Mass General Brigham

MASSACHUSETTS GENERAL HOSPITAL

# Endocervical AGCs and Worse

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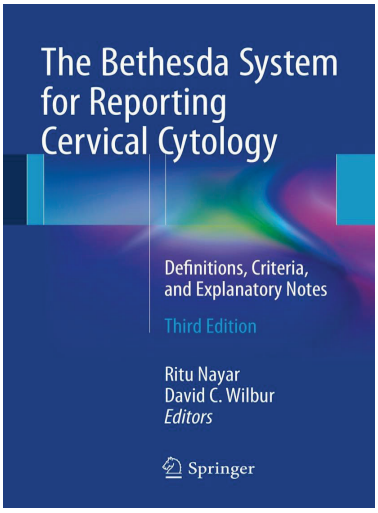
## Reporting Glandular Lesions using The Bethesda System

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



# Cervical Cytology for Glandular Lesions

- 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

# 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

# Histologic Correlates of Atypical Glandular Cells

**TABLE 2.** Histologic Follow-Up Results for Patients With Papanicolaou Tests Showing Different Types of AGC

| AGC Type | Cases, No. (%) | Cervical Squamous Lesions, No. (%) | | Cervical Glandular Lesions, No. (%) | | Endometrial Lesions, No. (%) | | MetCa, No. (%) | Negative, No. (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | CIN1 | CIN2/3 | AIS | ADCa | CAH | EmCa | | |
| AGC-NOS[a] | 1630 (54.2) | 196 (12.0) | 35 (2.2) | 13 (0.8) | 9 (0.6) | 14 (0.9) | 95 (5.8) | 11 (0.7) | 1259 (77.2) |
| AEC[a] | 760 (25.3) | 126 (16.6) | 21 (2.8) | 12 (1.6) | 3 (0.4) | 4 (0.5) | 3 (0.4) | 0 | 593 (78.0) |
| AMC | 211 (7.0) | 9 (4.3) | 0 | 1 (0.5) | 0 | 16 (7.6) | 47 (22.3) | 1 (0.5) | 137 (64.9) |
| AGC-FN[b] | 26 (0.9) | 0 | 3 (11.5) | 6 (23.1) | 2 (7.7) | 0 | 12 (46.2) | 1 (3.8) | 3 (11.5) |
| AGC/ASC-H[a] | 227 (7.6) | 63 (27.8) | 49 (21.6) | 6 (2.6) | 2 (0.9) | 2 (0.9) | 6 (2.6) | 1 (0.4) | 100 (44.1) |
| AGC/LSIL | 66 (2.2) | 42 (63.6) | 2 (3.0) | 0 | 1 (1.5) | 0 | 1 (1.5) | 0 | 20 (30.3) |
| AGC/HSIL | 87 (2.9) | 17 (19.5) | 57 (65.5) | 0 | 2 (2.3) | 0 | 2 (2.3) | 0 | 9 (10.3) |
| Total | 3007 | 453 (15.1) | 167 (5.6) | 38 (1.3) | 19 (0.6) | 36 (1.2) | 166 (5.5) | 14 (0.5) | 2121 (70.5) |

Abbreviations: ADCa, adenocarcinoma; AEC, atypical endocervical cells; AGC, atypical glandular cells; AGC-FN, atypical glandular cells, favor neoplastic; AGC-NOS, atypical glandular cells, not otherwise specified; AIS, adenocarcinoma in situ; AMC, atypical endometrial cells; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; CAH, complex atypical hyperplasia; CIN, cervical intraepithelial neoplasia; EmCa, endometrial carcinoma; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; MetCa, metastatic carcinoma.
For each case, only the severe lesion was recorded. However, for cases with CIN2/3 and AIS, both lesions were recorded (7 in all with both processes).
[a] Two cases in each subcategory had both CIN2/3 and AIS.
[b] One case had both CIN2/3 and AIS.

Pradhan D, et al. Cancer Cytopathol, 2016

# Histologic Correlates of Atypical Glandular Cells

**Table 2** Clinically significant histologic results of atypical glandular cells.

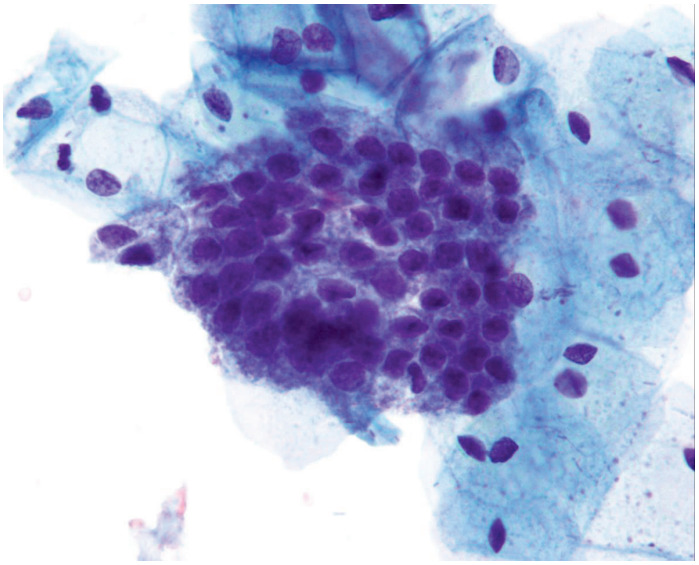
| Subcategories | Clinically significant histologic results | Benign or non-precancerous lesions |
|---|---|---|
| Atypical glandular cells, NOS | 128/443 (28.9%) | 315/443 (71.1%) |
| Atypical glandular cells, favor neoplastic | 23/28 (82.1%) | 5/28 (17.9%) |
| Atypical endometrial cells | 9/17 (52.9%) | 8/17 (47.1%) |
| Atypical endocervical cells, favor neoplastic | 12/19 (70.6%) | 7/19 (29.4%) |
| Atypical endocervical cells | 1/4 (25%) | 3/4 (75%) |

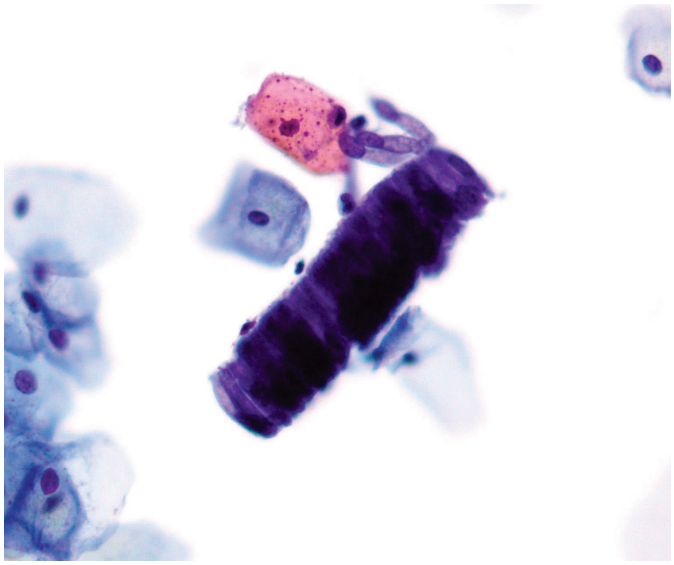
**Table 4** Malignancy rate and tumor types of follow-up histology.

| Subcategories | Malignancy rate | Type of malignancy | Number of cases |
|---|---|---|---|
| Atypical glandular cells, NOS | 19.2% (85/443) | Endometrial endometrioid carcinoma | 37/85 |
| | | Endometrial serous carcinoma | 14/85 |
| | | Endometrial mucinous carcinoma | 1/85 |
| | | Endocervical adenocarcinoma | 15/85 |
| | | Cervical squamous cell carcinoma | 13/85 |
| | | Endocervical adenocarcinoma in situ | 3/85 |
| | | Cervical neuroendocrine carcinoma | 1/85 |
| | | Ovarian mucinous borderline tumor | 1/85 |
| | | Invasive Paget's disease | 1/85 |
| Atypical glandular cells, favor neoplastic | 82.1% (23/28) | Endometrial endometrioid carcinoma | 11/23 |
| | | Endometrial serous carcinoma | 3/23 |
| | | Endometrial carcinosarcoma | 2/23 |
| | | Endometrial clear cell carcinoma | 1/23 |
| | | Endocervical adenocarcinoma | 6/23 |
| Atypical endocervical cells, favor neoplastic | 63.2% (12/19) | Endometrial endometrioid carcinoma | 3/12 |
| | | Endometrial carcinosarcoma | 2/12 |
| | | Endometrial serous carcinoma | 1/12 |
| | | Endocervical adenocarcinoma | 3/12 |
| | | Endocervical adenocarcinoma in situ | 2/12 |
| | | Cervical squamous cell carcinoma | 1/12 |
| Atypical endometrial cells | 52.9% (9/17) | Endometrial endometrioid carcinoma | 6/9 |
| | | Endometrial serous carcinoma | 3/9 |
| Atypical endocervical cells | 25% (1/4) | Endocervical adenocarcinoma | 1/1 |

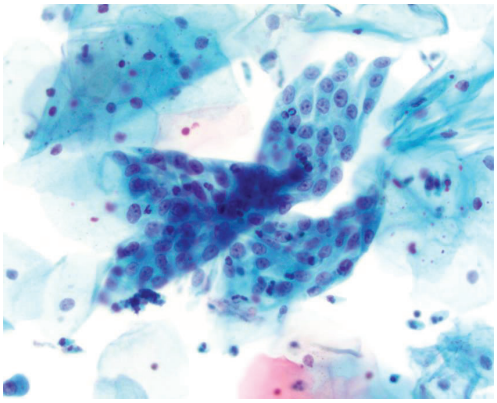
Hassan D, et al. J Am Soc Cytopathol, 2024

