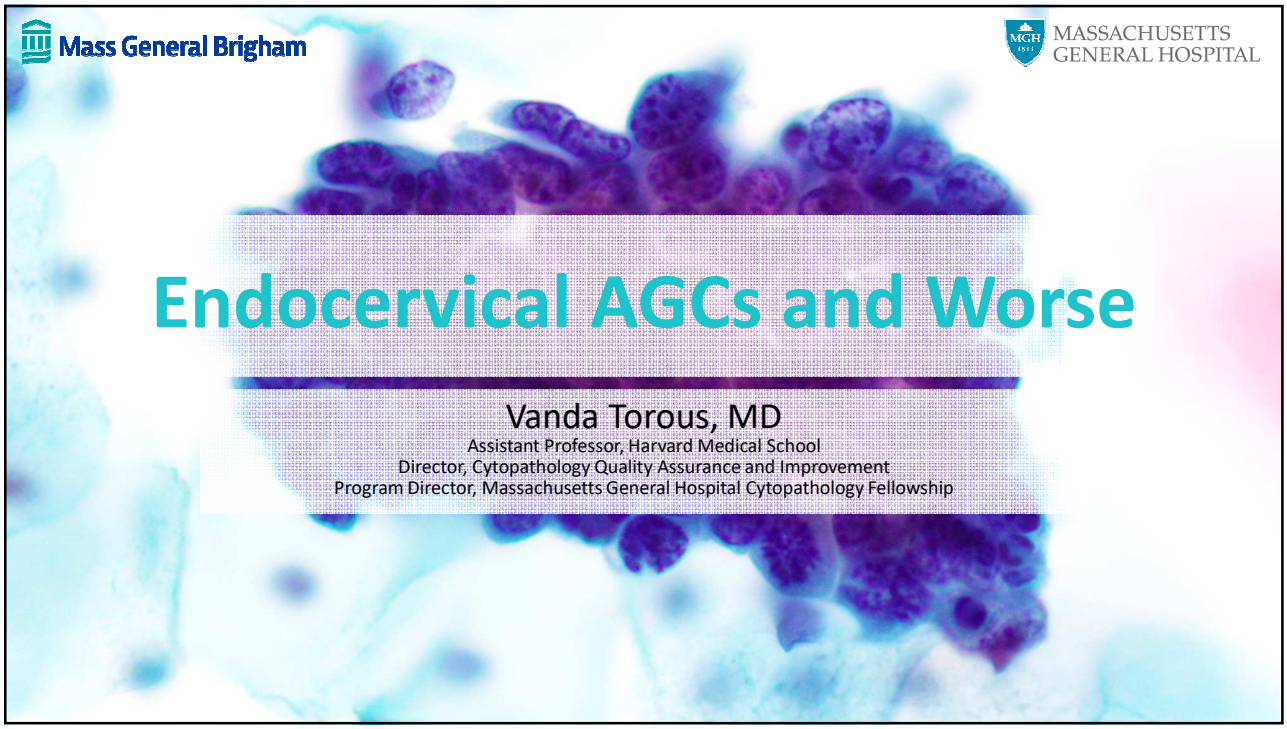


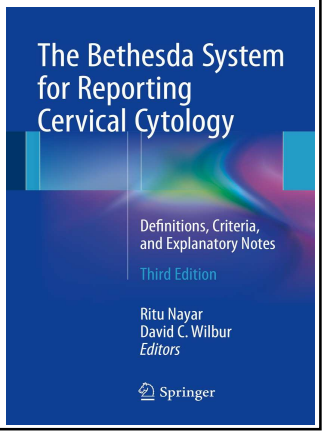

## Background

- Incidence of endocervical adenocarcinomas has increased, ~20-25% of cervical cancers
- Strength of the Pap test is with detection/diagnosis of squamous precursor lesions
  - Have not had the same level of success with glandular lesions
  - Sensitivity rates have increased from 45-76% to 88-92% but the false-negative rate of EA/AIS remains significantly higher relative to high-grade squamous lesions
- Several factors contribute to difficulty in detecting glandular lesions
- Methodical application of diagnostic criteria facilitates improved interpretation of glandular cell abnormalities

# Reporting Glandular Lesions using The Bethesda System

Epithelial cell abnormalities: Glandular Cell

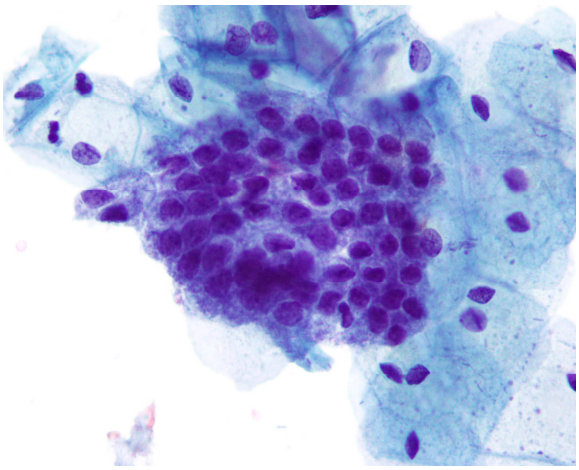
- Atypical
  - Endocervical cells (NOS or specify in comments)
  - Endometrial cells (NOS or specify in comments)
  - Glandular cells (NOS or specify in comments)
- Atypical
  - Endocervical cells, favor neoplastic
  - Glandular cells, favor neoplastic
- Endocervical adenocarcinoma in situ (AIS)
- Adenocarcinoma
  - Endocervical
  - Endometrial
  - Extrauterine
  - NOS

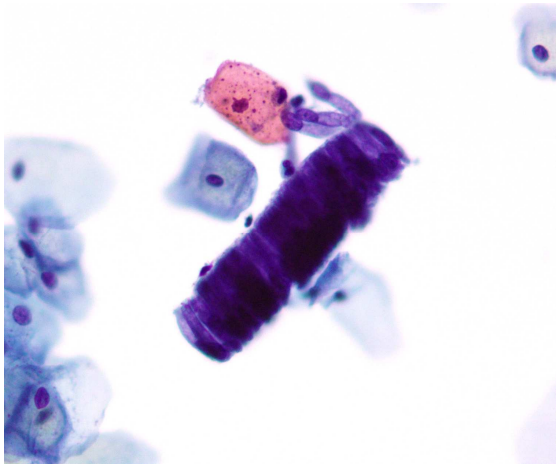



# Differential Diagnosis of "Glandular" Atypia

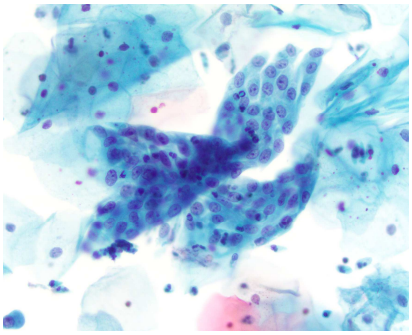
- Nonneoplastic
  - Reactive / nonspecific atypia
  - Lower uterine segment sampling
  - Menstrual endometrium
  - Tubal metaplasia
  - Intrauterine device effect
  - Endocervical / endometrial polyps
  - Radiation
  - Arias-Stella (pregnancy) change
  - Microglandular hyperplasia
- Neoplastic
  - High grade squamous intraepithelial lesion
    - HSIL involving endocervical glands
  - Endocervical adenocarcinoma in situ
  - Endocervical adenocarcinoma
  - Endometrial adenocarcinoma
  - Metastatic carcinoma

