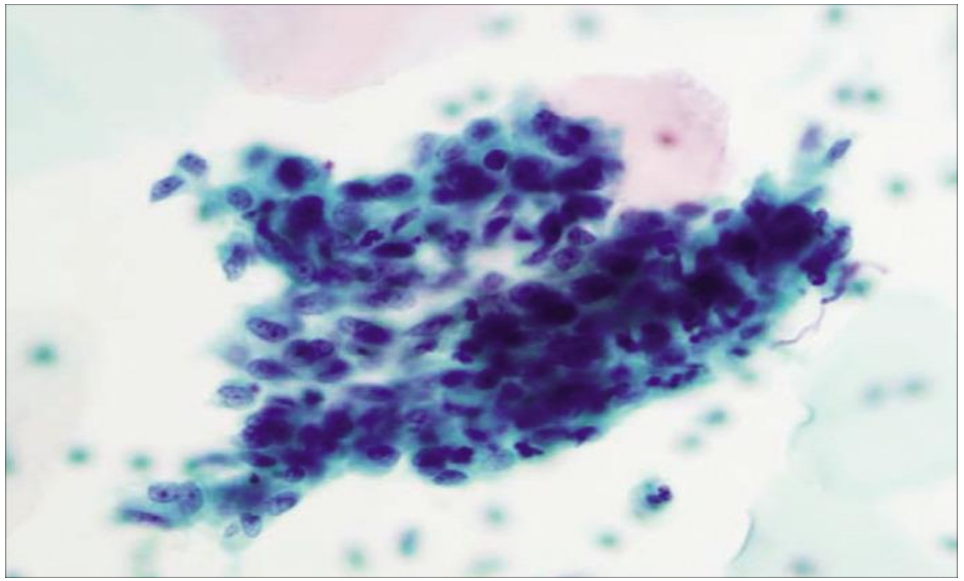
Atypical Repair vs. Endocervical AIS

Atypical repair and its lesional mimicker, endocervical AIS

Top: Atypical repair - HCG, nuclear pleomorphism, smudgy chromatin

Bottom: AIS - 3D, HCG, nuclear overlap, nucleoli





Khan MYA, Bandyopadhyay S, Alrajjal A, Choudhury MSR, Ali-Fehmi R, Shidham VB. Atypical glandular cells (AGC): Cytology of glandular lesions of the uterine cervix. Cytojournal. 2022 Apr 30;19:31

Diagnostic Approach to Differential of Repair

- Reparative changes can be a source of both FP and FN interpretations
- Streaming or pulled out architecture and nuclear nuclear chromatin texture favor reparative changes
- For challenging cases, an atypical category may be best for proper follow-up and treatment without overtreatment
- The Bethesda System (TBS) Categories:
  - Mild squamous atypia → atypical squamous cells of undetermined significance (ASC-US)
  - Marked squamous atypia raising concern for SqCC → atypical squamous cells cannot exclude an HSIL (ASC-H)
  - Glandular atypia → atypical endocervical cells not otherwise specified (AEC, NOS)

Glandular Abnormalities in Cervical Cytology

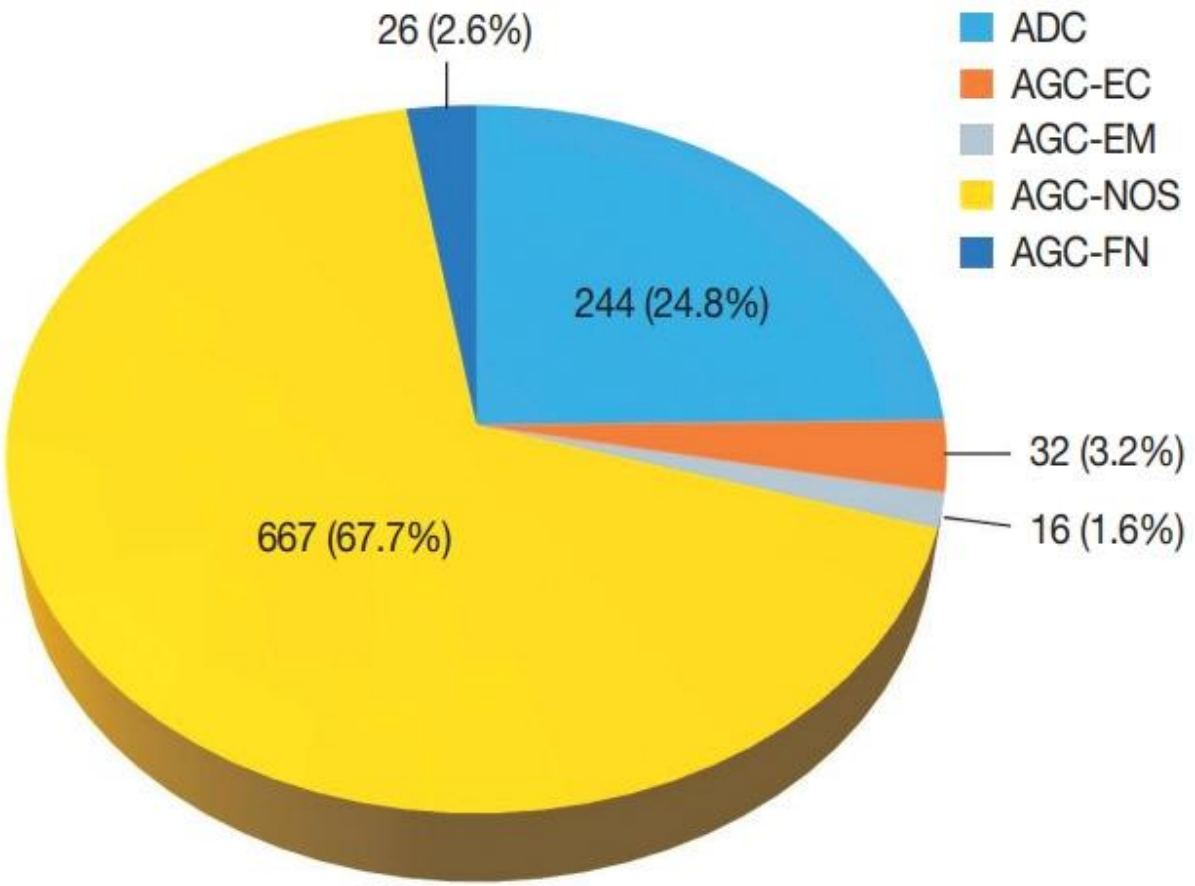
- Pap test is primarily considered a screening test for squamous cancer and its precursors
  - High specificity for squamous lesions
- Diagnostic accuracy of glandular cancers and their precursors is considered suboptimal in Pap tests
  - Relative rarity of glandular abnormalities
  - Sampling issue
  - Pathologists' lack of experience with cytomorphologic criteria
  - Reported interobserver variability
- Atypical glandular cells (AGC) is an uncommon cytologic interpretation used to interpret cases with glandular changes beyond reactive atypia that fall short of in situ/invasive carcinomas

Glandular Abnormalities in Cervical Cytology

- To reduce misinterpretation (discordance, FP, FNs) in Pap tests, it is necessary to identify:
  - Cytomorphologic differences between squamous and glandular abnormalities
  - Cytologic features observed in benign glandular lesions that could mimic malignancy
- AGC is often found to be a benign lesion or a squamous intraepithelial lesion rather than a glandular abnormality in follow-up histology
- HSIL with endocervical gland involvement could be misinterpreted as a glandular abnormality in cervical cytology

Distribution of Glandular Abnormalities in Cervical Cytology: 1995-2020

- Univ Hosp, Seoul Korea
- Of 683,197 Pap tests, **985** (0.144%) **glandular abnormalities**
- 923 patients: mean age, 49 years
- Histologic follow-up available in 657 cases (66.7%)