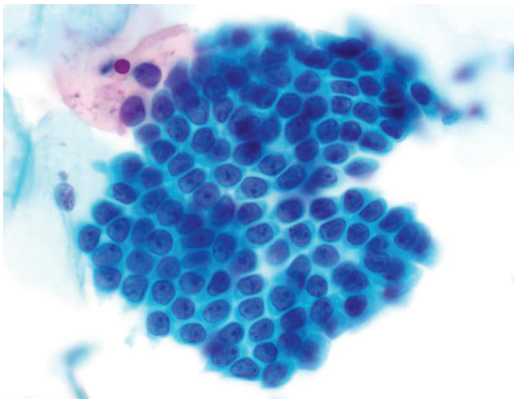
# Normal Endocervical Cells

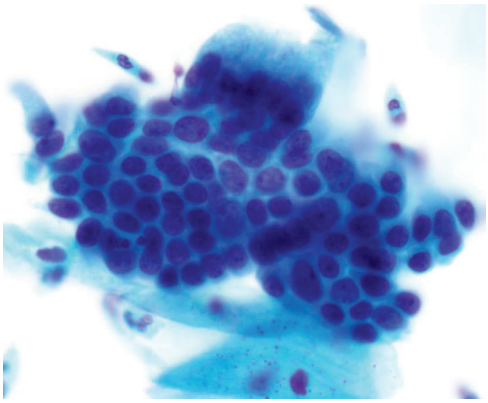


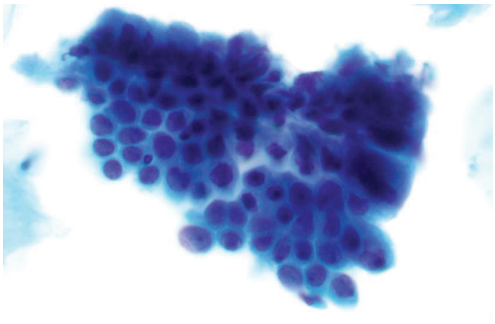


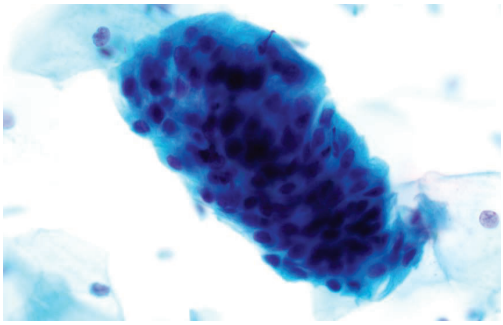
# Normal Endocervical Cells

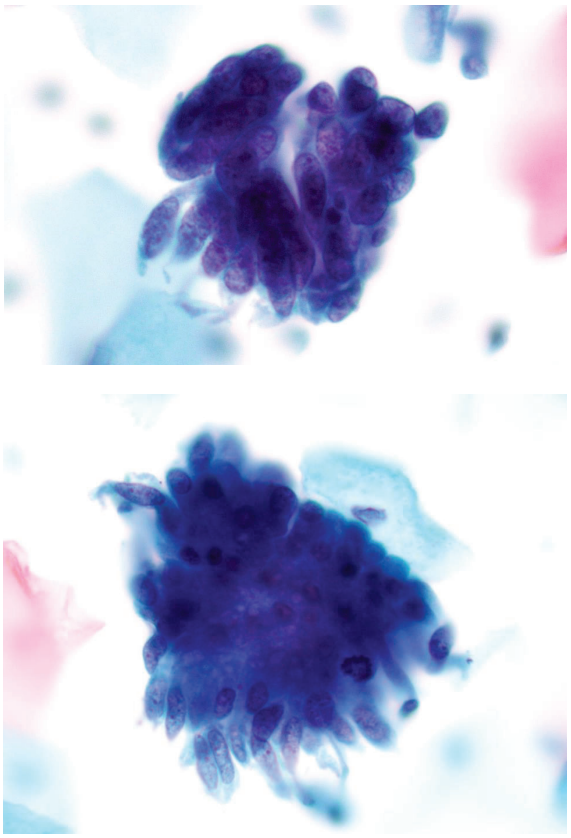


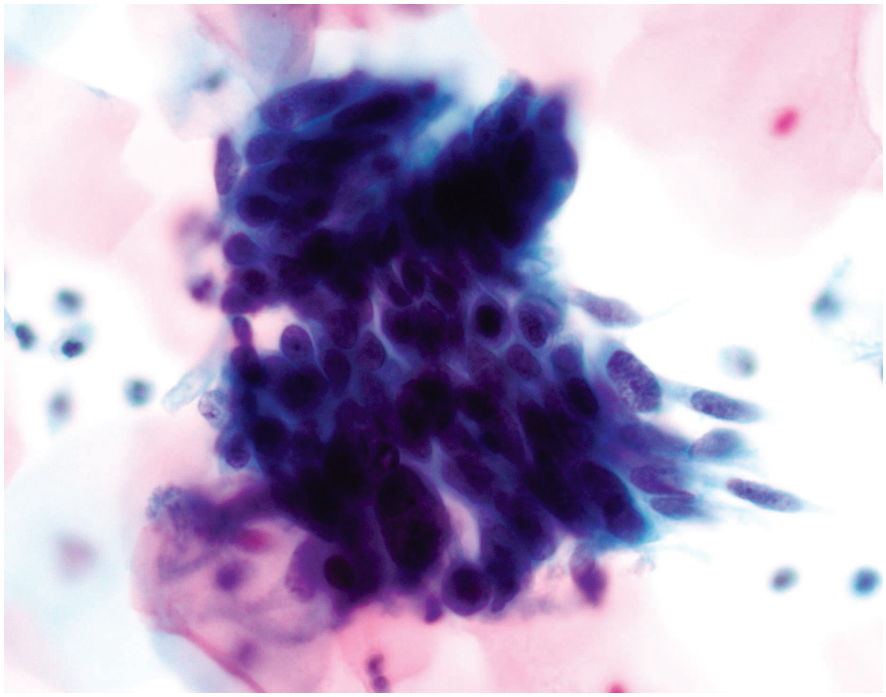






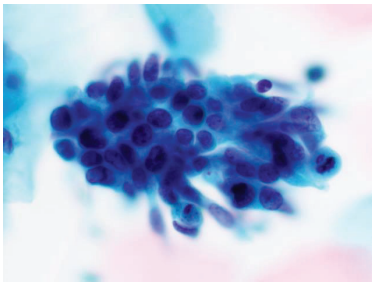


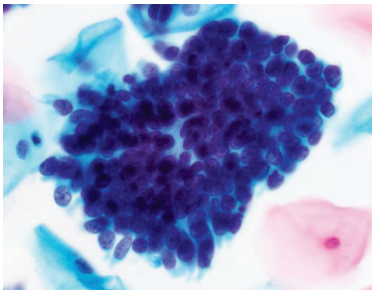




# Endocervical Adenocarcinoma In Situ

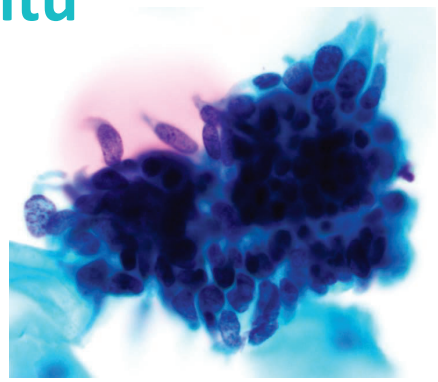
- The known precursor to endocervical adenocarcinoma
  - Women with AIS are on average 13 years younger than those with adenocarcinoma (39 vs 52 yo)
  - Morphologically similar and often found adjacent on histologic sections
  - HPV 16 and 18 are identified in similar proportions
- There has been a steady 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)