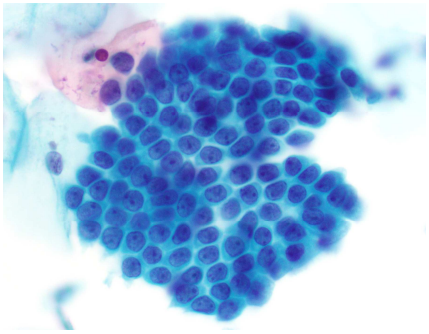
## Normal Endocervical Cells

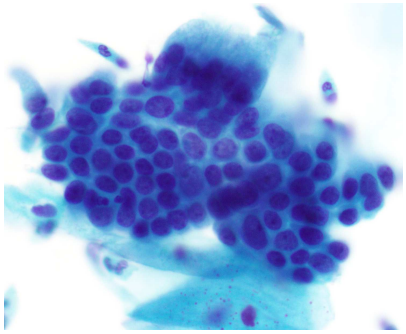


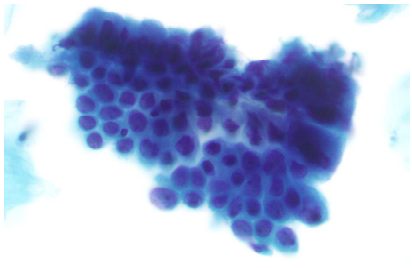


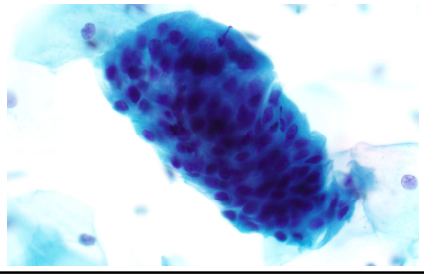
## Normal Endocervical Cells

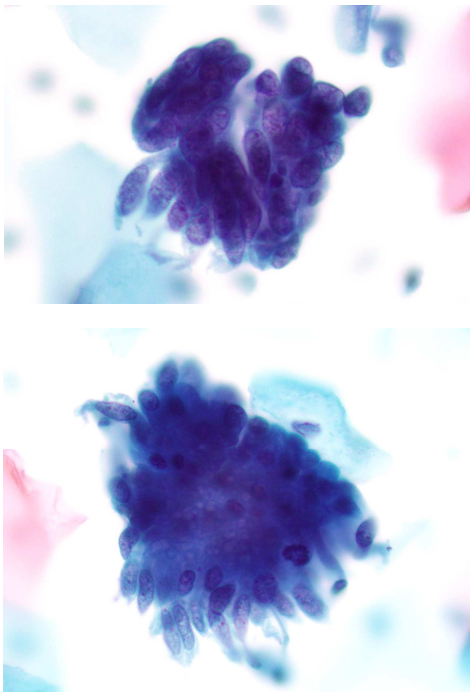


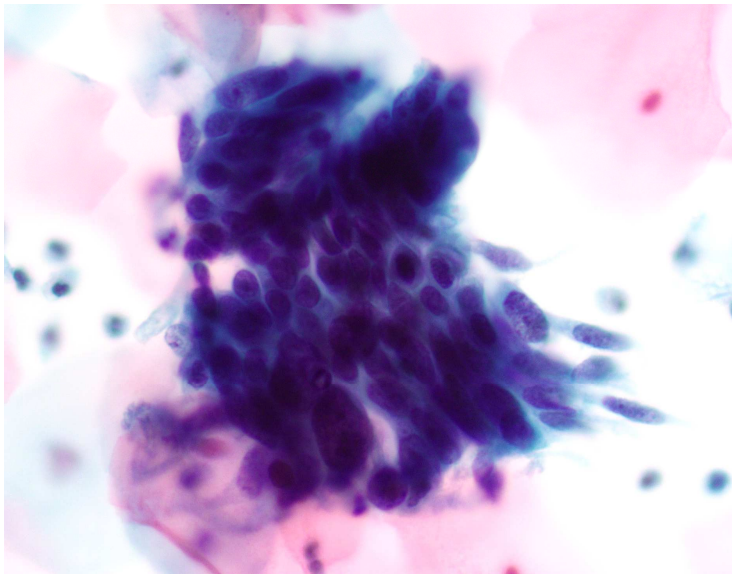






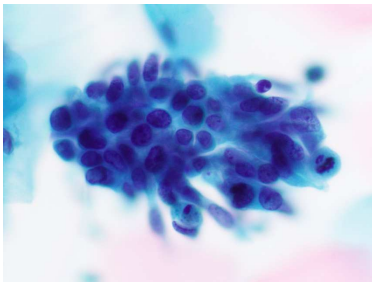


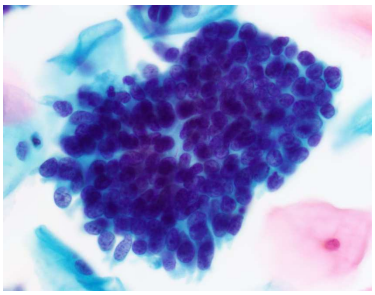




## Endocervical Adenocarcinoma In Situ

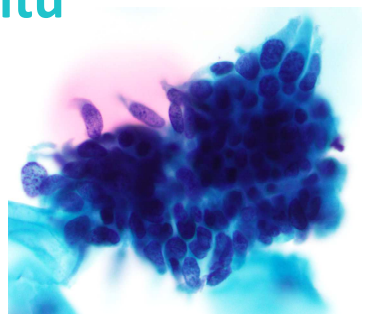
- The known precursor to endocervical adenocarcinoma
  - Women with AIS are on average 13 years younger than those with adenocarcinoma (39 vs 52 yo)
  - Morphologically similar and often found adjacent on histologic sections
  - HPV 16 and 18 are identified in similar proportions
- There has been a steady increase in the diagnosis of AIS
- Remains a diagnostically challenging lesion
  - Partially due to relatively low incidence: AIS 1.25/100,000 vs SCC in situ 44.4/100,000 (~1 case of AIS for every 36 cases of HSIL)

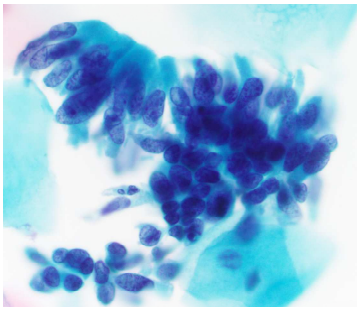


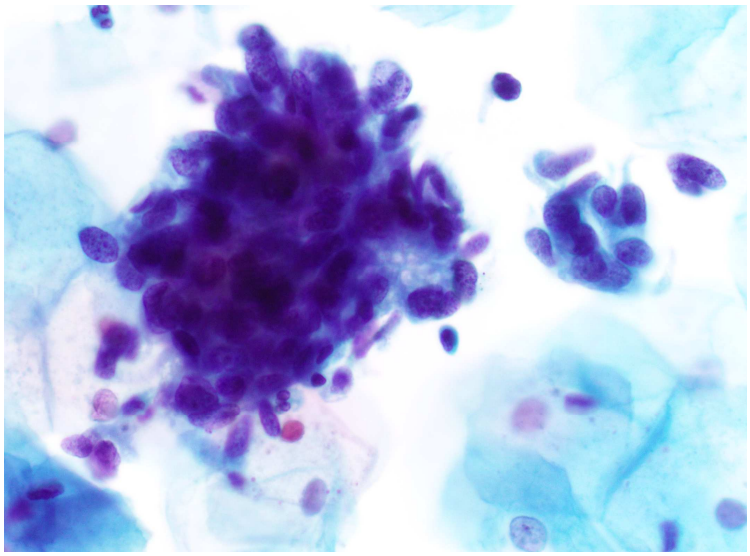


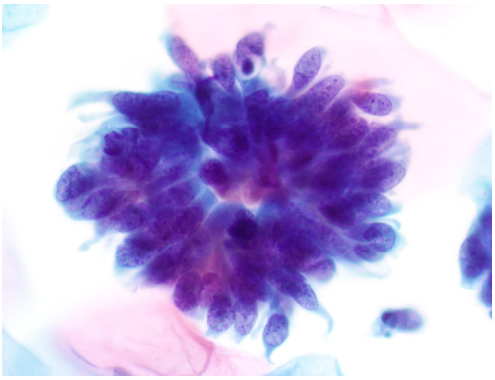
# Endocervical Adenocarcinoma In Situ

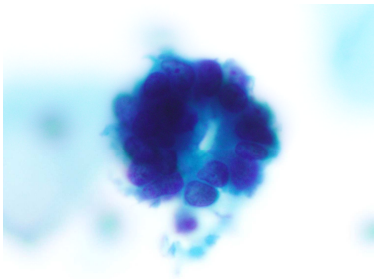
- Arranged in sheets, pseudostratified strips, and rosettes
- Glandular differentiation: columnar cells; peripheral feathering
- Oval or elongated nuclei with enlargement, size variation
- Hyperchromatic, evenly dispersed chromatin
- Nucleoli small/inconspicuous
- Increased N:C
- Mitoses, apoptoses common
- Clean background
- Variants (uncommon): mucinous, intestinal, clear cell, endometrioid