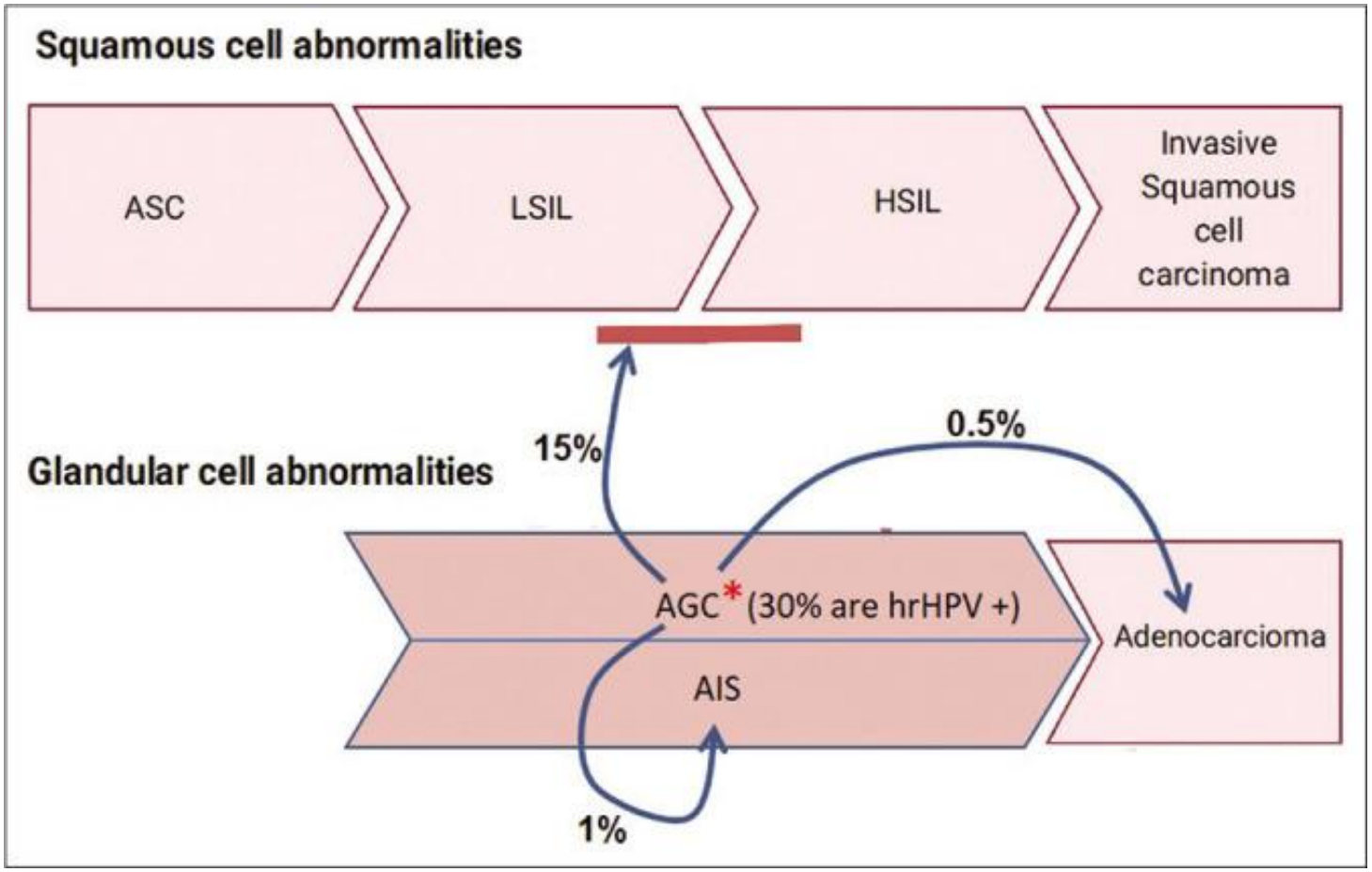
Sung JA, Nikas IP, Kim H, Ryu HS, Lee C. Diagnostic distribution and pitfalls of glandular abnormalities in cervical cytology: a 25-year single-center study. J Pathol Transl Med. 2022 Nov;56(6):354-360

Cytologic-Histologic Correlation of AGC

~30% of AGC are high-risk HPV (hrHPV) positive

On cyto-histo correlation ~15% are associated with LSIL or HSIL

1% are associated AIS and 0.5% with AdC



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Cyto-histologic Correlation of Glandular Abnormalities in Pap Tests

Of cases with histologic follow up:

- 188 (28.6%) fully concordant cases
  - Malignant in both cyto/histo
- 121 (18.4%) cases reported as AGC in cervical cytology
  - F/u revealed malignant tumors: majority EM, followed by cervix, and rare cases from ovary and metastatic sites
- 48 cases reported as AGC and 6 as ADC in cytology
  - follow-up histology - cervical squamous lesions including 4 squamous cell carcinomas
- Discordant cases
  - 213 reported as AGC and 9 as ADC in cytology
  - Follow-up histology benign

Sung JA, Nikas IP, Kim H, Ryu HS, Lee C. Diagnostic distribution and pitfalls of glandular abnormalities in cervical cytology: a 25-year single-center study. J Pathol Transl Med. 2022 Nov;56(6):354-360

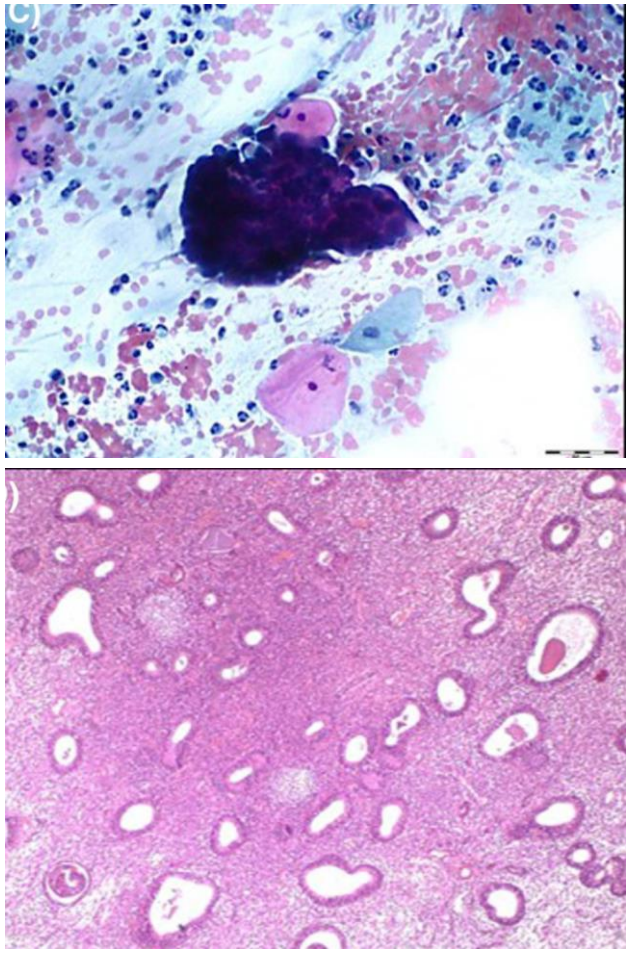
Pitfalls of Glandular Abnormalities in Cervical Cytology

- Non-neoplastic glandular entities that mimic malignancy
  - endometrial polyps, LUS sampling, tubal metaplasia, reactive change and microglandular hyperplasia
- Cytologic features in benign glandular lesions that mimic malignancy in cervical cytology
  - Crowded cellular groups
  - Hyperchromatic, overlapping nuclei
  - 3D cell clusters
- Most false-positive glandular lesions on Pap result from **endometrial polyps** (EMP) containing these features
- Second pathologist review, and experience can decrease the frequency of false-positive results

Yucel Polat A, Tepeoglu M, Tunca MZ, Ayva ES, Ozen O. Atypical glandular cells in Papanicolaou test: Which is more important in the detection of malignancy, architectural or nuclear features? Cytopathology. 2021 May;32(3):344-352

FP Glandular Lesions in Pap often from Endometrial Polyps

- Cytologic features in benign glandular lesions that mimic malignancy in cervical cytology
  - Crowded cellular groups
  - Hyperchromatic, overlapping nuclei
  - 3D cell clusters



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Endocervical AIS, AdC and Mimics

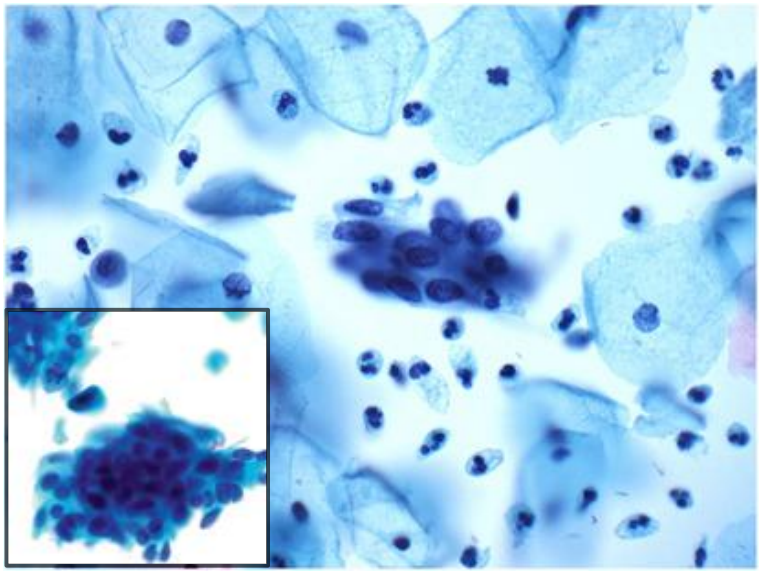
	AIS	INVASIVE ENDOCERVICAL ADENOCARCINOMA	TUBAL METAPLASIA	HSIL	DIRECTLY SAMPLED ENDOMETRIUM
Cellular groups	Yes	Yes	Yes	Yes	Yes
Pseudostratified strips	Yes	Yes	Yes	No	Yes
Feathering	Yes	Yes	No	No	No
Rosettes	Yes	Yes	No	No	No
Nuclear overlap	Yes	Yes	No	Yes	No
Coarse chromatin	Yes	Yes	No	Yes	No but can be with degeneration
Chromatin clearing	No	Yes	No	No	No
Mitoses	Yes	Yes	Rare	Yes	Yes in proliferative phase
Apoptosis	Yes	Yes	No	Yes	No
Organoid groups with attached stroma	No	No	No	No	Yes
Tumor diathesis	No	Yes	No	No	No