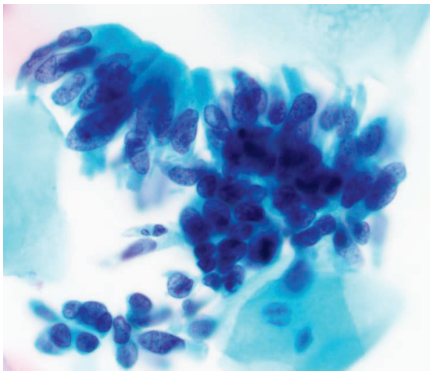


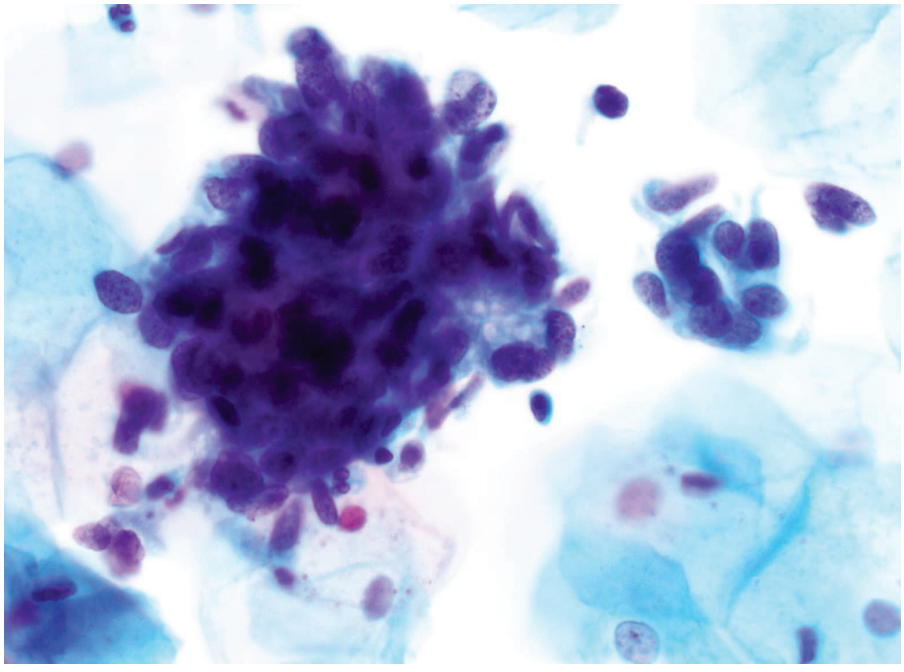


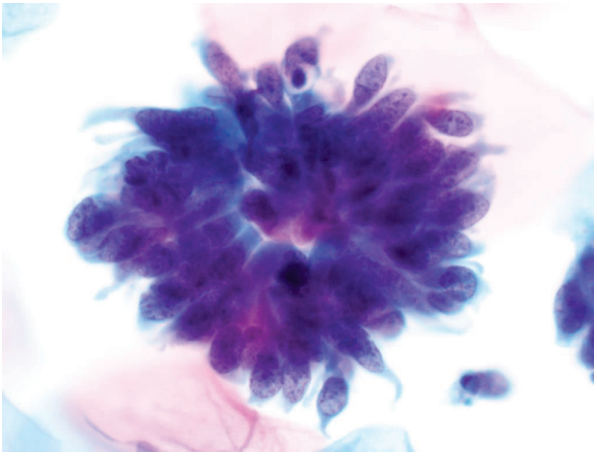
# Endocervical Adenocarcinoma In Situ

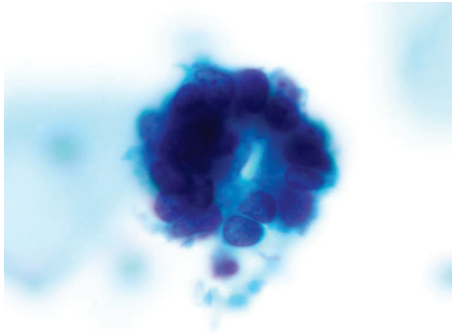
- Arranged in sheets, pseudostratified strips, and rosettes
- Glandular differentiation: columnar cells; peripheral feathering
- Oval or elongated nuclei with enlargement, size variation
- Hyperchromatic, evenly dispersed chromatin
- Nucleoli small/inconspicuous
- Increased N:C
- Mitoses, apoptoses common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

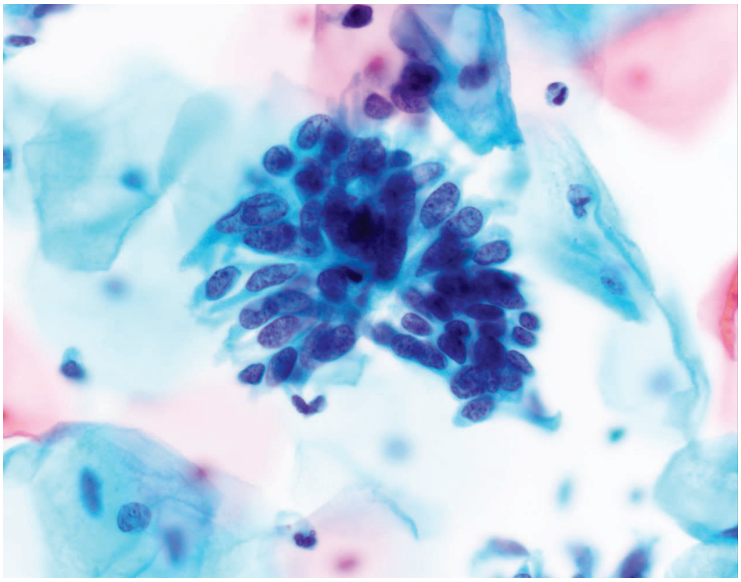










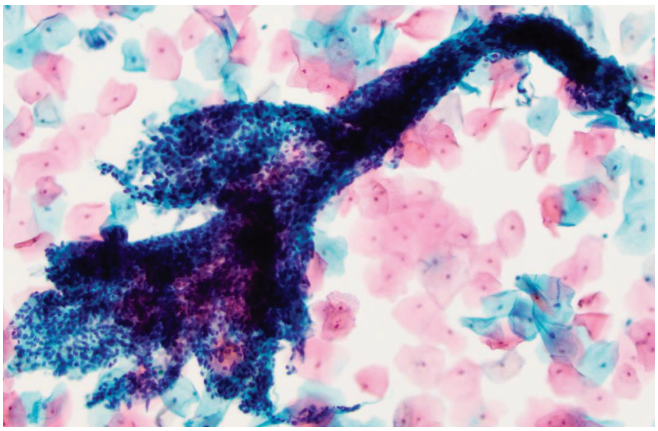


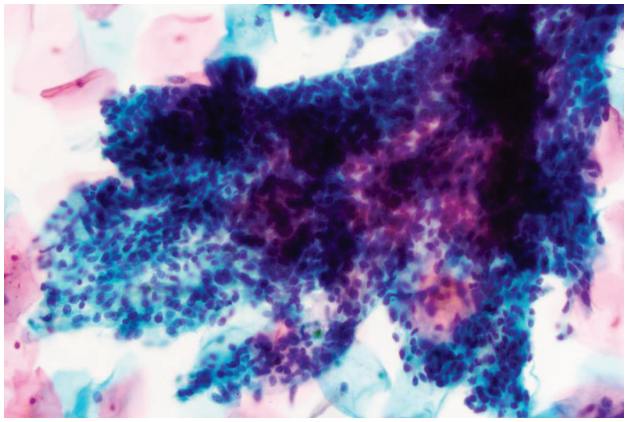
# Endocervical Adenocarcinoma In Situ

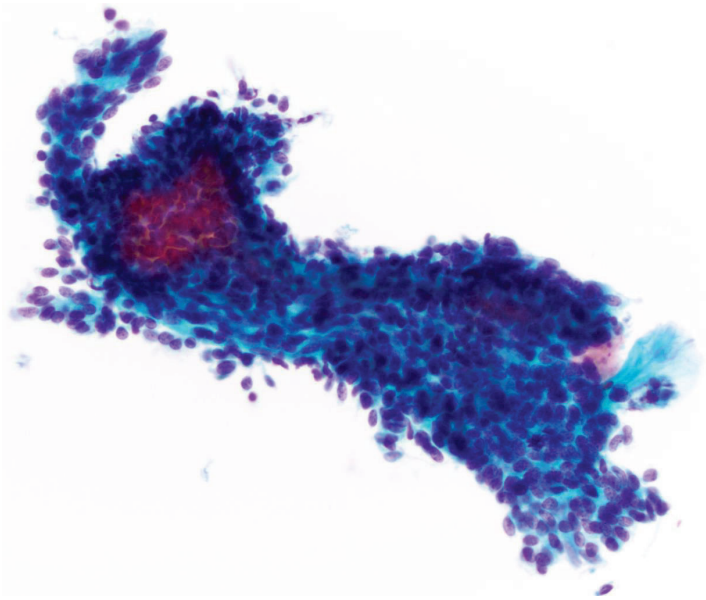
- Benign mimics
  - Lower uterine segment
  - Menstrual endometrium
  - Tubal metaplasia
- Neoplastic mimics
  - HSIL
  - Endocervical adenocarcinoma
  - Endometrial adenocarcinoma

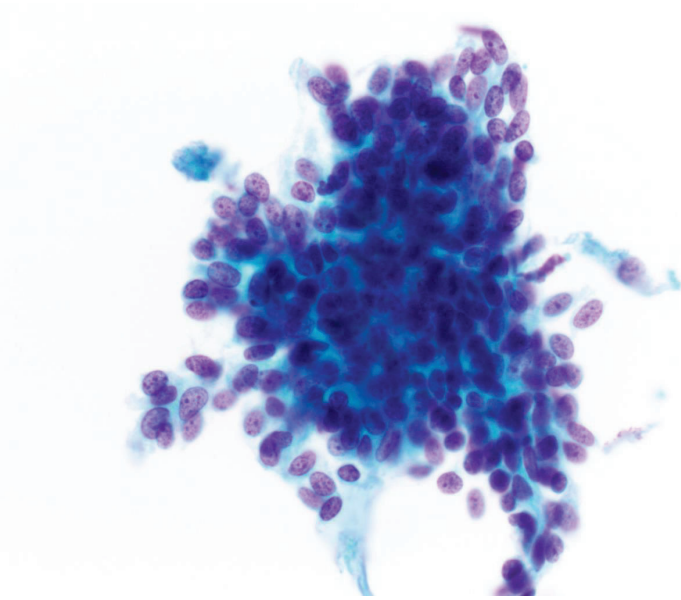
# Lower Uterine Segment

- 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





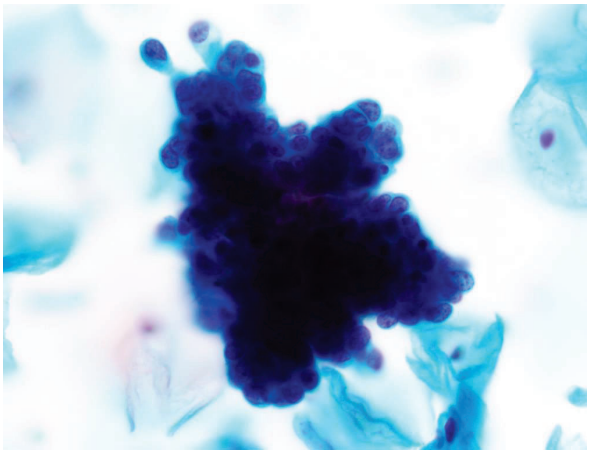


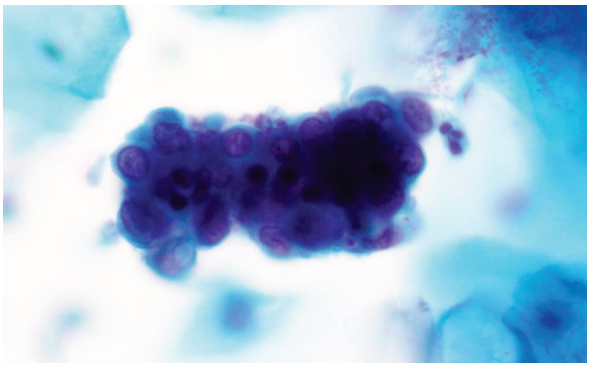


Lower uterine segment vs adenocarcinoma in situ?