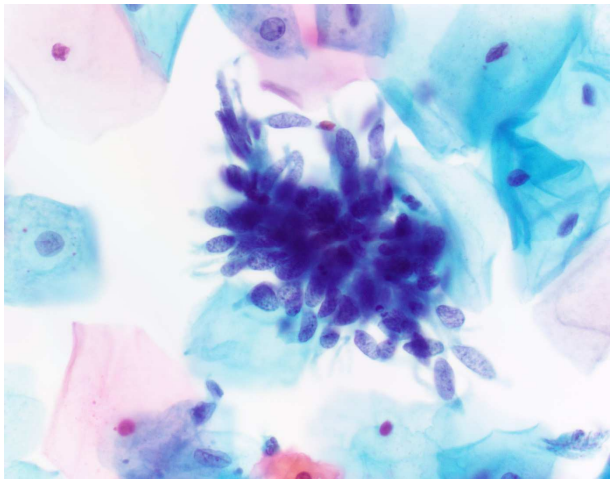


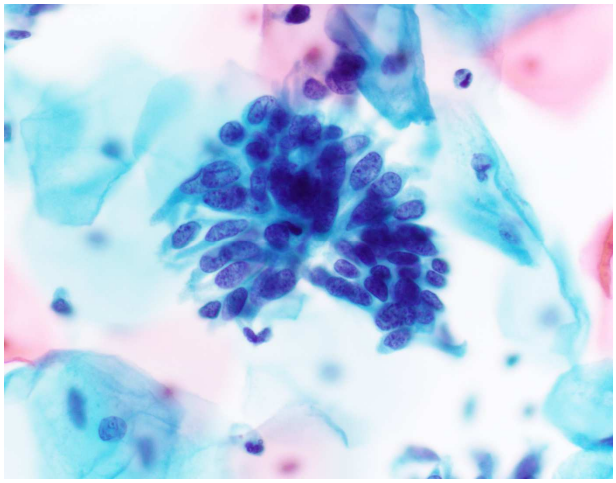










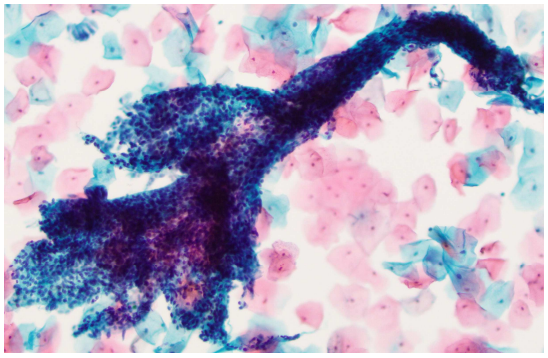


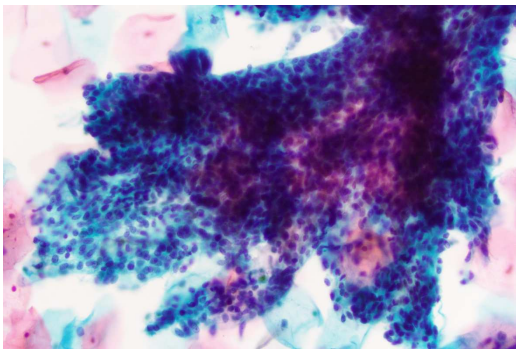
## Endocervical Adenocarcinoma In Situ

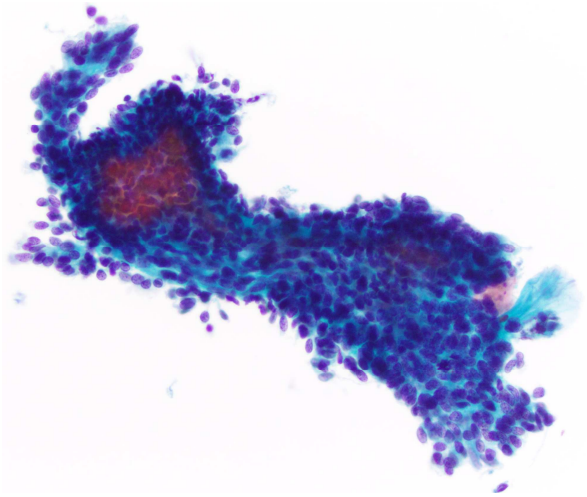
- Benign mimics
  - Lower uterine segment
  - Menstrual endometrium
  - Tubal metaplasia
- Neoplastic mimics
  - HSIL
  - Endocervical adenocarcinoma
  - Endometrial adenocarcinoma

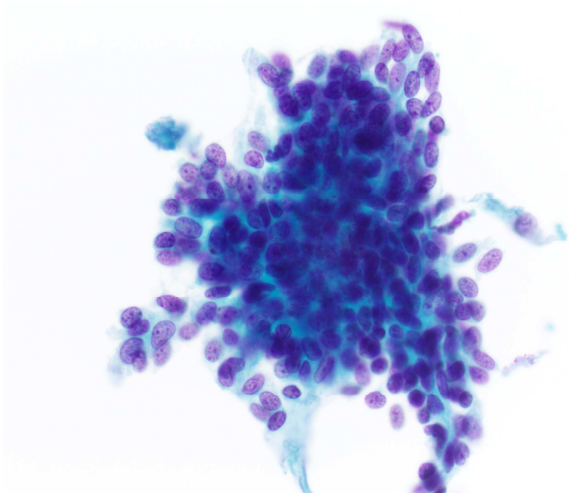
## Lower Uterine Segment

- Direct sampling from lower uterine segment can result in large, cellular, hyperchromatic groups
- Increased frequency with shortened endocervical canal (ie post cone bx or trachelectomy)
- Composed of both endometrial glandular and stromal cells
  - Glandular cells columnar with round-oval variably hyperchromatic nuclei; can see mitotic figures
  - Stromal groups more disorganized





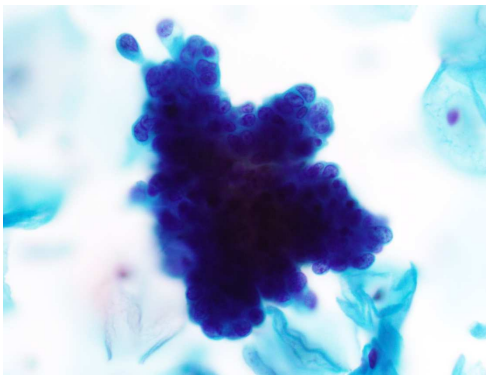


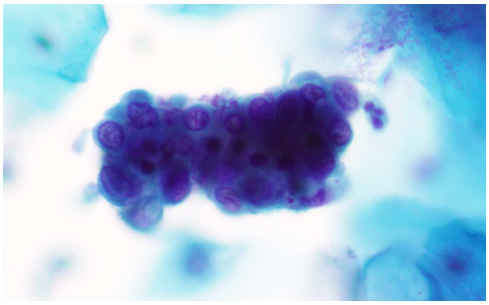


Lower uterine segment vs adenocarcinoma in situ?

# Menstrual Specimen

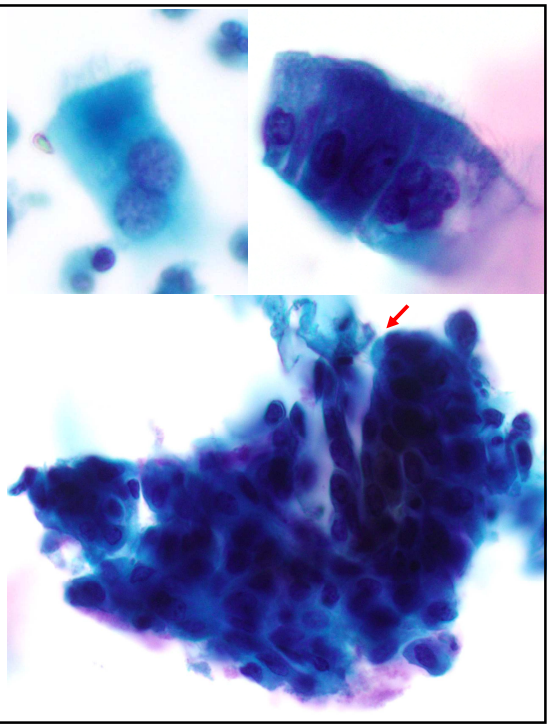
- Spontaneously exfoliated endometrial cells seen in first 12 days of menstrual cycle
- Most easily recognized when in spherical clusters
- Scant cytoplasm, dark nucleus
- Feathering, rosettes, and mitoses are virtually never seen in menstrual endometrium

