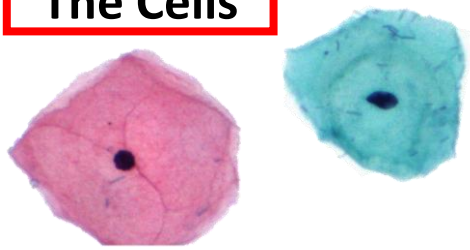
Last updated: 6/3/2025

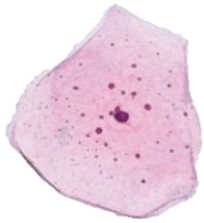
Prepared by Dr. Kurt Schaberg

Cervical Cytology

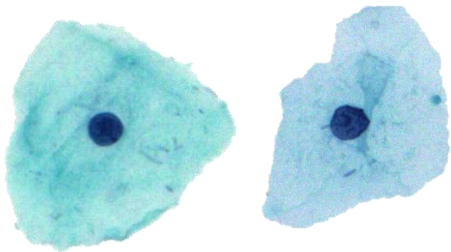
The Cells



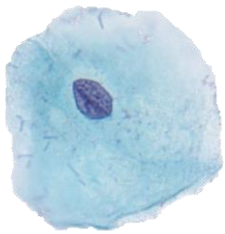
Superficial Cells:



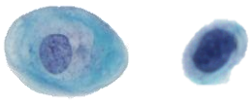
- Small, pyknotic, condensed nucleus
- Abundant cytoplasm (Often pink, can be blue)
- Polygonal shape
- Indicate abundant Estrogen
- Outermost epithelial cells



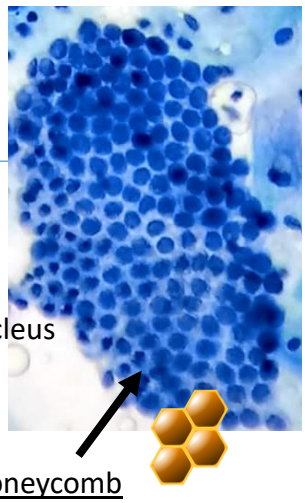
Intermediate Cells:



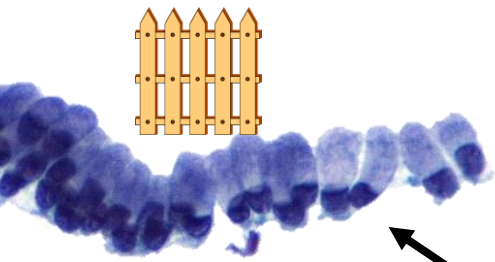
- Abundant blue cytoplasm, polygonal shape
- Larger, round to oval nuclei
- Finely granular, normochromatic nuclei
  - → Nuclei are important reference size
- Middle layer of squamous epithelium



Basal/Parabasal Cell:

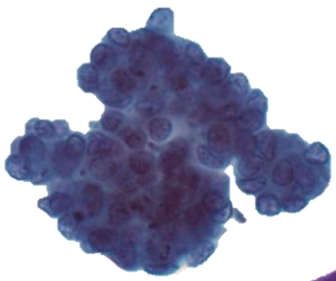


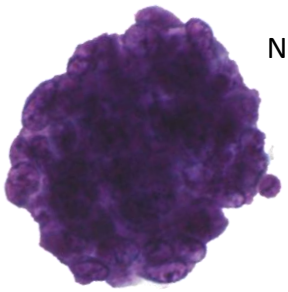
- Minimal and dense cytoplasm
- Round to oval nuclei
- Fine, but slightly darker chromatin
- Usually few in number, unless atrophic



Endocervical Cells:

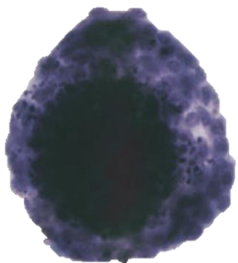
- Uniform, Columnar cells
- Polar, with round nucleus at one end
- Nucleus slightly bigger than intermediate nucleus
- Majority of cytoplasm occupied by mucin
  - → appears vacuolated to granular
- Appearance depends on orientation:
  - En face→ Arranged in flat sheets→ think "Honeycomb
  - From side→ Arranged in linear strips→ "Palisaded" or picket fence





Endometrial Cells:

- Small, High N:C ratio cells, monotonous (almost all nucleus!)
- Nucleus about the same size as an intermediate cell nucleus
- Round or cup-like nuclei with dense, heterogeneous chromatin
- Nucleoli are inconspicuous. Karyorrhexis common.
- Can be in large 3-D groups
  - "Exodus ball" → outside epithelium and in inside stroma
- Normal finding in first half of menstrual cycle if premenopausal (Report if >45 yrs old)



Exodus ball

The Bethesda System for Reporting Cervical Cytology

Findings are systematically reported in the following order:
1) *Specimen type* (conventional vs liquid-based, etc…)
2) *Specimen adequacy* (and quality indicators)
3) *General Categorization*: Negative for Intraepithelial Lesion or Malignancy ("*NILM*")
vs. Epithelial cell abnormality (see options below)
(with room for additional "interpretation/results," like infections)

Squamous cell lesions	Glandular lesions
Atypical squamous cells of undetermined significance (**ASC-US**)	Atypical Glandular (or endometrial/endocervical) cells, NOS
Atypical squamous cells cannot exclude HSIL (**ASC-H**)	Atypical Glandular (or endometrial/endocervical) cells, favor neoplastic
Low-grade squamous intraepithelial lesion (**LSIL**)	Endocervical adenocarcinoma in situ (**AIS**)
High-grade squamous intraepithelial lesion (**HSIL**)	Adenocarcinoma (endocervical, endometrial, extrauterine, NOS)
Squamous cell carcinoma (**SCC**)	

Adequacy Criteria

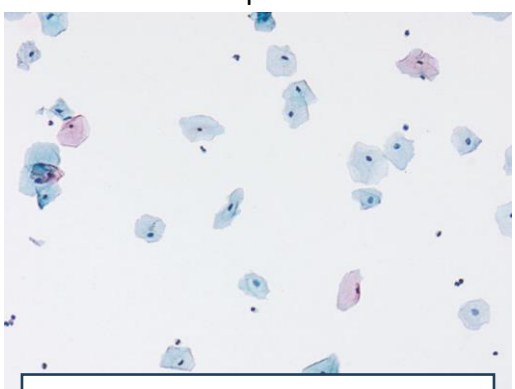
Minimum number of well-visualized and well-preserved squamous cells for adequacy.
Liquid-based preparation: 5,000 cells (ThinPrep and SurePath)
Conventional Preparation: 8,000 to 12,000 cells
(In practice, the number of cells is estimated based on sample photos, and the cells aren't counted).

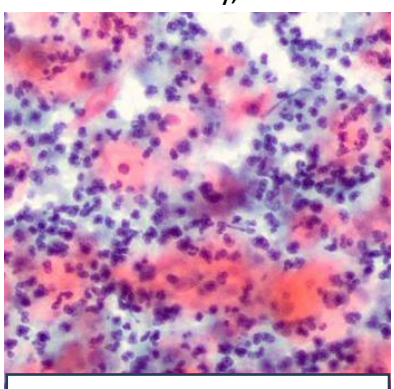
If obscuring elements cover >75% of epithelial cells → Unsatisfactory
Common obscuring elements: Blood, Lubricant, Acute inflammation, excessive drying artifact
If obscuring blood is the issue→ the lab can try reprocessing with glacial acetic acid (lyses RBCs)

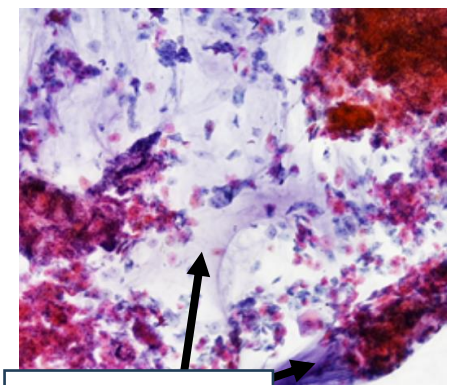
EC/TZ Quality indicator (Reported but not required)**:** Presence of ≥10 endocervical cells or squamous metaplastic cells (since SIL is thought to be most common at transition zone).

Any specimen with abnormal cells is considered adequate and should be reported!

Note: Women who have had chemo or radiation therapy, who are postmenopausal with atrophic changes, or who are post-hysterectomy may have samples with fewer than 5,000 cells, but they may still be considered adequate at the discretion of the laboratory, but must still have >2,000 cells.