# Menstrual Specimen

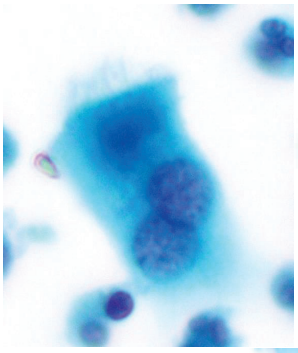
- Spontaneously exfoliated endometrial cells seen in first 12 days of menstrual cycle
- Most easily recognized when in spherical clusters
- Scant cytoplasm, dark nucleus
- Feathering, rosettes, and mitoses are virtually never seen in menstrual endometrium

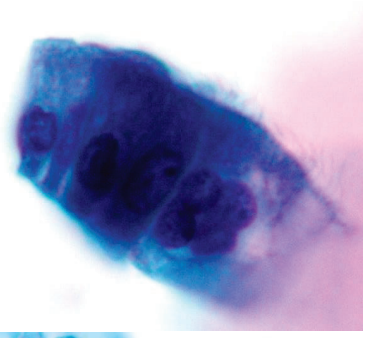


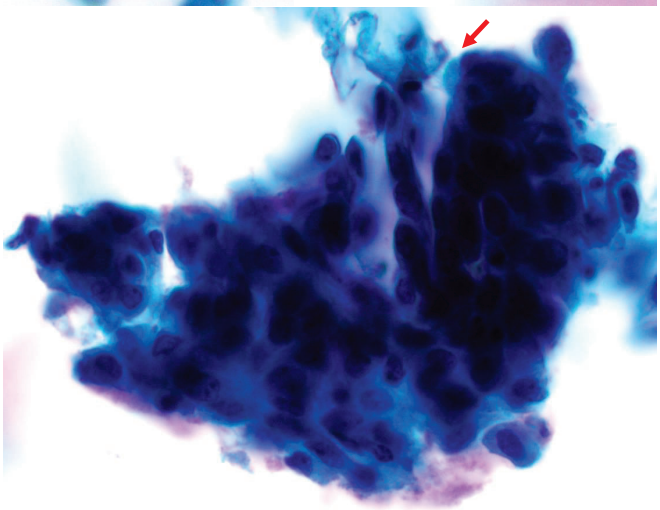


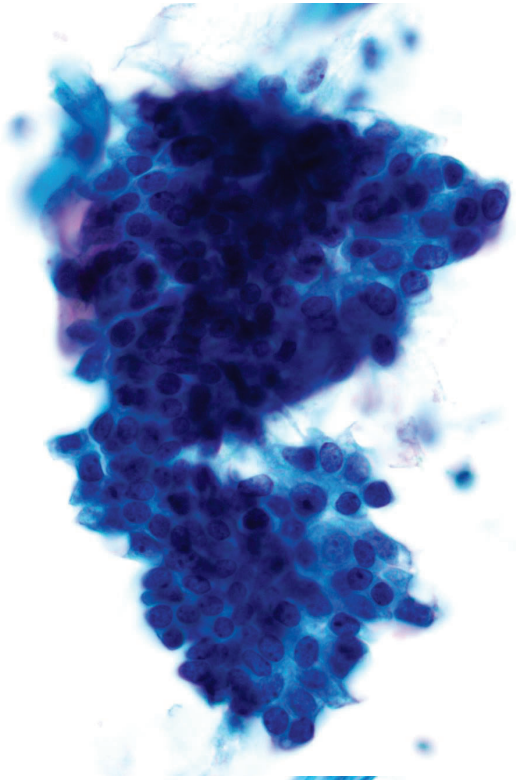
# Tubal Metaplasia

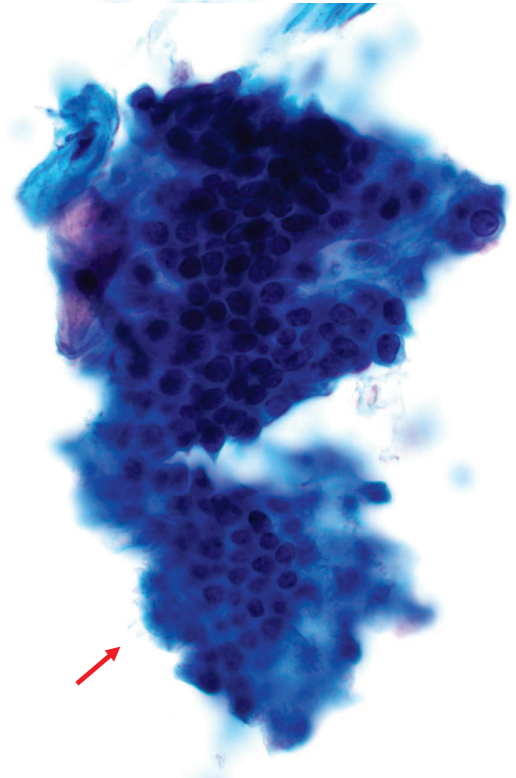
- Metaplastic change recapitulating fallopian tube epithelium
  - Not uncommon - about 1/3 of surgical pathology cases and ~10% of cervical cytology cases
- Characteristic feature: apical terminal bars and cilia
- Cellularity varies; found singly, in pseudostratified strips, flat sheets, or crowded clusters
- 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