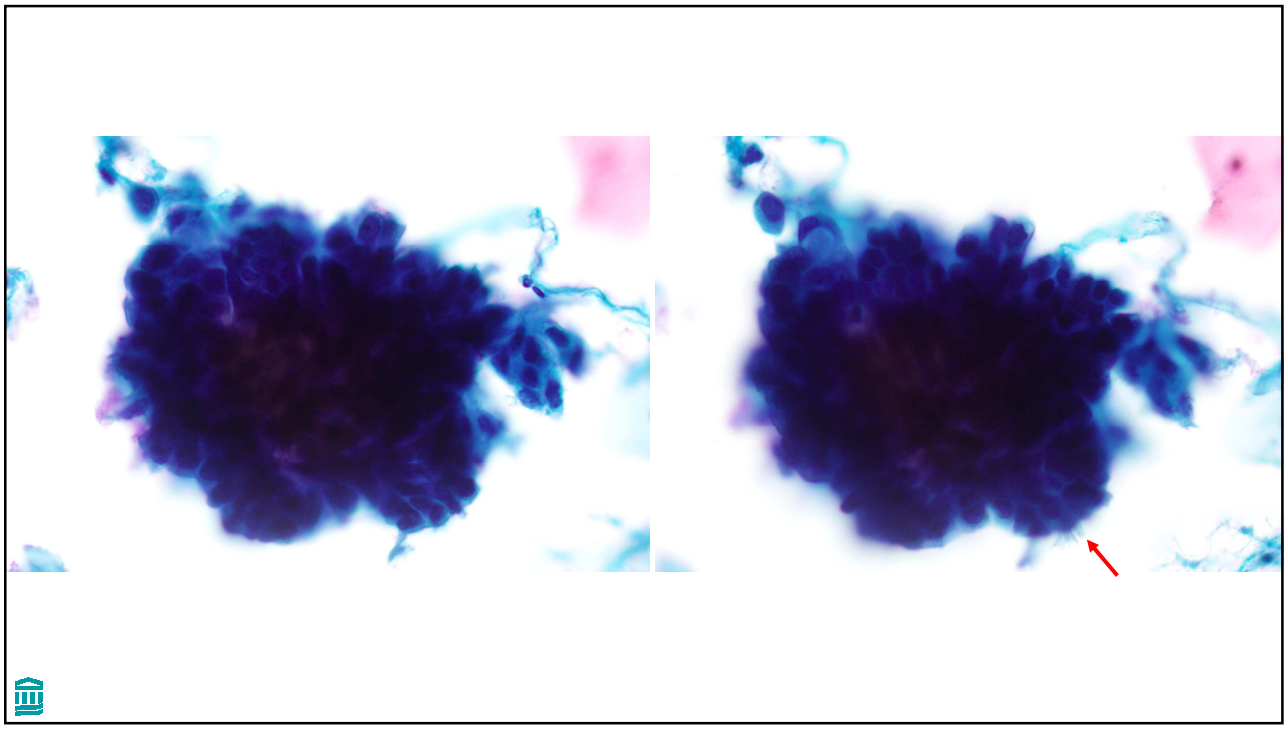


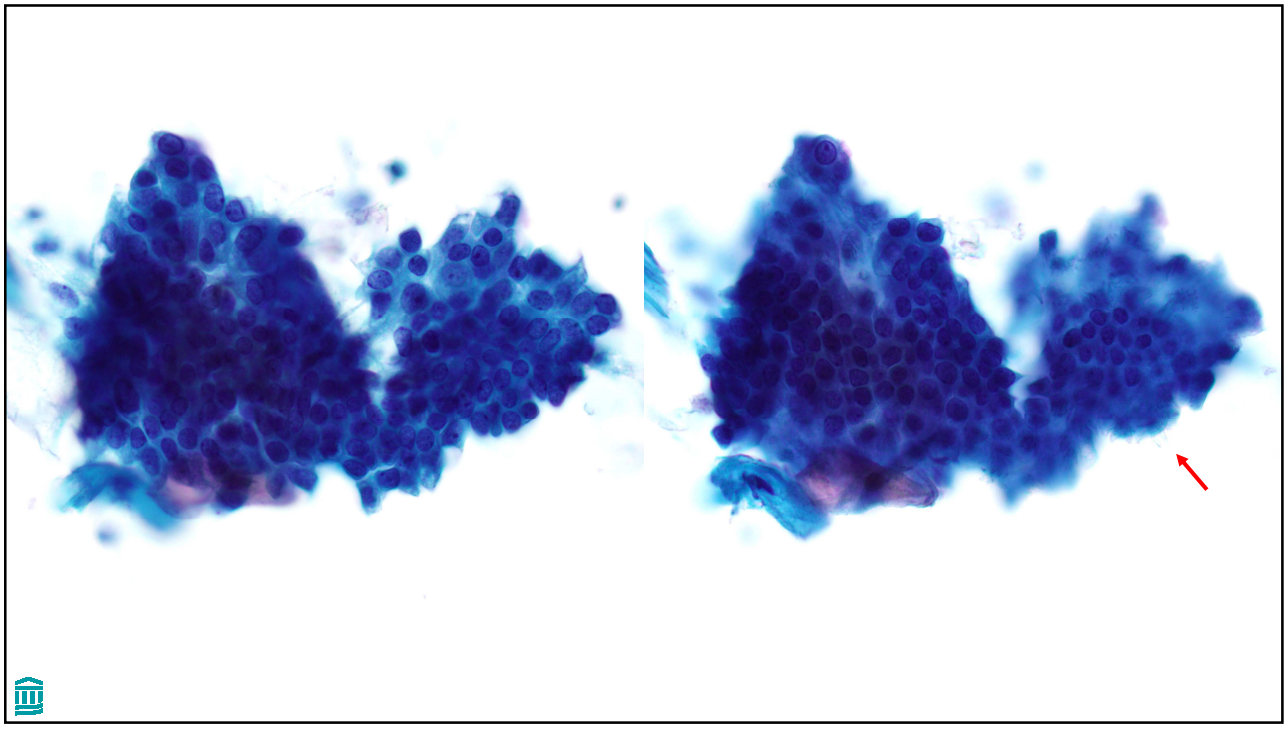


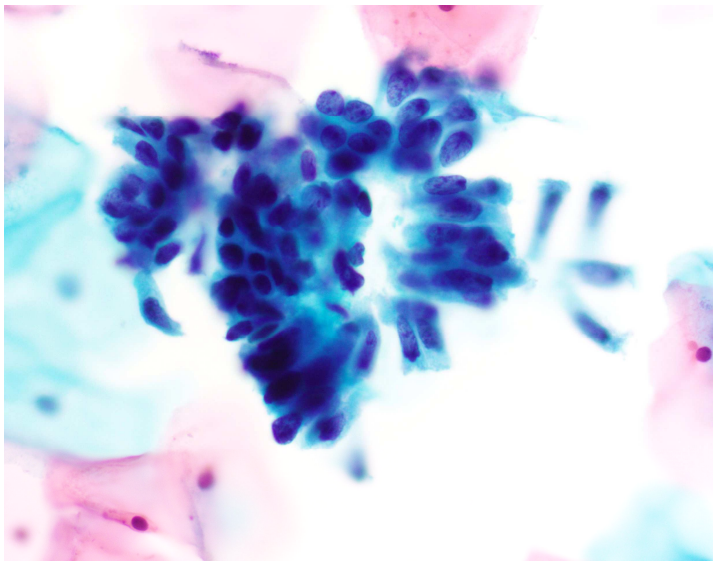
# Tubal Metaplasia

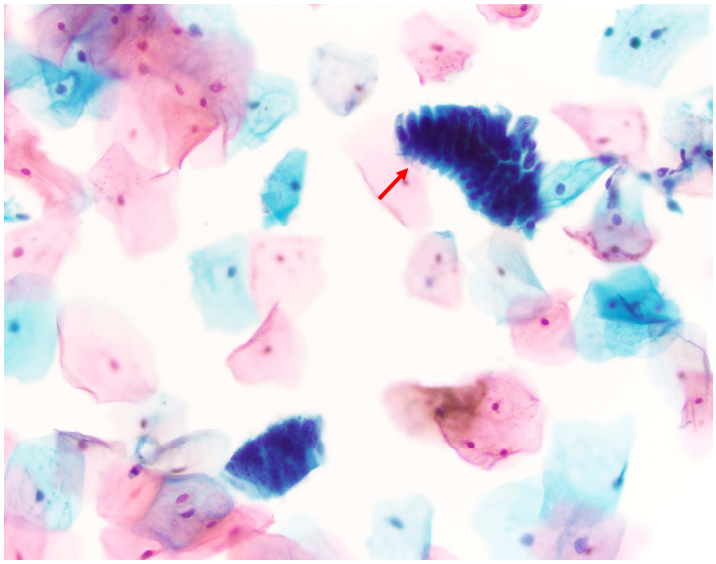
- Metaplastic change recapitulating fallopian tube epithelium
  - Not uncommon - about 1/3 of surgical pathology cases and ~10% of cervical cytology cases
- Characteristic feature: apical terminal bars and cilia
- Cellularity varies; found singly, in pseudostratified strips, flat sheets, or crowded clusters
- Round to oval nuclei, may be mildly enlarged and mildly crowded; without peripheral feathering
- Chromatin usually fine; nucleoli usually inconspicuous
- Mitotic figures can be seen
- No apoptosis or tumor diathesis
- Careful attention to details leads to correct diagnosis in majority of cases Novotny DB et al, Acta Cytol 1992