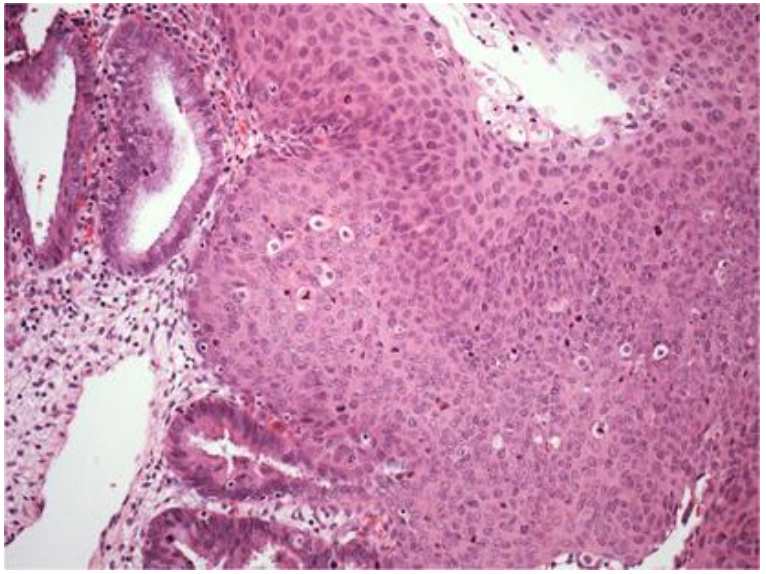
Practical issues related to uterine pathology: *in situ* and invasive cervical glandular lesions and their benign mimics: emphasis on cytology–histology correlation and interpretive pitfalls. Wilbur, David C. Modern Pathology, Volume 29, S1 - S11

Cytologic Mimics of Endocervical Neoplasia

HSIL:

- **Pap -** hyperchromatic crowded groups with focal columnar configurations when HSIL involves the endocervical glands
- **H&E -** HSIL involving endocervical gland





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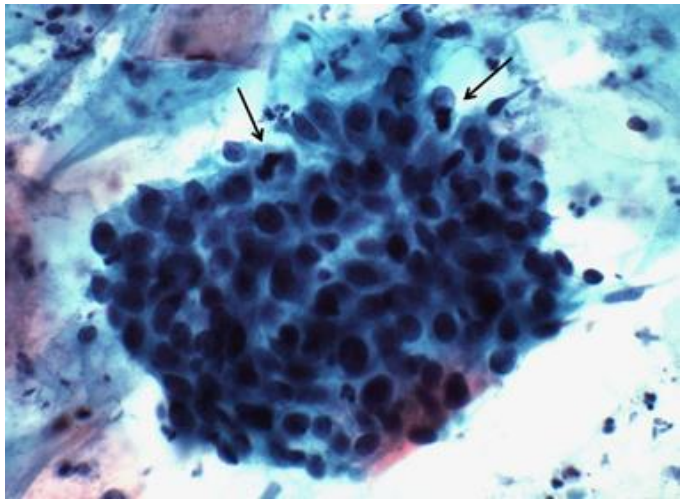
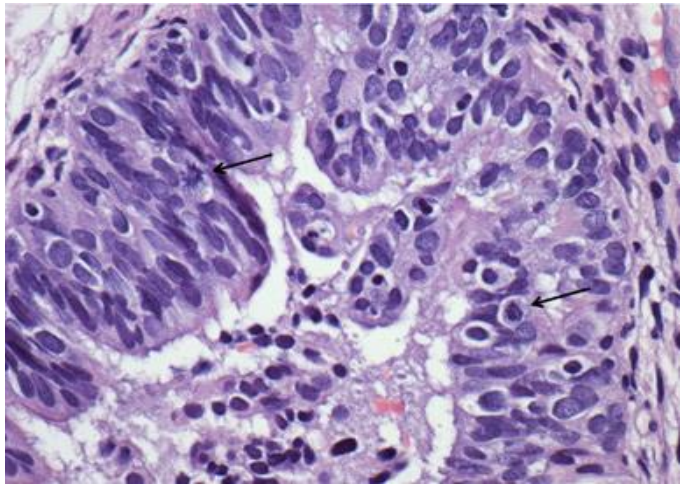
Cytologic Mimics of Endocervical Neoplasia

Tubal metaplasia:

- **Pap** - hyperchromatic crowded group with mitotic figures (arrows)
- **Pap** - chromatin pattern of the nuclei is smooth and evenly distributed in contrast to AIS
- **H&E** - several mitotic figures present (arrow)





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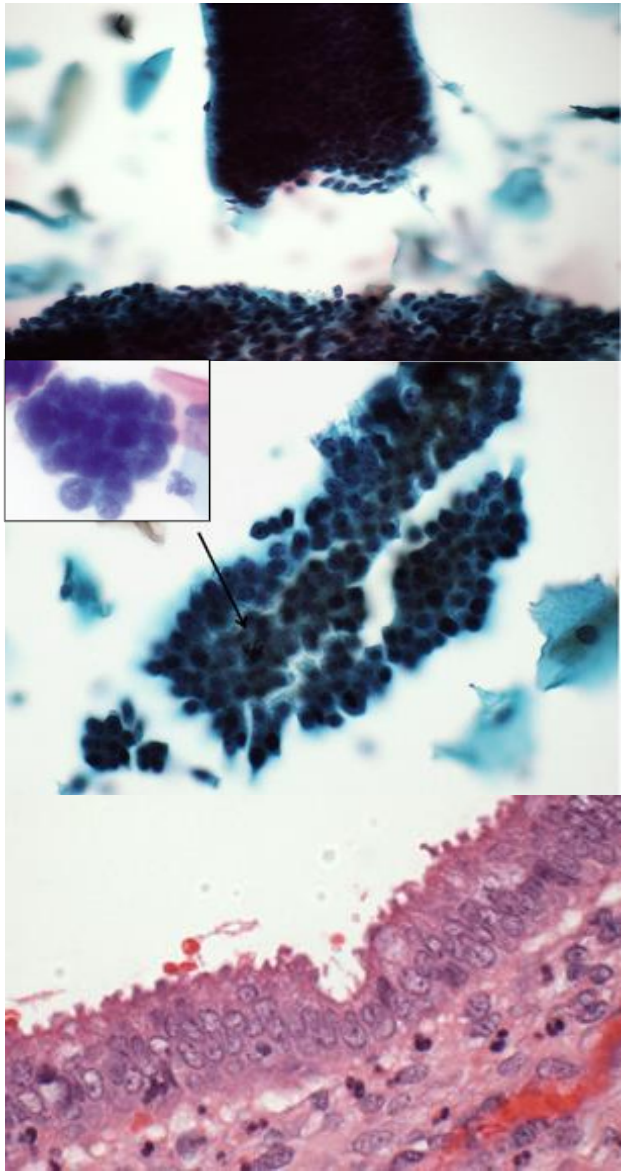
Cytologic Mimics of Endocervical Neoplasia

Pap – Directly Sampled Endometrium/LUS

- Sharply defined hollow organoid tube of endometrial epithelial cells; group of endometrial stromal cells at the bottom; usseful in differing from AIS
- Mitotic figures noted in the proliferative phase (arrow); note small nuclear size and the presence of endometrial stromal fragments

H&E – high endocervix/LUS

- Endocervical epithelium merges with LUS endometrial epithelium, it becomes pseudostratified and mucous cap is depleted
- Can cause over interpretations of atypical glandular cells because of their similarities with AIS