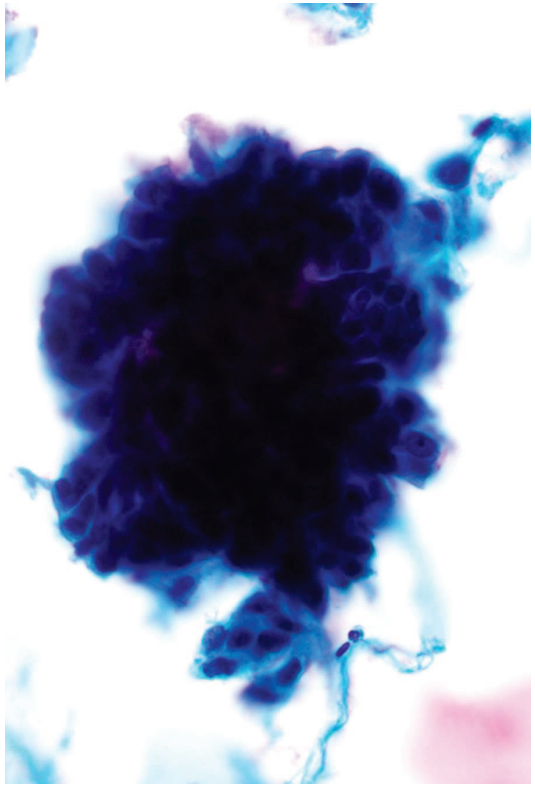


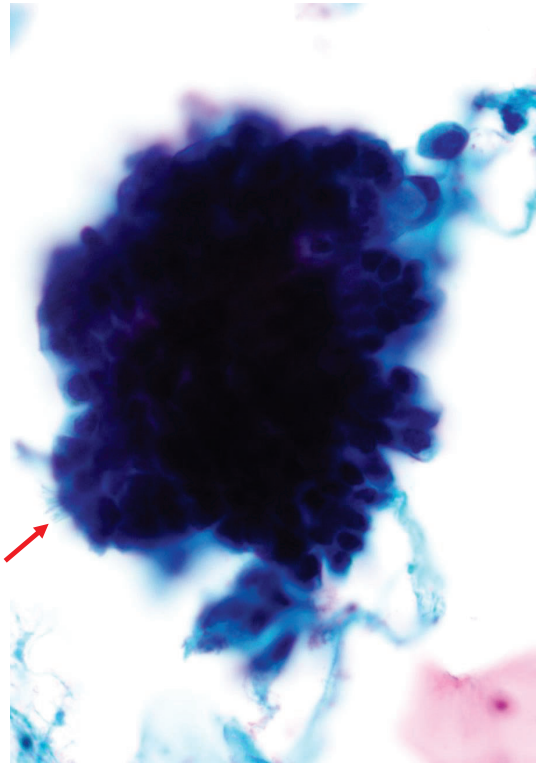




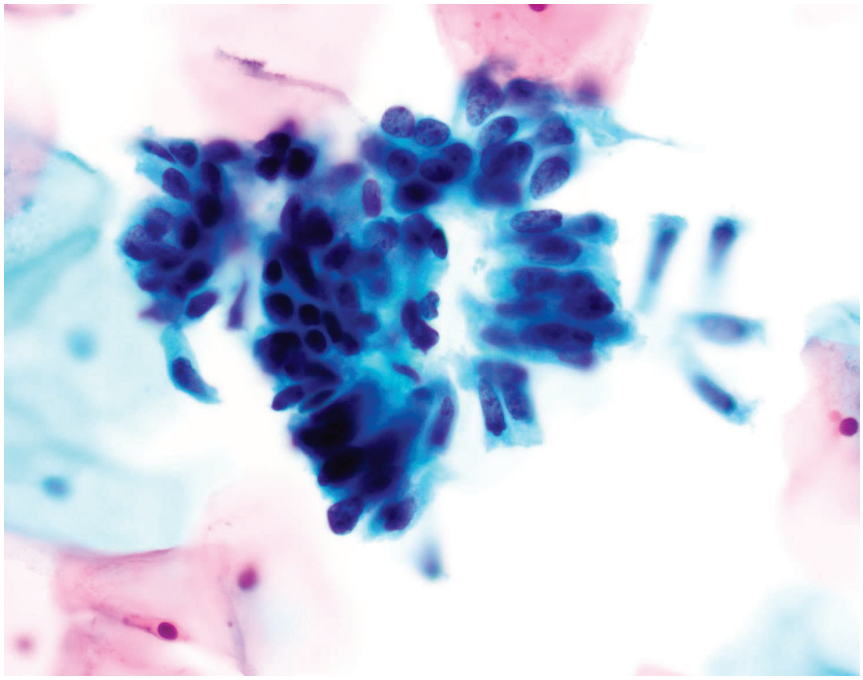


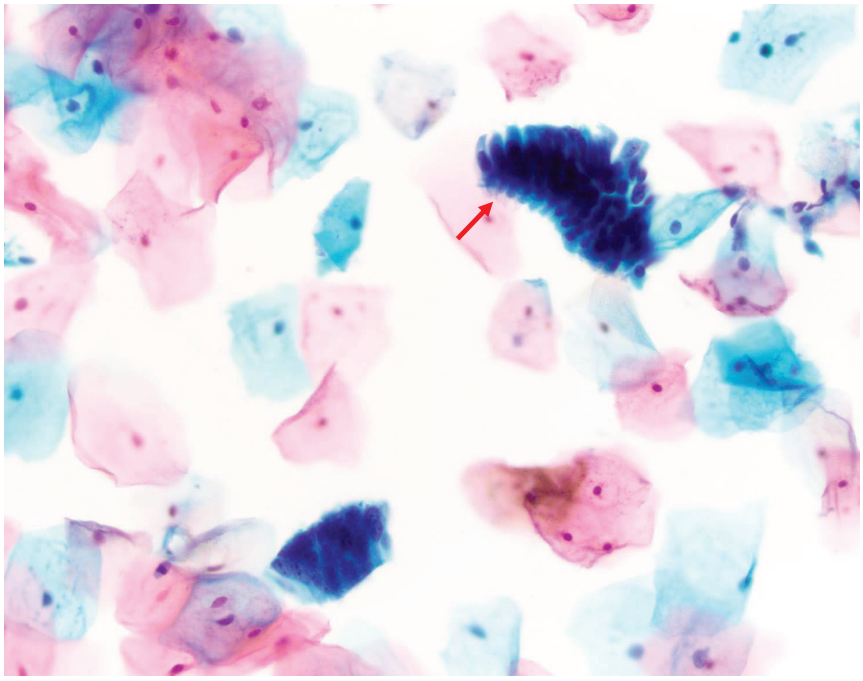




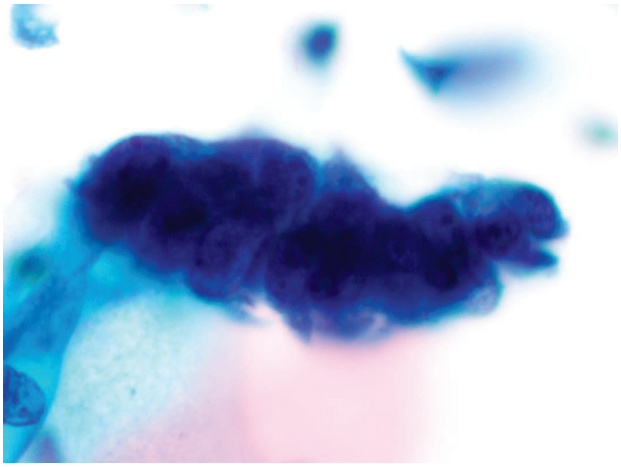


44 year old F with h/o severe squamous dysplasia status post LEEP

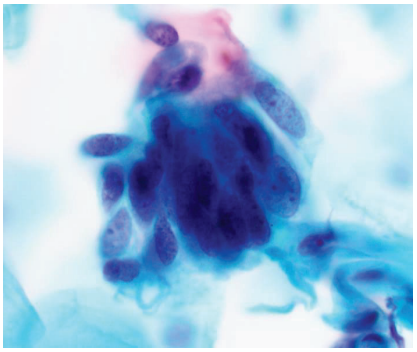




Tubal Metaplasia



Endocervical Adenocarcinoma In Situ

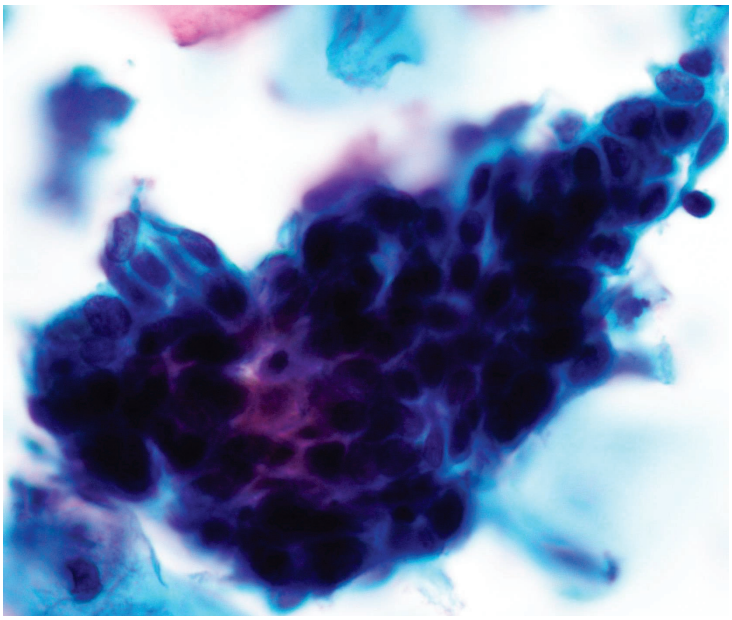


Tubal Metaplasia

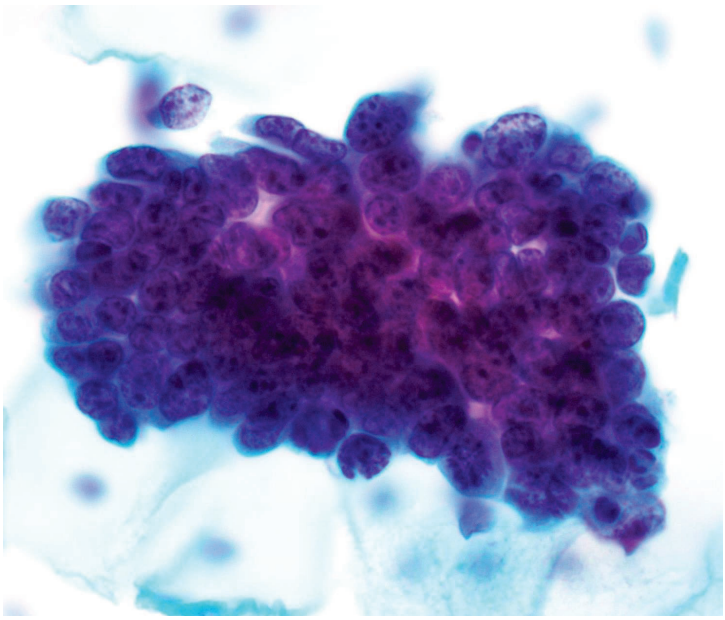
| | Tubal Metaplasia | Endocervical Adenocarcinoma In Situ |
|---|---|---|
| **Clinical** | Any age | Any age but avg of late 30's |
| **Cellular pattern** | Low to high cellularity; found singly or in strips, flat sheets, or crowded clusters | Usually cellular; hyperchromatic crowded groups or strips |
| **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 |