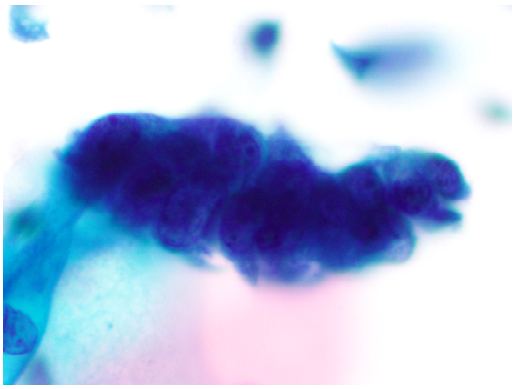




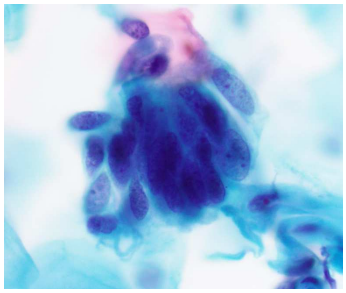




Tubal Metaplasia



Endocervical Adenocarcinoma In Situ



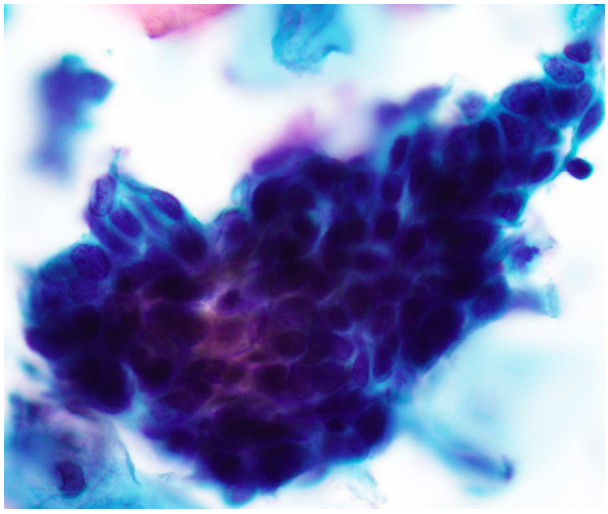
Tubal Metaplasia

| | Tubal Metaplasia | Endocervical Adenocarcinoma In Situ |
|---|---|---|
| **Clinical** | Any age | Any age but avg of late 30's |
| **Cellular pattern** | Low to high cellularity; found singly or in strips, flat sheets, or crowded clusters | Usually cellular; hyperchromatic crowded groups or strips |
| **Cytomorphology** | Feathering only rarely seen; rosettes not seen<br>Without significant nuclear atypia<br>May see mild mild nuclear crowding and mild hyperchromasia; finely granular chromatin; nucleoli inconspicuous; mitoses rarely seen | Feathering will be present; rosettes are characteristic<br>Nuclear atypia will be present including enlargement, crowding/overlapping, hyperchromasia, and chromatin coarseness; nucleoli will usually be inconspicuous; mitoses can be seen |
| **Distinguishing characteristics** | Apical terminal bars and cilia are characteristic (although may be poorly preserved)<br>Cells at periphery of groups tend to retain their cytoplasm (lack peripheral feathering)<br>Chromatin fine granularity<br>Mitotic figures rare, no apoptosis<br>p16 patchy positive | One should search for feathering and rosettes, which are not typical of tubal metaplasia<br>Chromatin will show coarse granularity<br>Mitotic figures and apoptosis will be more readily identified than in benign processes<br>p16 block positivity |

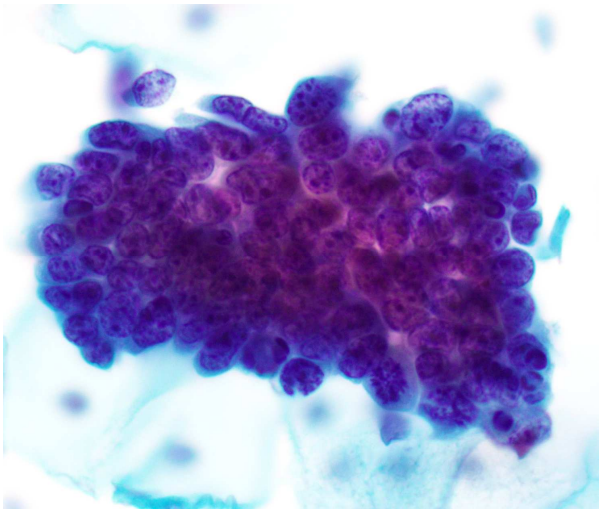
Torous VF and Pitman MP, JASC 2021

## HSIL vs AIS

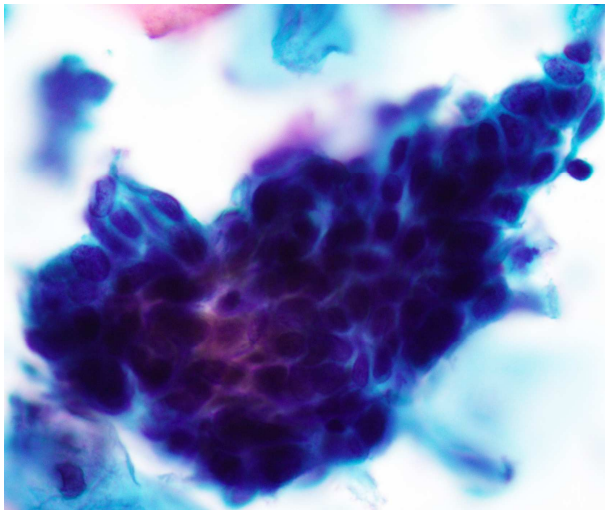
- Can resemble each other almost to perfection
- “Atypical glandular cells” are more frequently HSIL than AIS
- Both: hyperchromatic crowded groups, mitoses, apoptosis, coarse chromatin
- Call AIS when there is clear columnar glandular differentiation (ie strips of columnar cells, rosettes, feathering)
- Cell blocks can help in difficult cases
- Challenging cases exist…



High Grade Squamous Intraepithelial Lesion
(involving endocervical glands)



Endocervical Adenocarcinoma In Situ



High Grade Squamous Intraepithelial Lesion
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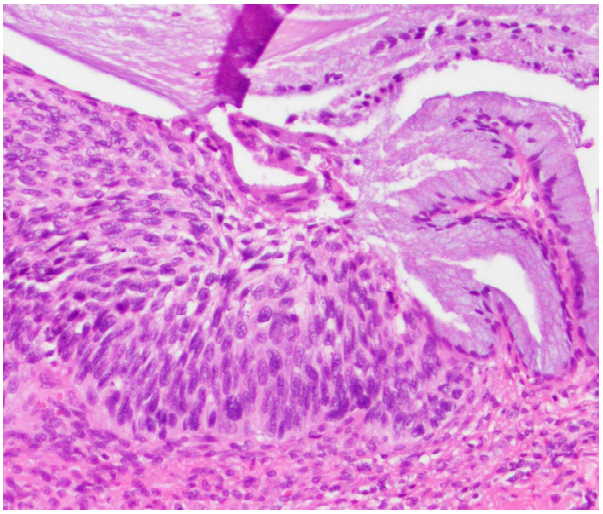
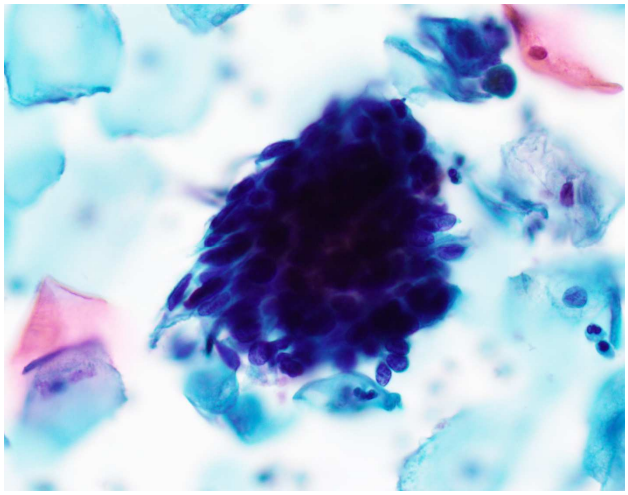
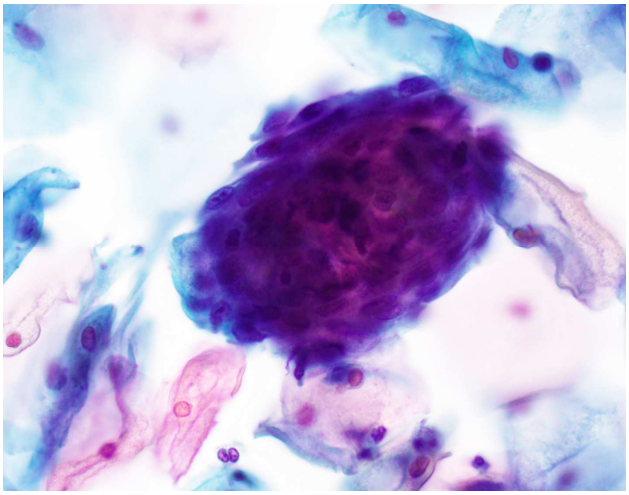