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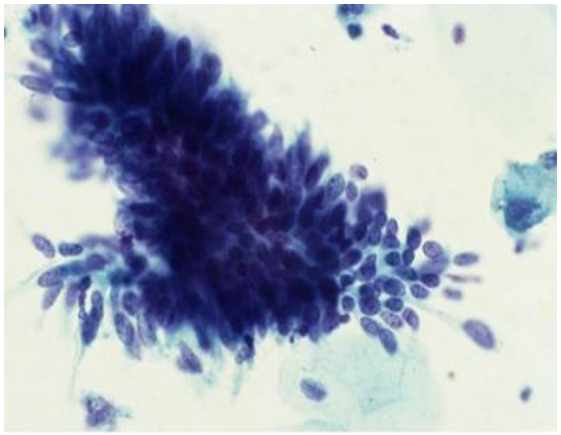
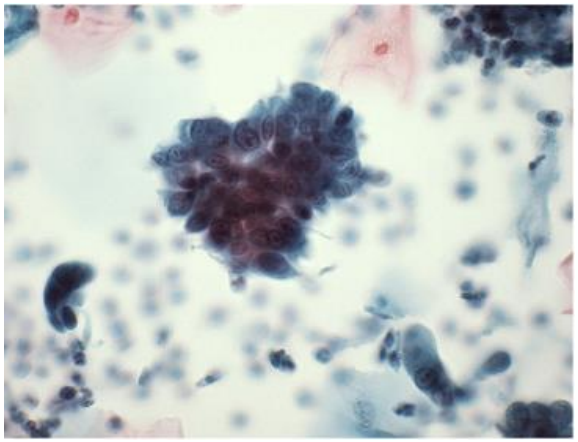
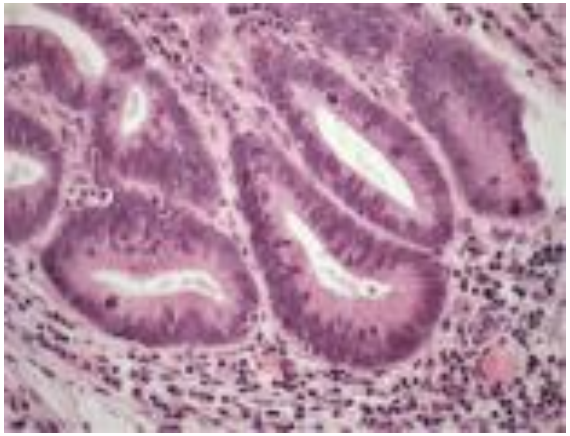
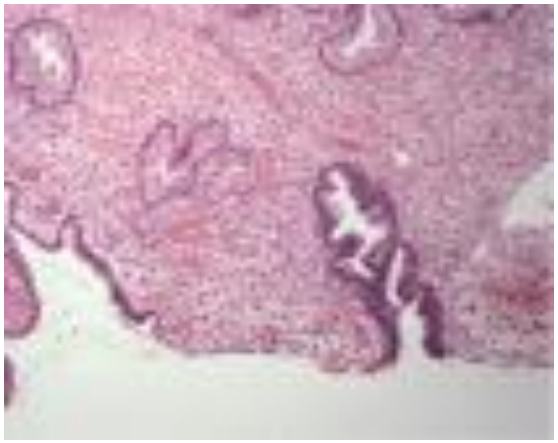
Endocervical AIS

- **Pap** - nuclear protrusion at the margins of the group => “feathering”
- **Pap** - coarse chromatin granularity and irregularity of the nuclear envelope
- **H&E** - pseudostratified nuclei with numerous mitotic figures present
- **H&E** - AIS involving a single gland. The gland in the lower right shows hyperchromatic epithelium in contrast to the normal endocervical epithelium lining the non-involved glands









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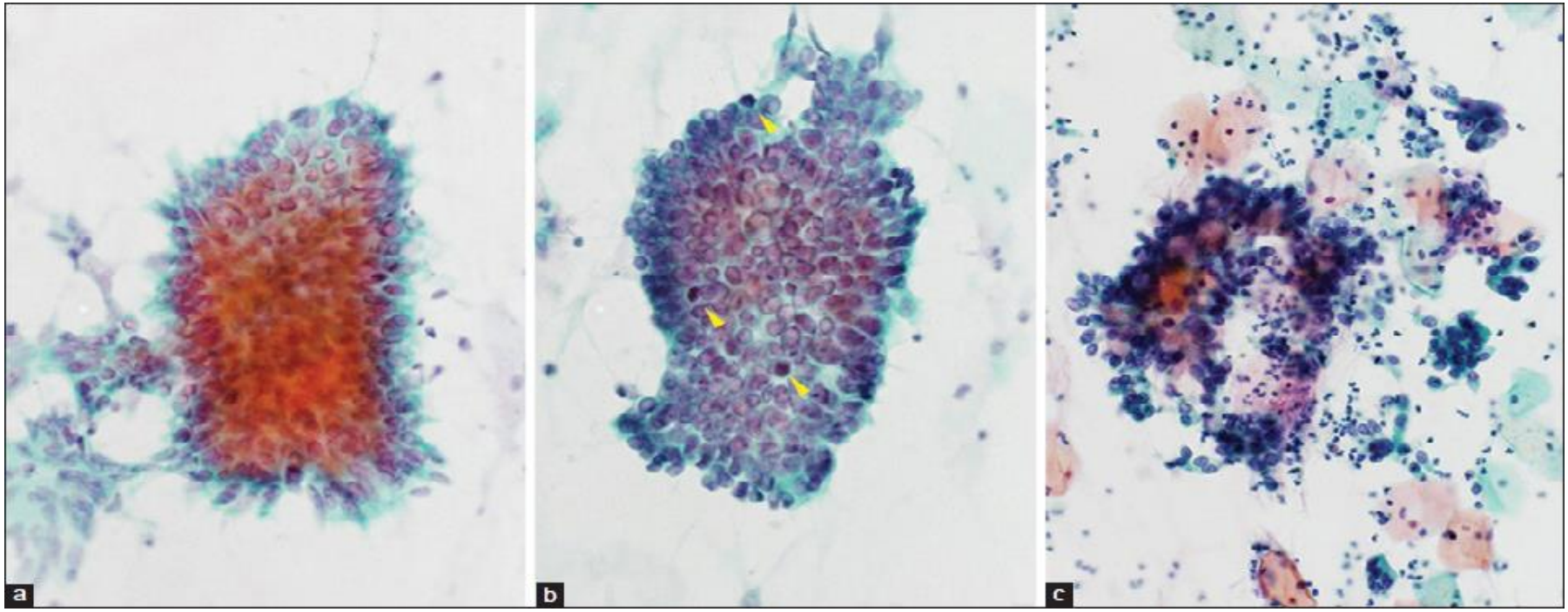
Adenocarcinoma In Situ – EC, EM, IT



AIS subtypes (Pap, conventional smear)

(a) Endocervical (EC) subtype - pseudostratified, crowding, feathering

(b) Endometrioid (EM) subtype - pseudostratified, crowding, mitotic figures

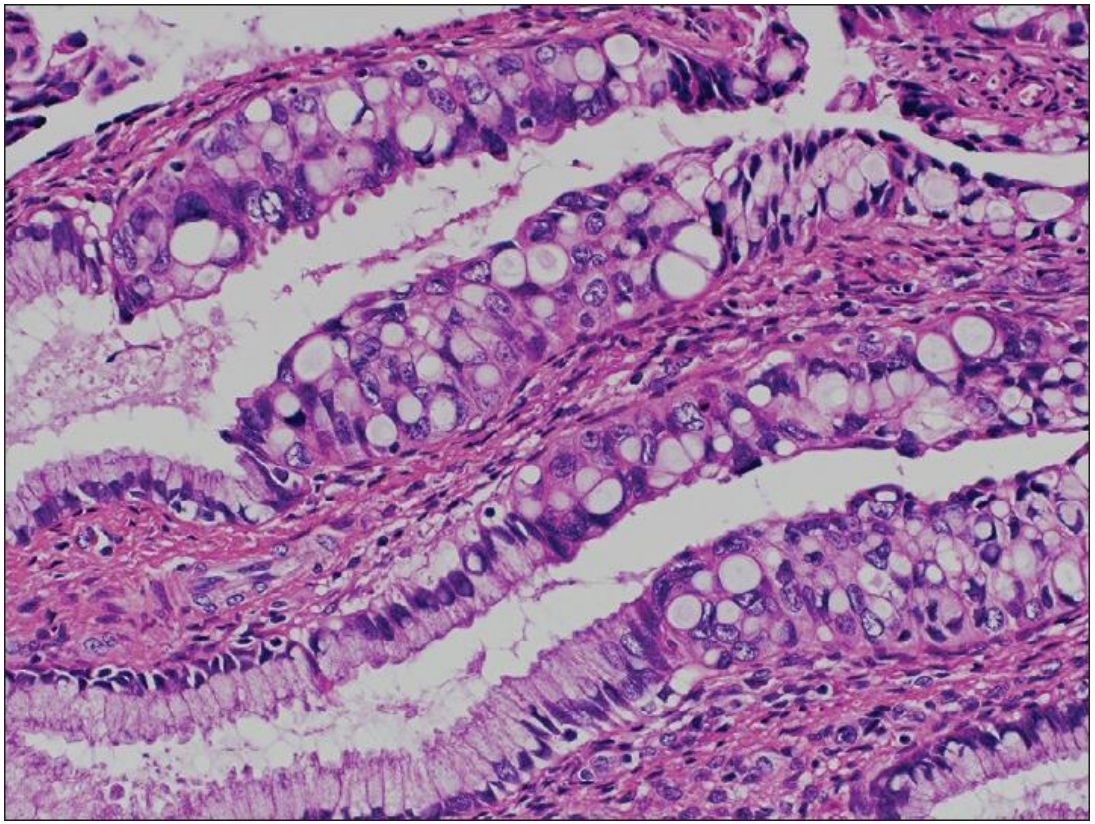
(c) Intestinal subtype (IT) - mucin droplets in the clusters (Goblet cells)