# HSIL vs AIS

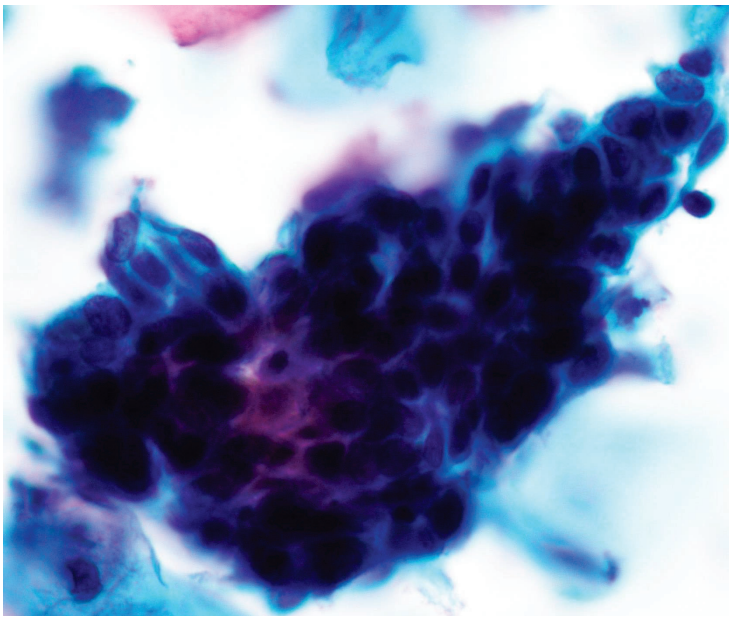
- Can resemble each other almost to perfection
- “Atypical glandular cells” are more frequently HSIL than AIS
- Both: hyperchromatic crowded groups, mitoses, apoptosis, coarse chromatin
- Call AIS when there is clear columnar glandular differentiation (ie strips of columnar cells, rosettes, feathering)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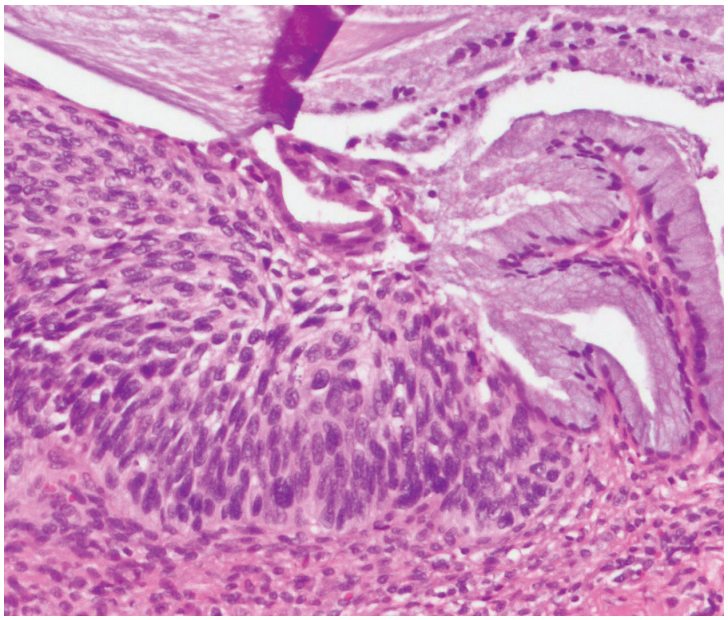
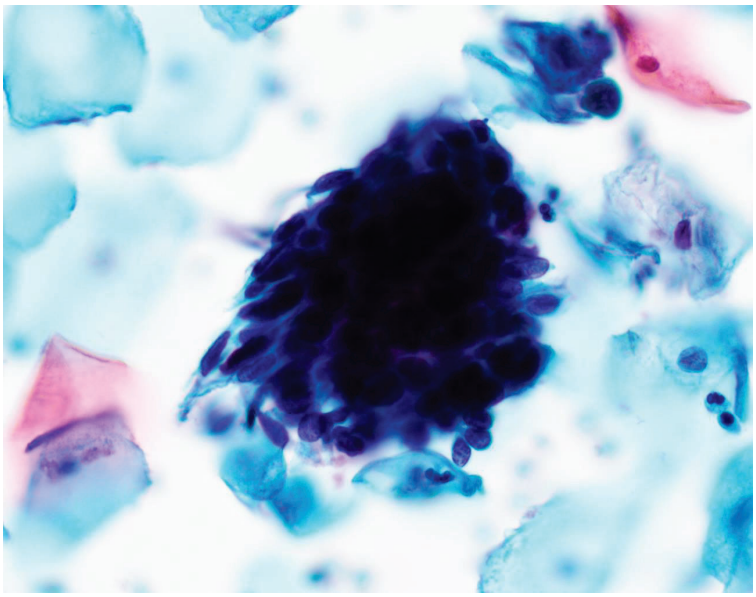
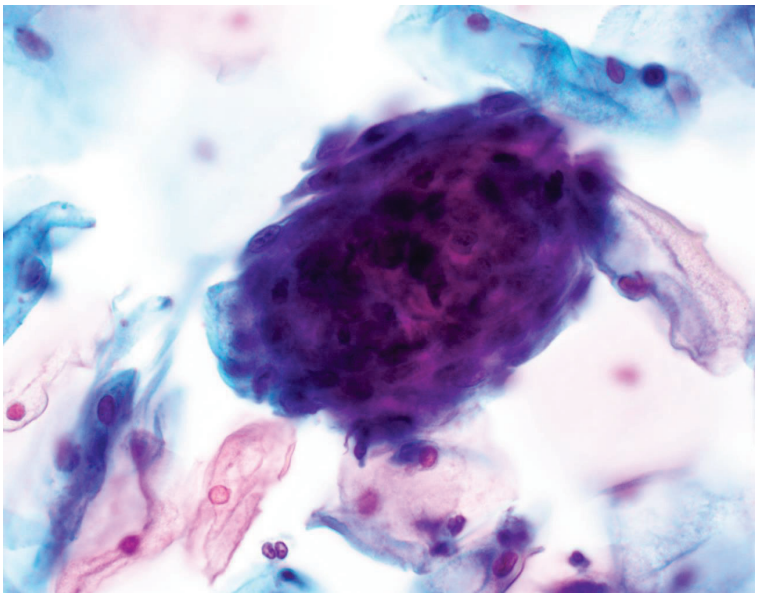


High Grade Squamous Intraepithelial Lesion
(involving endocervical glands)



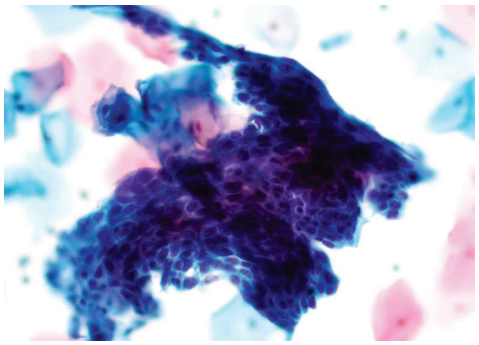
High Grade Squamous Intraepithelial Lesion
(involving endocervical glands)

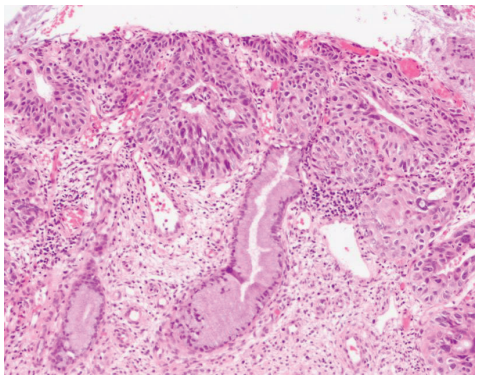


High Grade Squamous Intraepithelial Lesion
(involving endocervical glands)

# 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

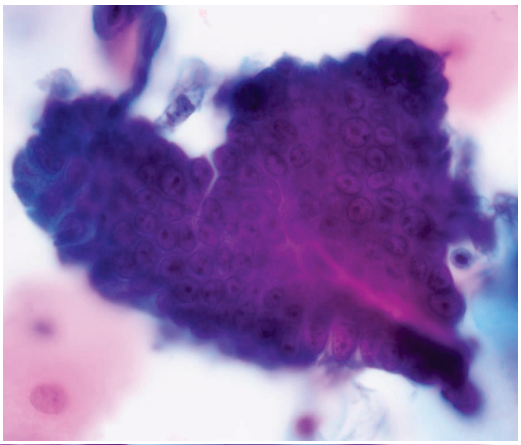
# A note...

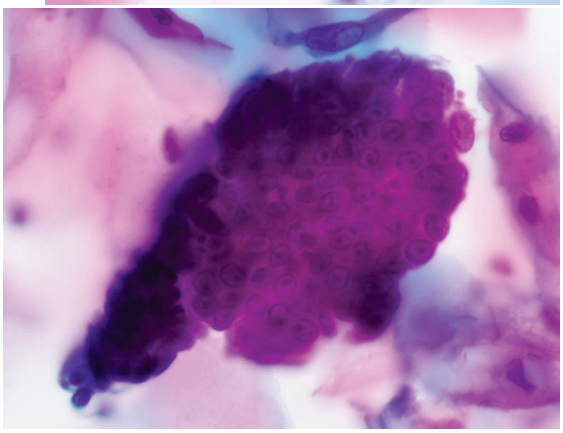
- When a combined diagnosis describing both a squamous lesion and glandular abnormality is given, follow-up pathology often reveals a squamous lesion and rarely a combined lesion
- Glandular and squamous lesions can coexist, but squamous lesions are far more common
- In some studies, up to half of AIS 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