High Grade Squamous Intraepithelial Lesion
(involving endocervical glands)



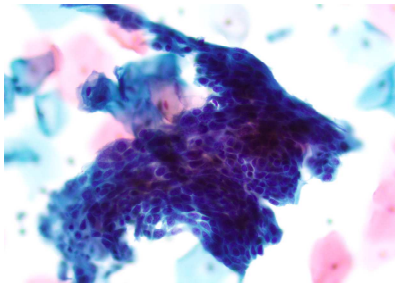
High Grade Squamous Intraepithelial Lesion
(involving endocervical glands)

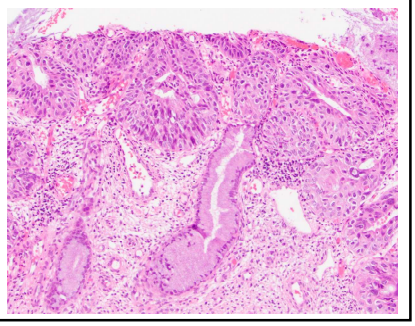


High Grade Squamous Intraepithelial Lesion
(involving endocervical glands)

# HSIL with Endocervical Gland Involvement (EGI)

- EGI diagnosed on surgical pathology has been associated with higher rates of residual or recurrent dysplasia
  - Clinical significance of diagnosis on cytology not known
- Diagnosis relatively straightforward on surgical path, but complicated by poor sensitivity and interobserver concordance on cytology
- Features suggestive of HSIL with EGI on cytology:
  - Centrally whorled or spindled cells with flattening of nuclei at the periphery of the cluster





# HSIL with Endocervical Gland Involvement (EGI)

**Table 4** Comparison of cytology-surgical pathology concordance, interobserver concordance, and interobserver variation in percentage of cases diagnosed as HSIL with endocervical glandular involvement.

| SP Dx | Attending 1 | Attending 2 | Fellow 1 | Fellow 2 | Tech 1 | Tech 2 | Cyto-SP Concordance | Inter-observer concordance |
|---|---|---|---|---|---|---|---|---|
| HSIL | HSIL EGI | HSIL EGI | HSIL EGI | HSIL EGI | HSIL EGI | HSIL EGI | 0.0% | 100.0% |
| HSIL EGI | HSIL | HSIL | HSIL | HSIL | HSIL | HSIL | 0.0% | 100.0% |
| HSIL EGI | HSIL | HSIL EGI | HSIL | HSIL | HSIL | HSIL | 16.7% | 83.3% |
| HSIL | HSIL EGI | HSIL EGI | HSIL | HSIL EGI | HSIL EGI | HSIL EGI | 16.7% | 83.3% |
| HSIL EGI | HSIL | HSIL EGI | HSIL | HSIL | HSIL | HSIL | 16.7% | 83.3% |
| HSIL EGI | HSIL | HSIL EGI | HSIL | HSIL EGI | HSIL | HSIL | 33.3% | 66.7% |
| HSIL | HSIL | HSIL EGI | HSIL | HSIL EGI | HSIL EGI | HSIL | 50.0% | 50.0% |
| HSIL | HSIL | HSIL EGI | HSIL | HSIL EGI | HSIL | HSIL EGI | 50.0% | 50.0% |
| HSIL EGI | HSIL | HSIL EGI | HSIL EGI | HSIL EGI | HSIL | HSIL EGI | 66.7% | 66.7% |
| HSIL EGI | HSIL EGI | HSIL EGI | HSIL | HSIL | HSIL EGI | HSIL EGI | 66.7% | 66.7% |
| HSIL EGI | HSIL EGI | HSIL EGI | HSIL | HSIL EGI | HSIL EGI | HSIL | 66.7% | 66.7% |
| HSIL | HSIL | HSIL EGI | HSIL | HSIL EGI | HSIL | HSIL | 66.7% | 66.7% |
| HSIL EGI | HSIL | HSIL | HSIL EGI | HSIL EGI | HSIL EGI | HSIL EGI | 66.7% | 66.7% |
| HSIL | HSIL | HSIL EGI | HSIL | HSIL | HSIL | HSIL EGI | 66.7% | 66.7% |
| HSIL EGI | HSIL EGI | HSIL | HSIL EGI | HSIL | HSIL EGI | HSIL EGI | 66.7% | 66.7% |
| HSIL | HSIL | HSIL EGI | HSIL | HSIL | HSIL | HSIL | 83.3% | 83.3% |
| HSIL EGI | HSIL | HSIL EGI | HSIL EGI | HSIL EGI | HSIL EGI | HSIL EGI | 83.3% | 83.3% |
| HSIL | HSIL EGI | HSIL | HSIL | HSIL | HSIL | HSIL | 83.3% | 83.3% |
| HSIL | HSIL | HSIL | HSIL | HSIL | HSIL | HSIL | 100.0% | 100.0% |
| HSIL | HSIL | HSIL | HSIL | HSIL | HSIL | HSIL | 100.0% | 100.0% |
| | 30.0% | 70.0% | 25.0% | 50.0% | 40.0% | 45.0% | 55.0% | 76.7% |
| | Percent of Total Cases Diagnosed as HSIL EGI | | | | | | Total Concordance Rate | |

The diagnosis of HSIL-EGI on Pap tests is complicated by poor sensitivity and interobserver concordance

Jones R et al, JASC 2020

| | Endocervical Adenocarcinoma In Situ | High Grade Squamous Intraepithelial Lesions |
|---|---|---|
| **Clinical** | Any age but avg of late 30's | Any age but usually younger women, peak in mid to late 30's |
| **Cellular pattern** | Usually cellular; hyperchromatic crowded groups or strips | Usually cellular; hyperchromatic crowded groups (or singly) |
| **Cytomorphology** | Feathering will be present; rosettes are characteristic<br>Nuclear atypia will be present including enlargement, crowding/overlapping, hyperchromasia, and chromatin coarseness; nucleoli will usually be inconspicuous; mitoses can be seen | Feathering is possible (due to glandular involvement); rosettes will not be seen<br>Nuclear atypia will be present with enlargement, contour irregularity, hyperchromasia, and chromatin coarseness; nucleoli will usually be inconspicuous; mitoses can be seen |
| **Distinguishing characteristics** | One should search for feathering and rosettes, which are not typical of tubal metaplasia<br>Chromatin will show coarse granularity<br>Mitotic figures and apoptosis will be more readily identified than in benign processes<br>p16 block positivity | Spindling or whorling of centrally located cells which can appear as central piling of cell groups<br>Flattening of nuclei at the periphery will give the cell clusters smooth, rounded borders; however, due to glandular involvement, peripheral palisading and nuclear stratification could still be present<br>One should search for discrete single atypical cells in the background<br>p16 block positivity |

Torous VF and Pitman MP, JASC 2021

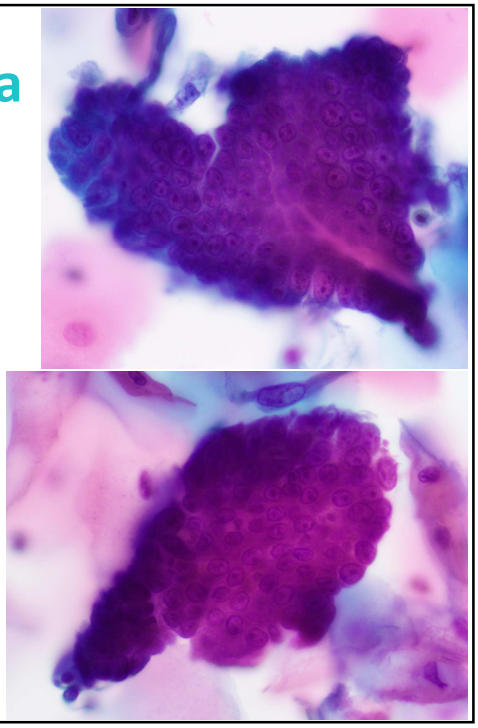
# A note...