Umezawa T, Umemori M, Horiguchi A, Nomura K, Takahashi H, Yamada K, Ochiai K, Okamoto A, Ikegami M, Sawabe M. Cytological variations and typical diagnostic features of endocervical adenocarcinoma in situ: A retrospective study of 74 cases. Cytojournal. 2015 Apr 29;12:8

Adenocarcinoma In Situ – EC, IT

H&E, mixed AIS subtypes

- Endocervical subtype with columnar cells with abundant mucin, “picket fence”
- Intestinal subtype with intracytoplasmic mucin droplets (Goblet cells)
- Recognition of subtypes leads to improved initial Pap interpretation



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Cytomorphologic Findings in AGC Associated with Malignant Histology

- Review of 74 **AGC Pap** tests to identify cytomorphologic differences between the cases confirmed as benign and malignant by histology
- Features significantly associated with malignancy in f/u were:
  - irregular nuclear membranes
  - three-dimensional clusters
  - single-cell pattern
  - presence of mitoses
- In contrast, no significant association was identified with:
  - increased N/C ratio
  - nuclear hyperchromasia or overlap
  - prominent nucleoli (reactive change confounding)
- Concordant with reported literature

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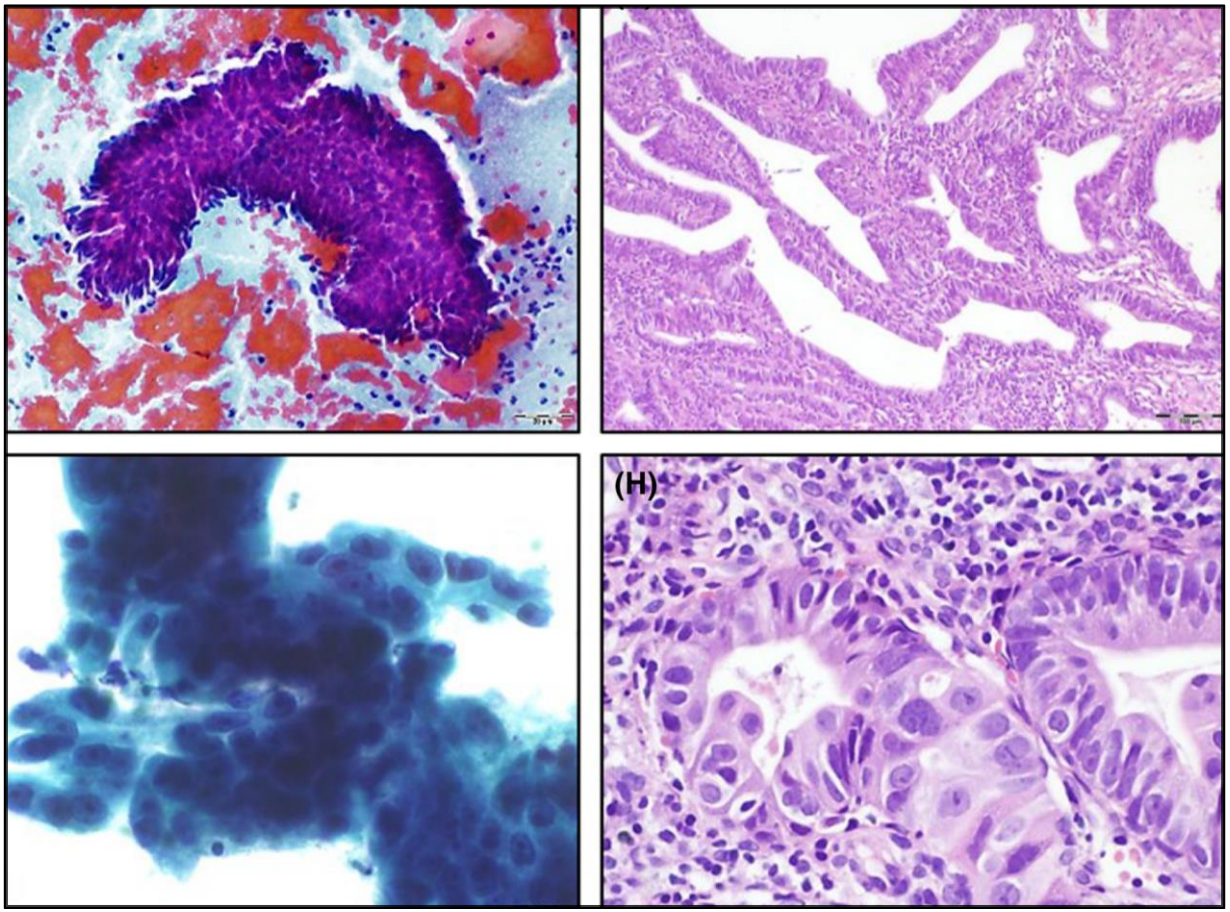
Cytomorphologic Findings in AGC Associated with Malignant Histology

Significant ($p < .05$):

- irregular nuclear membrane, macronucleoli, loss of polarity, papillary pattern, **3D formation**

Not significant:

- rosette formation, increased N/C ratio, and hyperchromasia
- **Paps** – AGC, FN
- **H&Es** – top: cervical AdC; bottom: serous endometrial AdC



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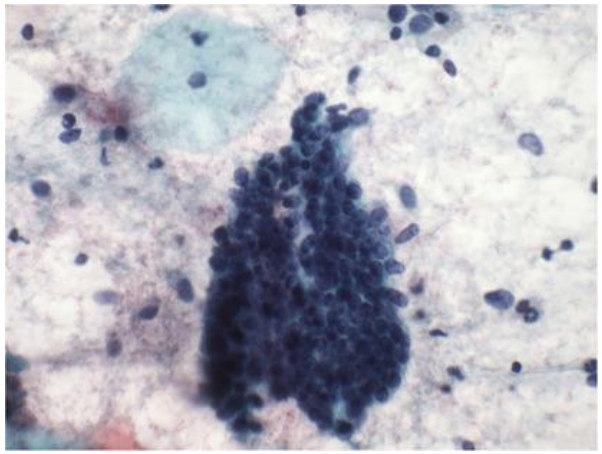
Invasive Endocervical Adenocarcinoma

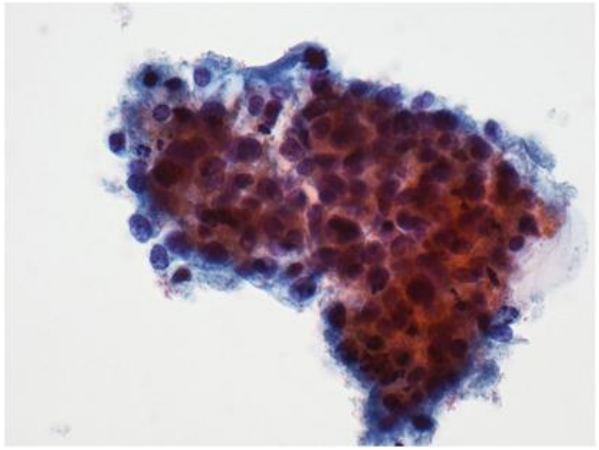
Top left - Endocervical adenocarcinoma, endometrioid variant