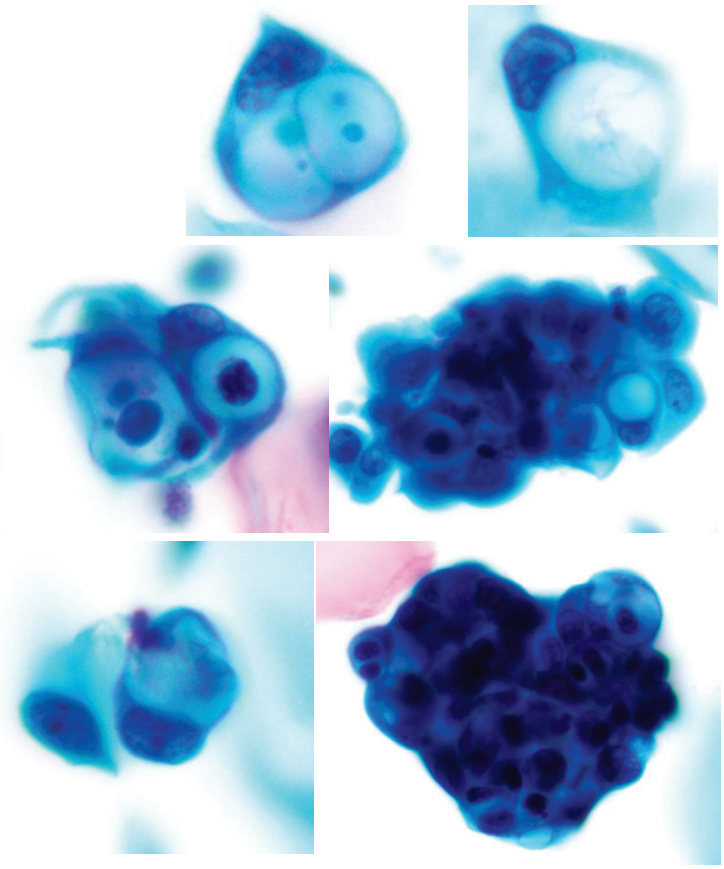


# Endocervical Adenocarcinoma

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

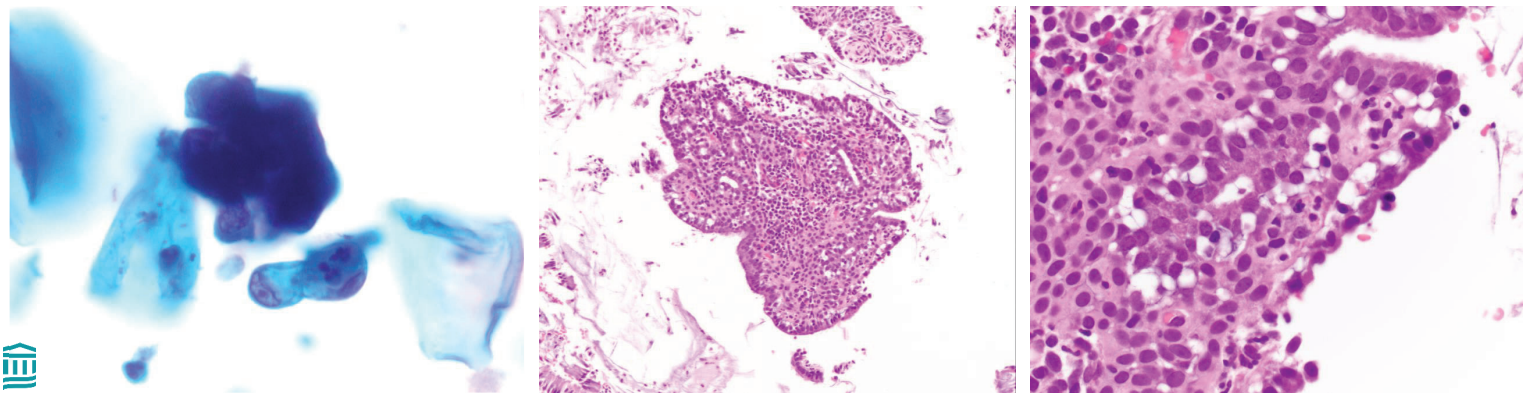
# IUD – Vacuolated Cells

- 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



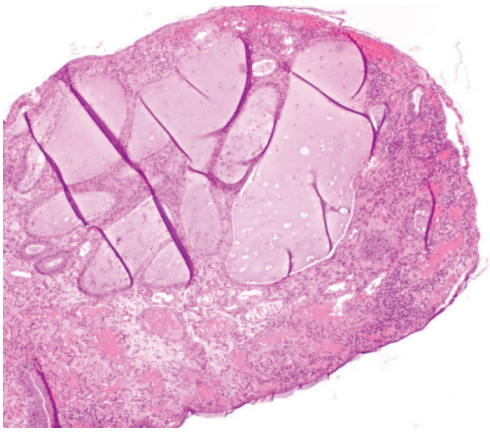
# Microglandular hyperplasia

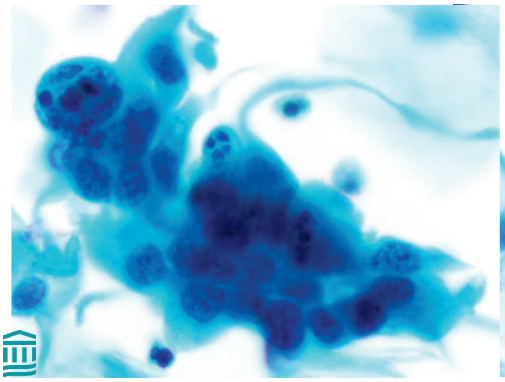
- Cervical lesion commonly incidentally identified on surgical specimens
  - Reproductive age women, contraceptive use or pregnancy
- Histologic findings include closely packed tubular or irregular glands lined by cuboidal to low columnar mucinous epithelium with characteristic sub-and supranuclear vacuoles and with associated inflammation
- A well-recognized diagnostic pitfall on small/fragmented biopsies/curettage specimens

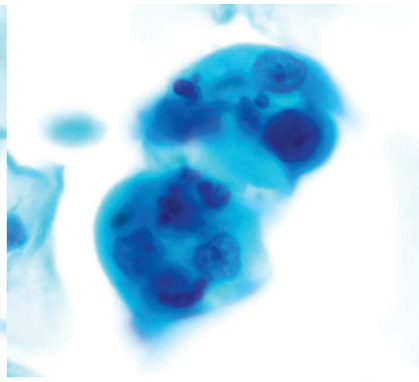


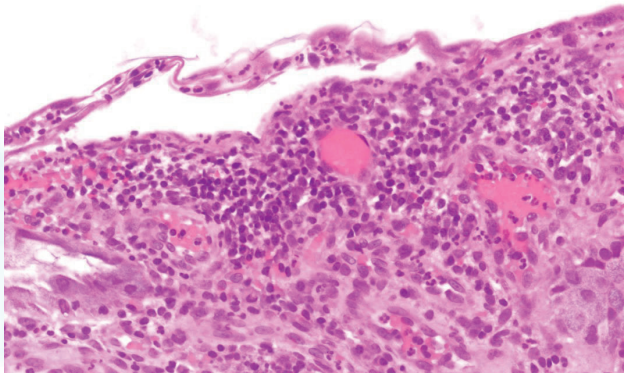
# Polyps

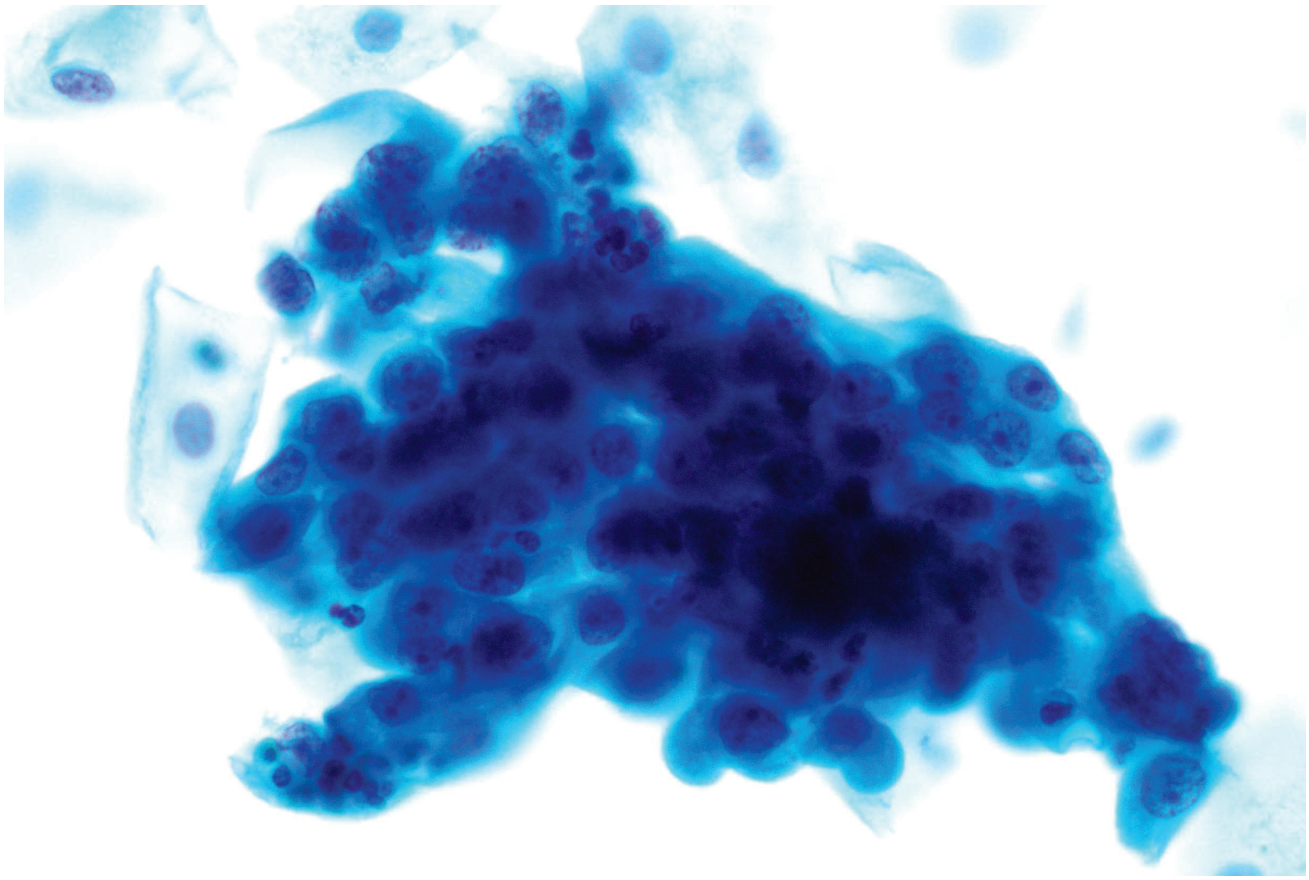
- Morphologic overlap with adenocarcinomas possible, particularly when inflamed
  - "Bag of polys" not specific
- May not be possible to distinguish from adenocarcinoma without prior clinical knowledge