- When a combined diagnosis describing both a squamous lesion and glandular abnormality is given, follow-up pathology often reveals a squamous lesion and rarely a combined lesion
- Glandular and squamous lesions can coexist, but squamous lesions are far more common
- In some studies, up to half of AIS lesions have a coexisting SIL
- It may not be possible to distinguish glandular from squamous lesions on cytology

Khor et al, Cancer Cytopathol 2014
Harbhajanka A, Chahar S, Michael CW, Diagn Cytopathol 2019
Jones R et al, JASC 2020

## Endocervical Adenocarcinoma

- Cytologic overlap with AIS
- Enlarged, pleomorphic nuclei with irregular nuclear contours and uneven chromatin distribution
- Macronucleoli
- Finely vacuolated cytoplasm
- Tumor diathesis

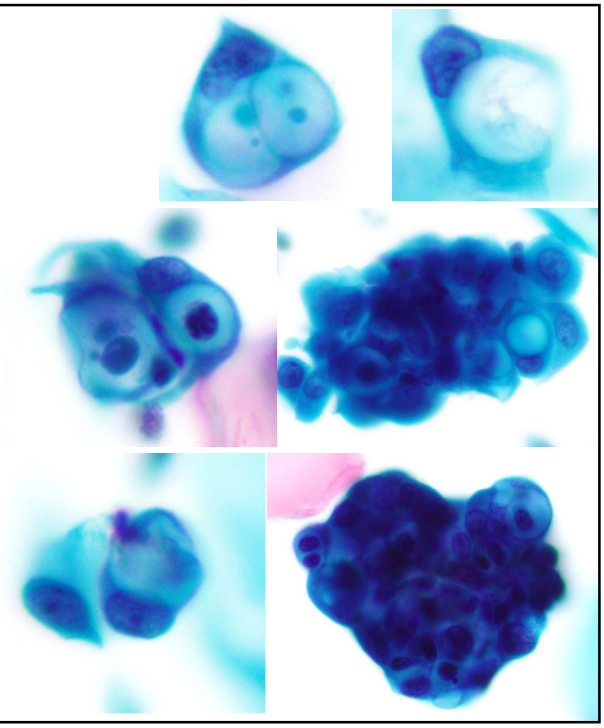


## Endocervical Adenocarcinoma

- Benign mimics
  - Reactive changes
  - IUD (vacuolated cells)
  - Polyps
  - Aria Stella reaction
  - Microglandular hyperplasia
- Neoplastic mimics
  - Endometrial adenocarcinoma

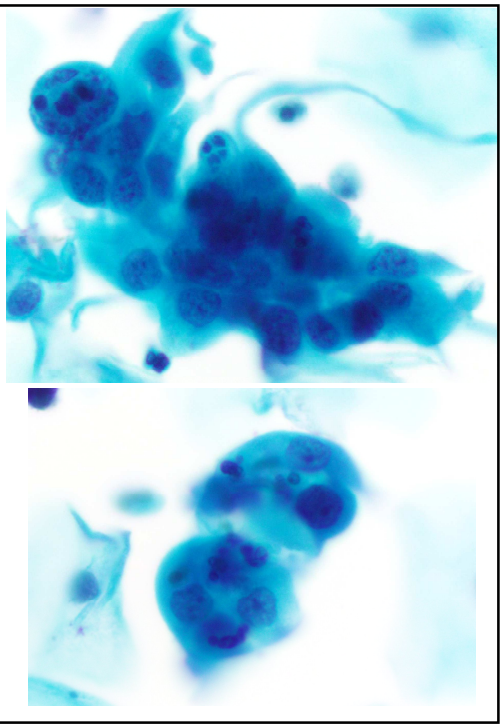
## IUD – Vacuolated Cells

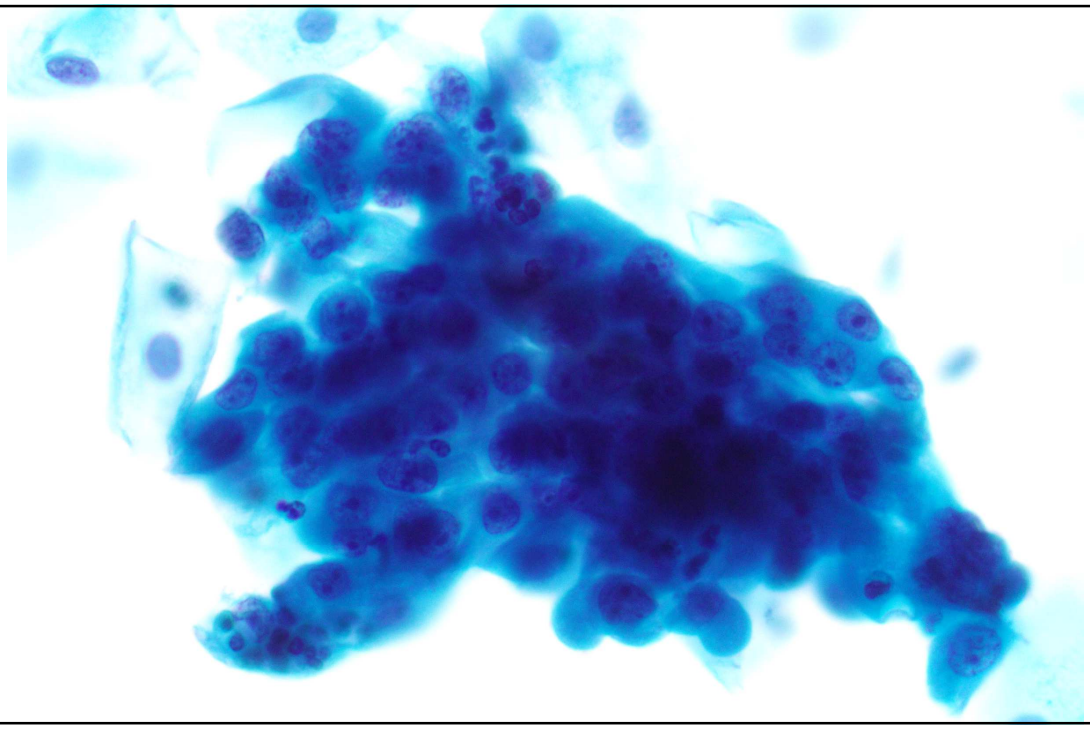
- Can be found in clusters and singly
- Variable size of vacuoles
  - Large vacuoles can displace nucleus, impart signet-ring appearance
- Nucleoli may be present
- May mimic adenocarcinoma
  - A diagnosis of adenocarcinoma should be made only with great caution the presence of an IUD



## Polyps

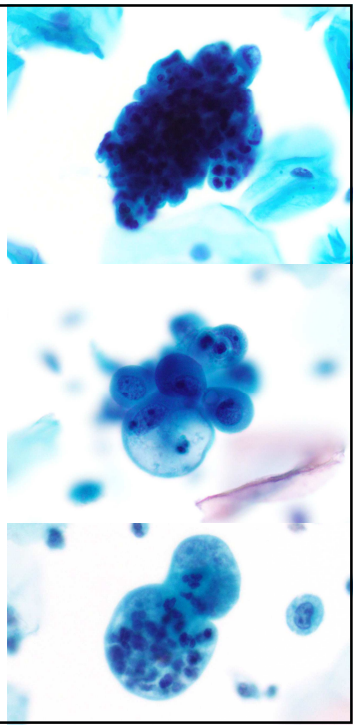
- Morphologic overlap with adenocarcinomas possible, particularly when inflamed
  - "Bag of polys" not specific
- May not be possible to distinguish from adenocarcinoma without prior clinical knowledge