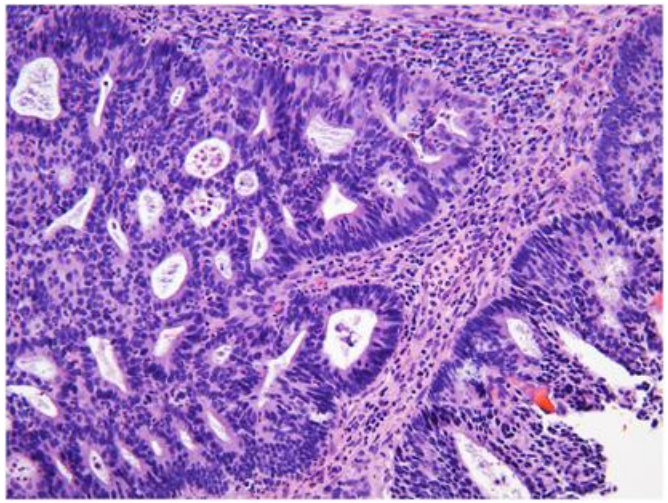
- Challenging due small size of the nuclei, feathering evident; misinterpreted as benign endometrial sampling or exfoliation

Top right - Endocervical adenocarcinoma, usual type; nuclear atypia is greater; prominent diathesis (necrosis, blood)

Bottom - Endocervical adenocarcinoma, usual type







Practical issues related to uterine pathology: *in situ* and invasive cervical glandular lesions and their benign mimics: emphasis on cytology–histology correlation and interpretive pitfalls. Wilbur, David C. Modern Pathology, Volume 29, S1 - S11

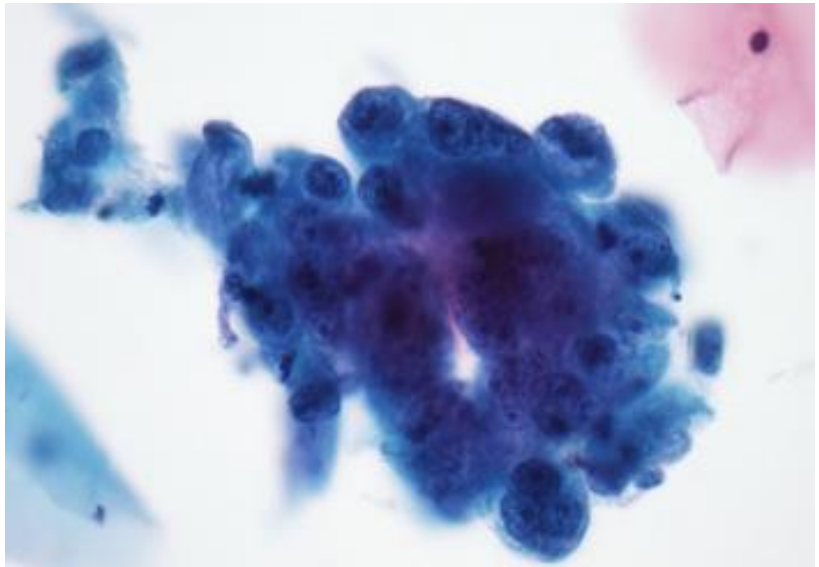
Invasive Endocervical Adenocarcinoma

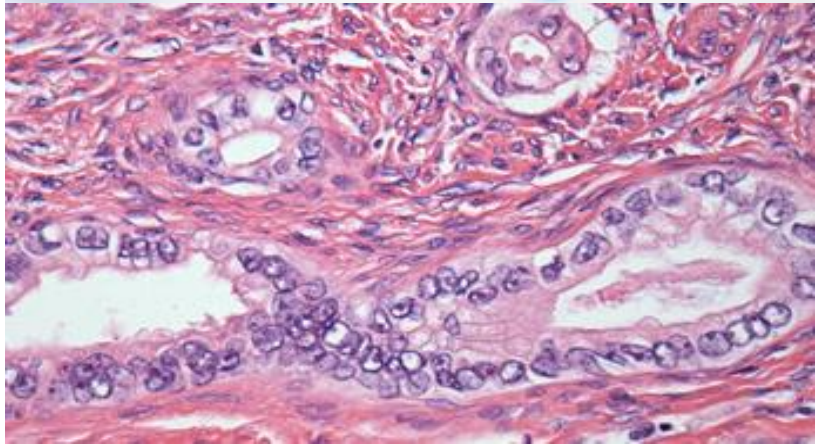
Pap - Mucinous endocervical adenocarcinoma

- Abundant frothy mucinous cytoplasm, architectural groups groups, marked nuclear atypia

H&E – Mucinous endocervical adenocarcinoma

- Atypical mucinous epithelial cells arranged in glandular units invading the cervical wall with little stromal reaction





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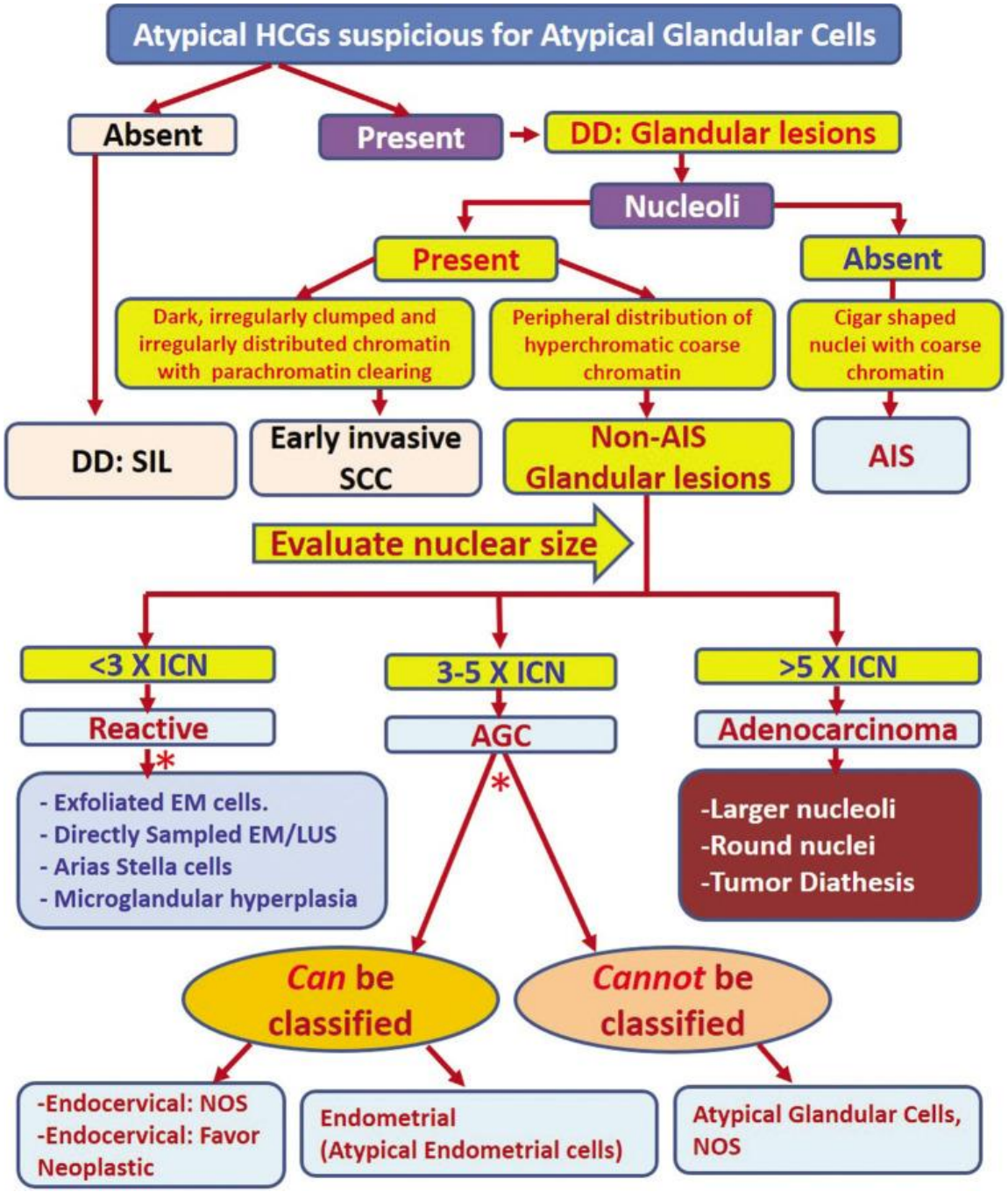
Approach to Glandular Lesions

Atypical hyperchromatic crowded groups suspicious for AGC



Khan MYA, Bandyopadhyay S, Alrajjal A, Choudhury MSR, Ali-Fehmi R, Shidham VB. Atypical glandular cells (AGC): Cytology of glandular lesions of the uterine cervix. Cytojournal. 2022 Apr 30;19:31

Endocervical Neoplasia: Approach to Differential Diagnosis

- Endocervical neoplasia is increasing compared to squamous
  - Relative and absolute incidence
  - Less likely to show symptoms or be colposcopically evident
  - Cytology and pHPV testing have important role in detection
- AGCs require colposcopy
  - Triage to colposcopy by HPV testing not recommended
  - Some endocervical carcinomas are HPV-negative (mucinous)
- Numerous **common cytologic mimics**
  - Lead to FP interpretations: TM, LUS
- Careful attention to differential diagnostic features helpful
- In challenging cases, best to classify as AGC to prompt further investigation

Wilbur DC. Practical issues related to uterine pathology: in situ and invasive cervical glandular lesions and their benign mimics: emphasis on cytology-histology correlation and interpretive pitfalls. Mod Pathol. 2016 Jan;29 Suppl 1:S1-11.