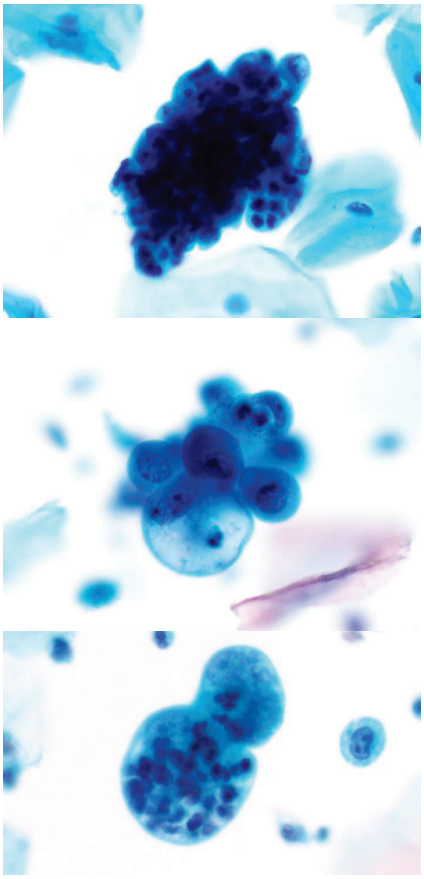


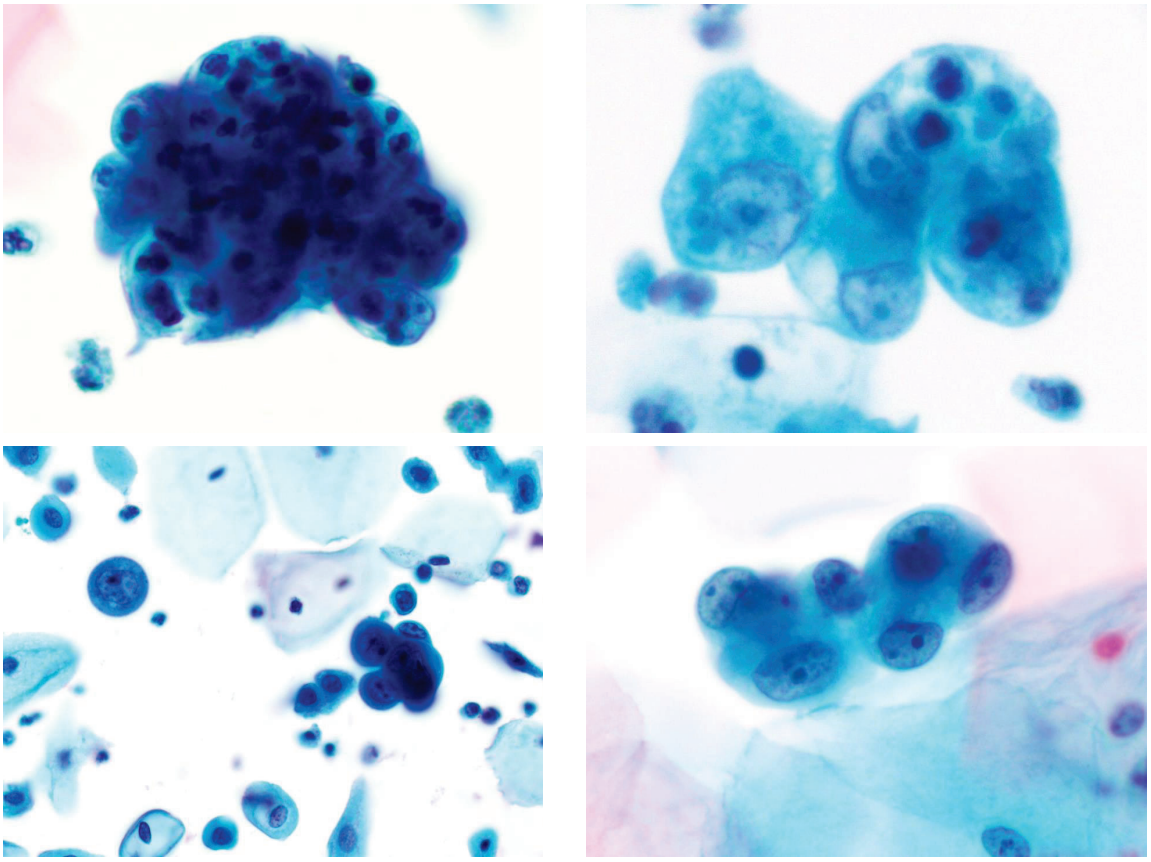


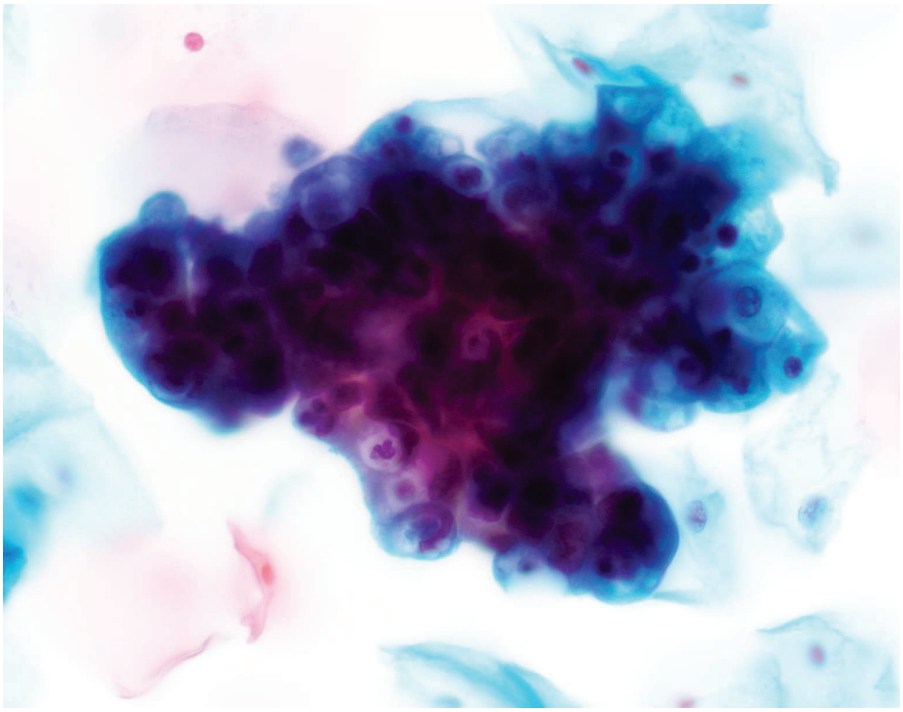


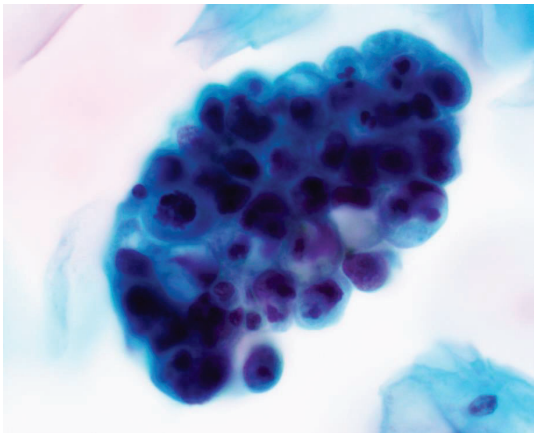
# Endometrial Adenocarcinoma

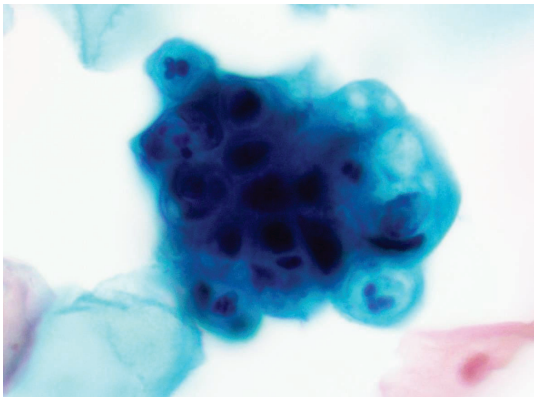
- Findings largely dependent on grade of tumor
- Arranged singly or as small tight clusters
- Round cells
- Variably sized nuclei with loss of nuclear polarity
- Hyperchromatic
- Small to prominent nucleoli
- Scant or abundant vacuolated cytoplasm
- Intracytoplasmic neutrophils (“bag of polys”)
- Variable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



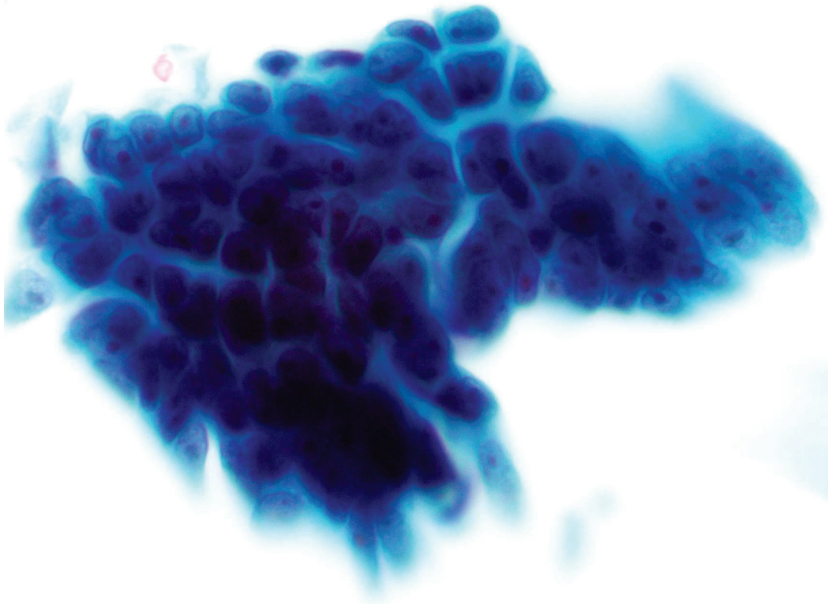


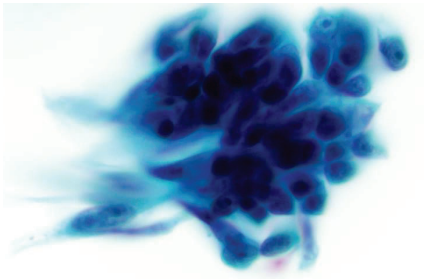


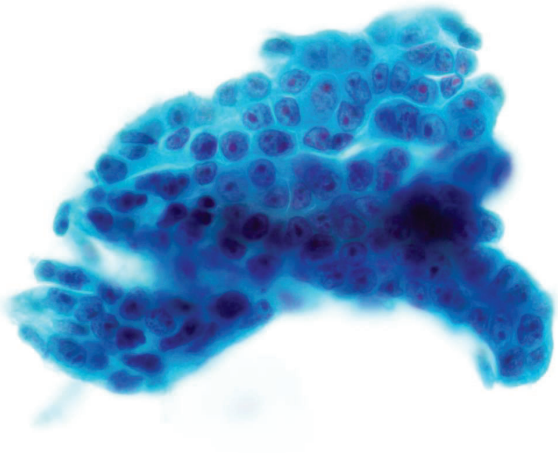




Invasive Endocervical Mucinous Adenocarcinoma







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 with glandular lesions causing particular challenge
- 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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- @VandaTorousMD