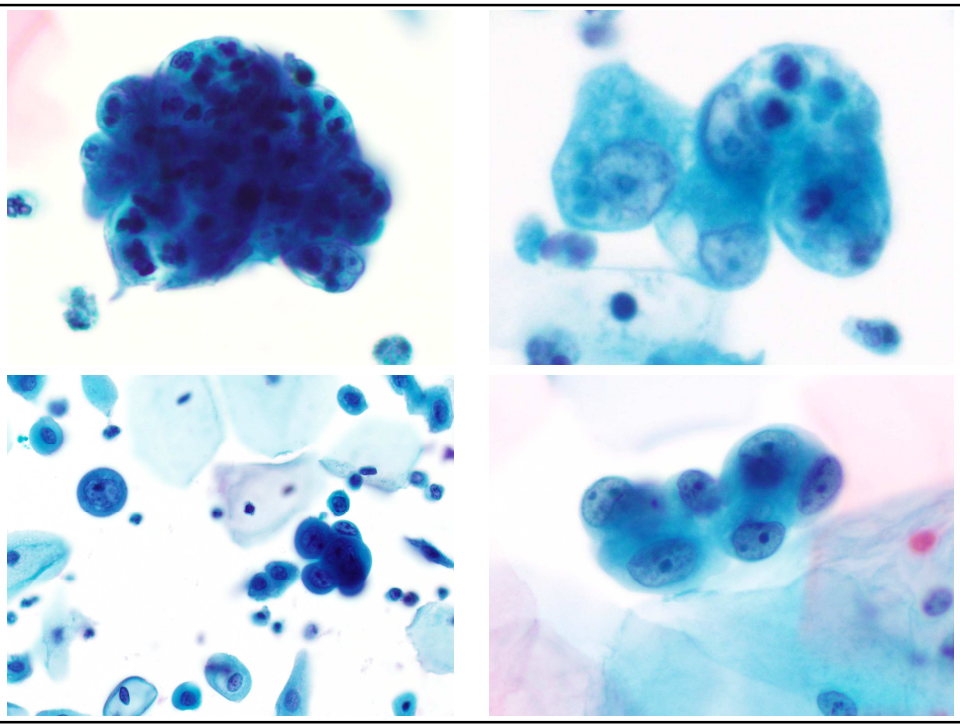


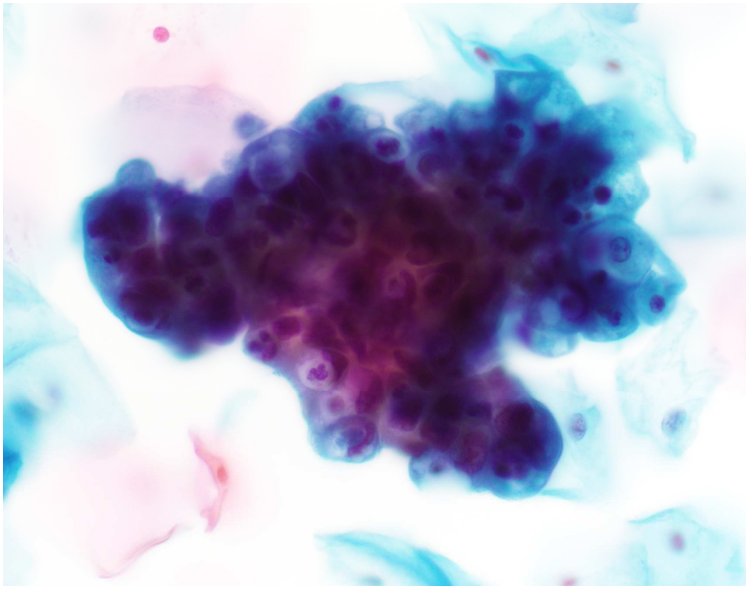


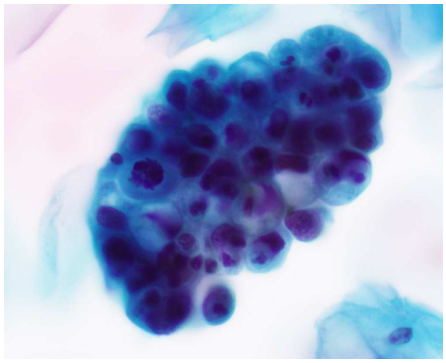
## Endometrial Adenocarcinoma

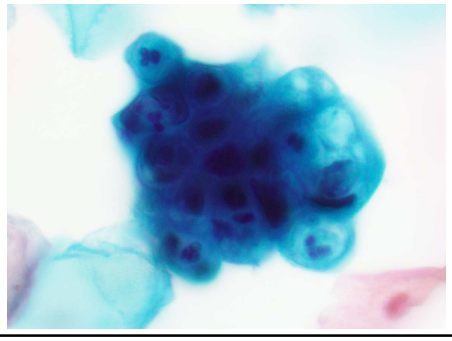
- Findings largely dependent on grade of tumor
- Arranged singly or as small tight clusters
- Round cells
- Variably sized nuclei with loss of nuclear polarity
- Hyperchromatic
- Small to prominent nucleoli
- Scant or abundant vacuolated cytoplasm
- Intracytoplasmic neutrophils (“bag of polys”)
- Variable “watery” tumor diathesis



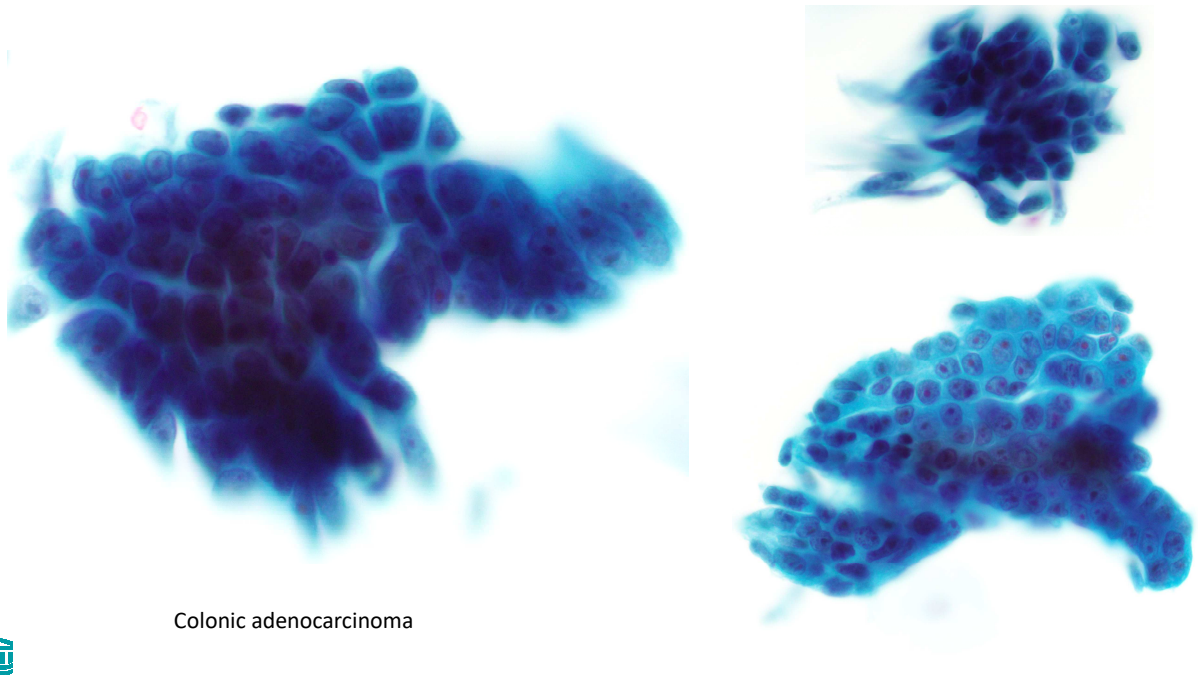








Invasive Endocervical Mucinous Adenocarcinoma



Colonic adenocarcinoma

| Features | Endocervical Carcinoma | Endometrial Carcinoma | Extrauterine Carcinoma |
|---|---|---|---|
| Cellularity | Hypercellular | Low cellularity usually | Rare cells (unless direct extension / mets) |
| Pattern | Strips, rosettes, sheets with feathering, single malignant cells | Small clusters, rarely papillae, single cells | Varies depending upon primary and mode of spread |
| Diathesis | Visible, type varies by preparation | Variable, watery or subtle or absent | Usually absent unless direct spread or mets |
| Cell shapes | Oval, columnar, pleomorphic | Round, irregular, usually smaller | Variable, do not belong |
| Nuclei | Oval, elongated, pleomorphic, vesicular | Round, irregular in higher grade | Variable |
| Cytoplasm | Mucin + | Degenerative vacuoles | Variable |
| SIL or Sq Ca | Present in >50% | Absent | Absent |
| High-risk HPV | Positive in most | Negative | Negative |
| p16 | Block positive | Patchy / focal except in high grade / serous | Variable, depends on type |

Bethesda System, 3rd ed

## Summary

- Benign and reactive processes in cervical cytology can be problematic given their morphologic overlap with various neoplastic processes
- Attention to morphologic clues may be helpful in distinguishing between benign and neoplastic processes
- Knowledge of diagnostic pitfalls can help avoid over diagnosis

## Thanks!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