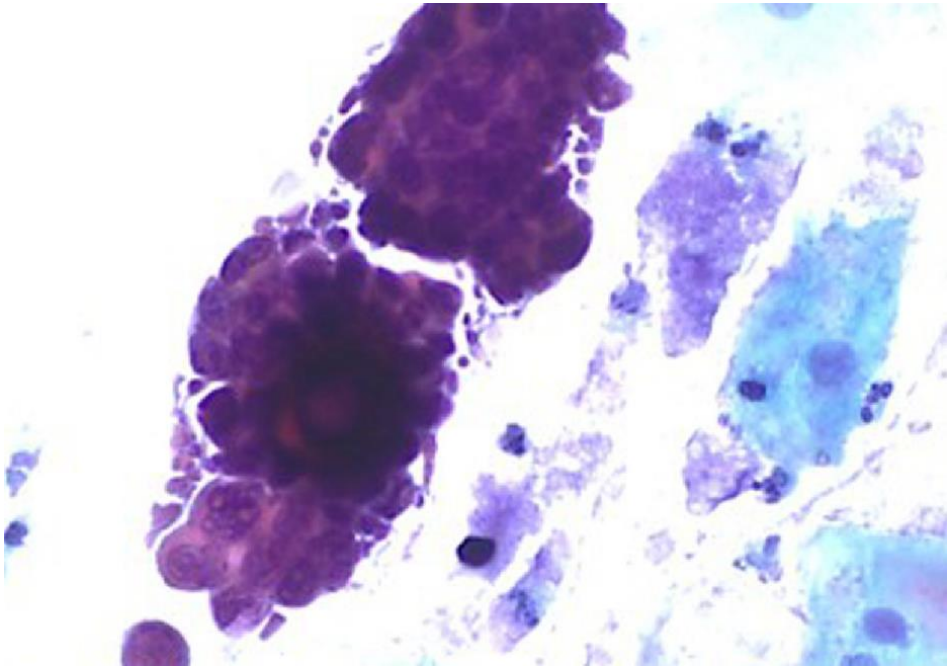
Cervical Cytology in the Diagnosis of Extrauterine Malignancies

- 104 (of 11,674) Paps bet 2019-23 from tertiary care center in India
- Most common extrauterine malignancy was adenocarcinoma (AdC)
  - Ovarian, colorectal, and vaginal
- Other epithelial malignancies
  - Urothelial carcinoma and breast carcinoma
- Non-epithelial malignancies
  - Melanoma, vaginal and cutaneous
  - Acute leukemia and anaplastic large cell lymphoma

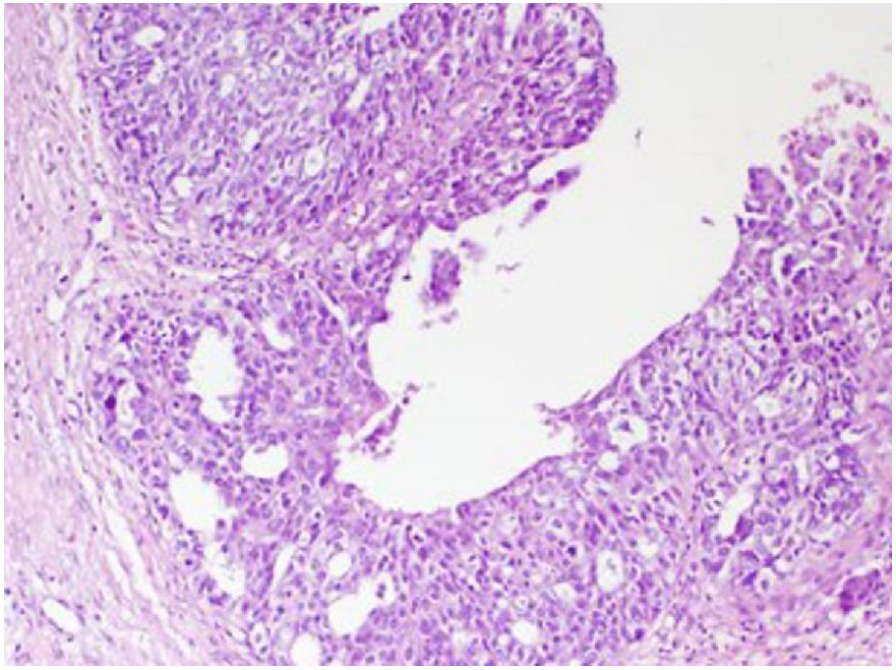
Tandon A, Kaur K, Shah M, Trivedi P. Role of Pap Smear Cervical Cytology in the Diagnosis of Extrauterine Malignancies: Largest Study of 104 Cases from Tertiary Care Cancer Centre in India. Acta Cytol. 2024;68(4):319-326

Ovarian Carcinoma in Cervicovaginal Cytology

Papillary formation with psammoma body and clean background



Serous carcinoma of ovary



Yucel Polat A, Tepeoglu M, Tunca MZ, Ayva ES, Ozen O. Atypical glandular cells in Papanicolaou test: Which is more important in the detection of malignancy, architectural or nuclear features? Cytopathology. 2021 May;32(3):344-352

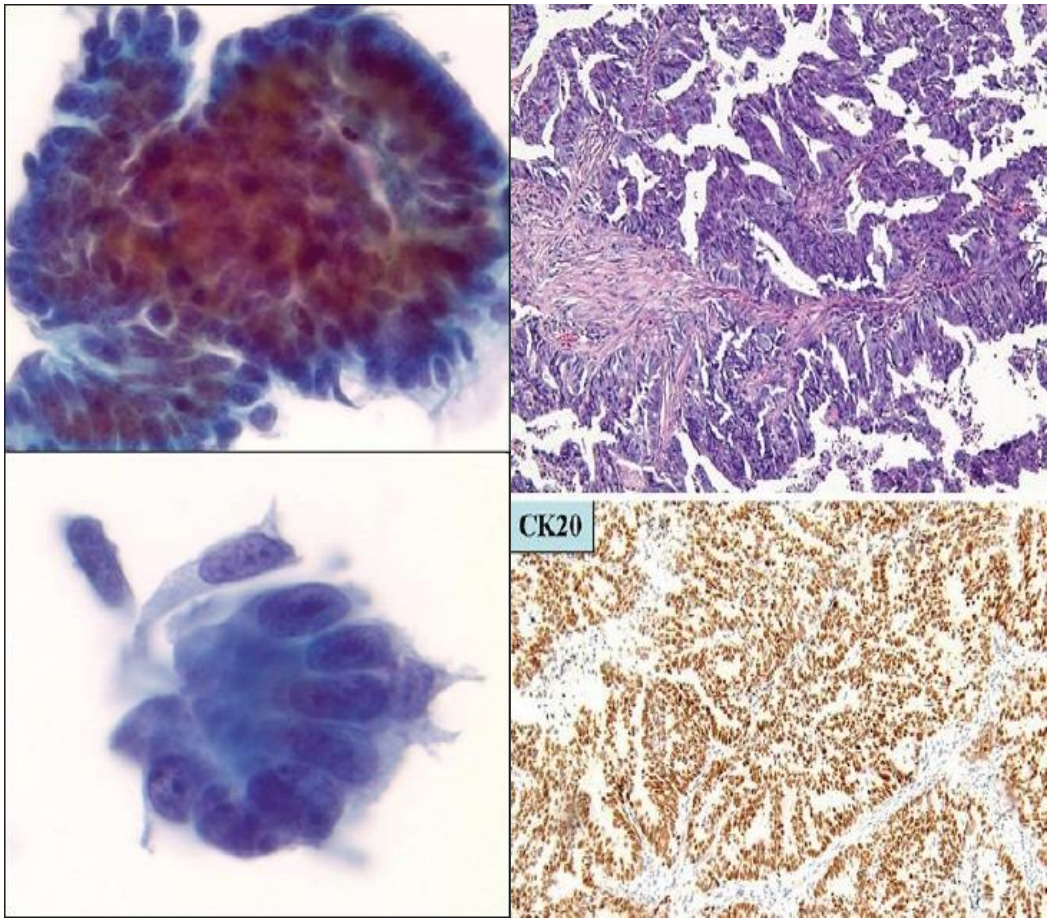
Metastatic Colorectal Adenocarcinoma in Cervicovaginal Cytology

Pap

- Groups of columnar cells
- Picket fence arrangement
- Increased nuclear to cytoplasmic ratio
- Nuclear pleomorphism
- Hyperchromasia
- Prominent nucleoli

Histology/IHC

- AdC positive for CK20/CDX2



Zulfiqar M, Liu S, Shi D, Madan S, Jacques S, King L, Shidham V, Giorgadze T. Metastatic colorectal adenocarcinoma in cervicovaginal cytology specimens confirmed by immunocytochemical stains on liquid base specimens: Two study cases with review of the literature. Cytojournal. 2013 May 20;10:9

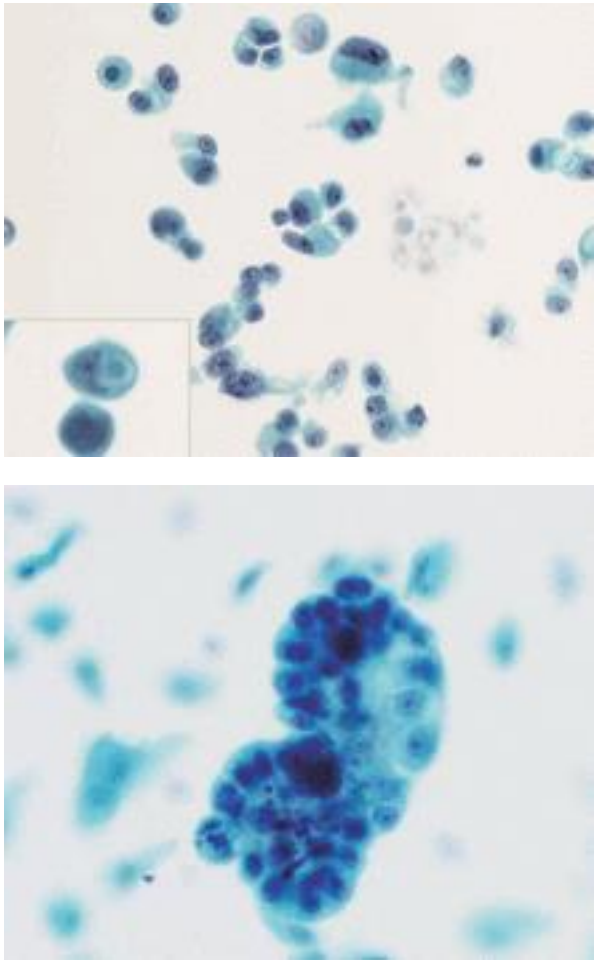
Metastatic Breast Carcinoma in Cervicovaginal Cytology

Top Pap with metastatic lobular carcinoma

- Highly cellular
- Single cell population; single file arrangement
- Eccentrically placed nuclei
- Cytoplasmic vacuoles with intracytoplasmic round bodies

Bottom Pap with metastatic ductal carcinoma

- 3D clusters and
- Prominent nucleoli

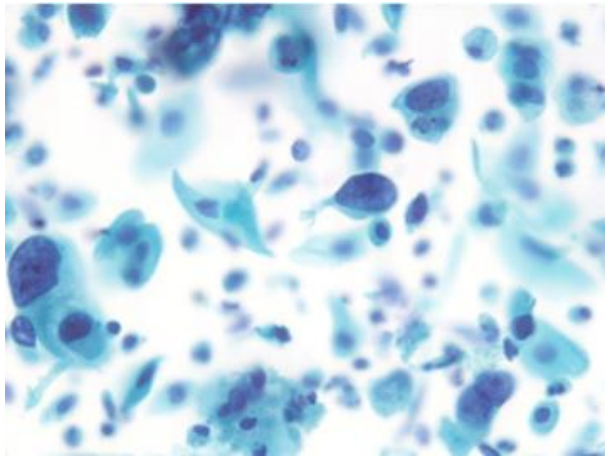


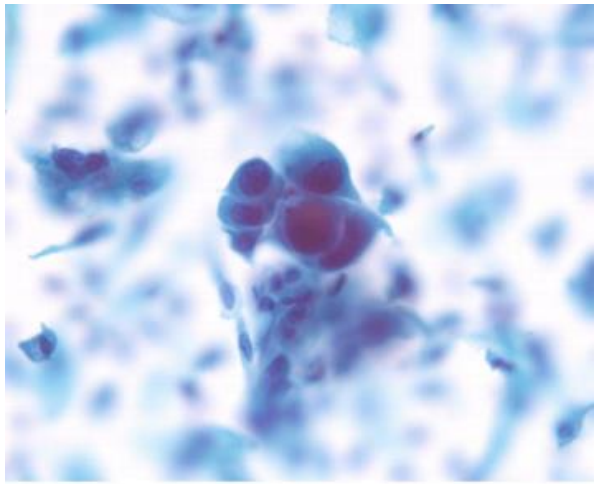
Gupta, N., Dudding, N. and Smith, J.H.F. (2013), Cytomorphological features of extra-genital metastases in SurePath™ cervical liquid-based cytology: a series of eight cases. Cytopathology, 24: 123-128

Metastatic Urothelial Carcinoma in Cervicovaginal Cytology

High Grade Urothelial Carcinoma

- Single atypical cells
- Large nuclei
- Anisonucleosis
- Hyperchromasia
- Dense cytoplasm
- Small groups of malignant cells with large, hyperchromatic nuclei and dense cytoplasm





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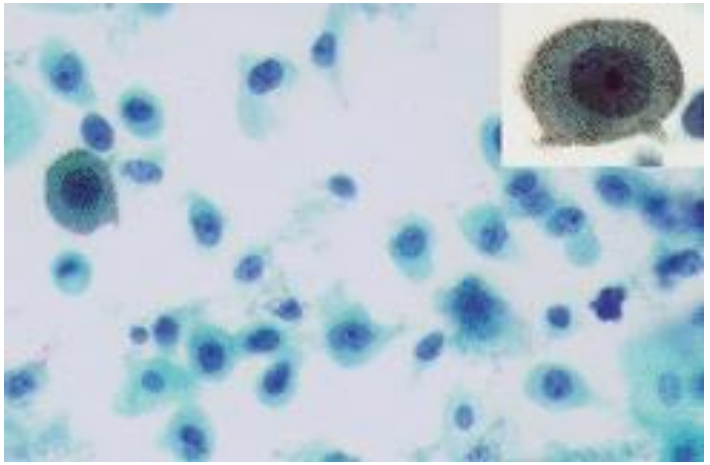
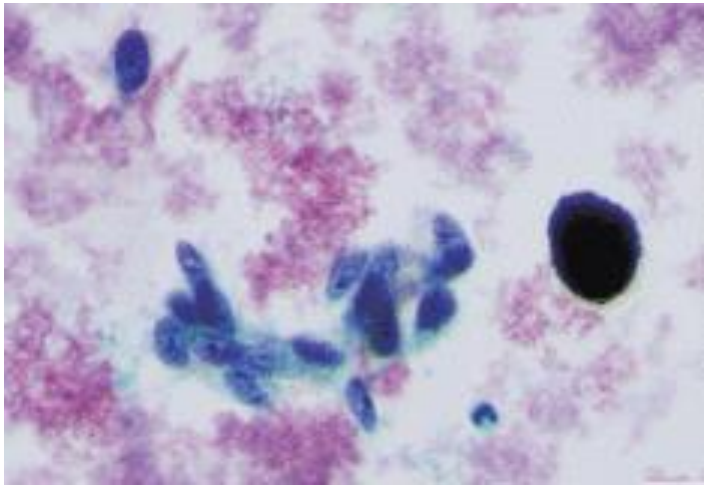
Metastatic Melanoma in Cervicovaginal Cytology

- Single cell population of round to plasmacytoid cells
- intracytoplasmic melanin (inset)
- cluster of oval- to spindle-shaped cells
- conspicuous nucleoli
- pigment-laden histiocytes
- hemorrhagic background





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Conclusions

- Select benign and reactive processes in cervical cytology were described that while common are particularly problematic given their morphologic overlap with various neoplastic processes
- Select glandular entities were described with histologic correlates spanning a broad differential from benign to malignant
- Morphologic clues to the potential pitfalls and suggestions for how to approach the interpretation of these challenging cases were reviewed

Conclusions

- Knowledge of these diagnostic pitfalls and the differential diagnosis can help to avoid misinterpretation
- Quality control review and cyto-histo correlation of Pap tests with FP and FN diagnoses is important in the cytology laboratory, and must be considered an educational and experience-building procedure and opportunity

Conclusions

- Awareness of pitfalls in cervical cytology is important for cytopathologists and clinicians to avoid errors in both under and over interpretation leading to over or under treatment
- Review of challenging cases with experienced Cytopathologists is important for best classification in significant diagnoses