Satisfactory, but borderline, cellularity

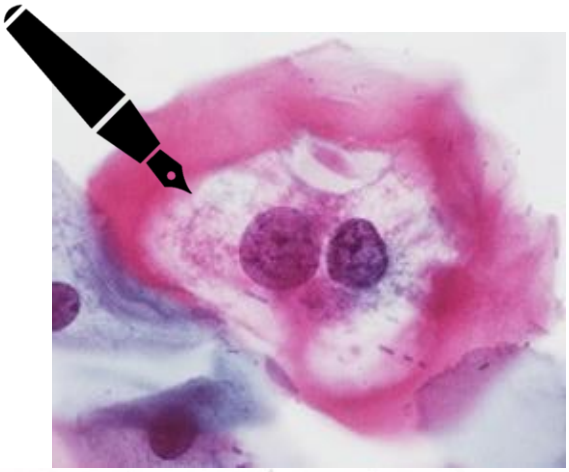

Obscuring acute inflammation


Obscuring lubricant

Squamous Lesions

Low-Grade Squamous Intraepithelial Lesion (LSIL)

aka "mild dysplasia." Correlates with CIN1

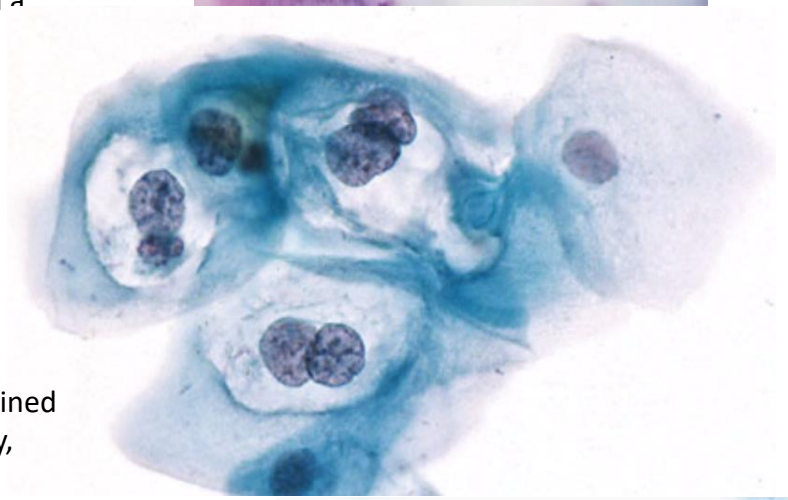


Mature Keratinocytes (intermediate/superficial cells with lots of squamous cytoplasm) AND:

- **Enlarged nuclei (≥3x normal** intermediate cells)

Optional:

- Nuclear **membrane irregularities**
- **Hyperchromasia** ("Rasinoid")
- Perinuclear **Halos = Koilocytes**
  - Large, irregular clearing
  - Thick borders, like it was drawn with a calligraphy pen
- **Multinucleation**
- NO nucleoli

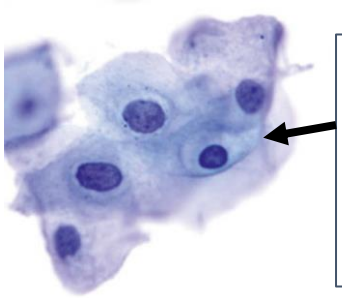


Caused by High and Low-risk HPV
May regress spontaneously!

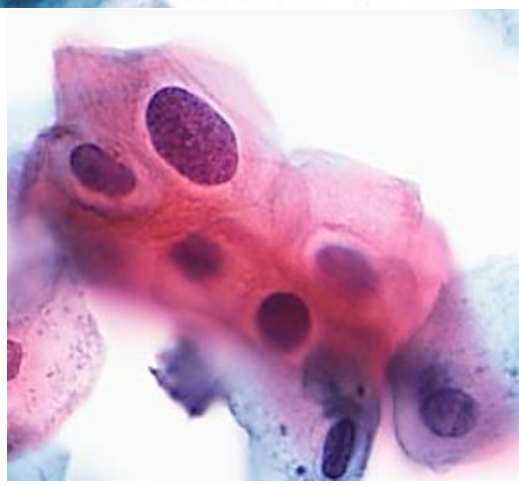
Some findings, but "not enough"?

Consider "Atypical Squamous Cells of Undetermined Significance" (**ASC-US**), which means, essentially, changes *suggestive* of SIL

Can be either Quantitative (i.e., only rare atypical cells) or Qualitative (e.g., only ~2.5x nuclear enlargement)

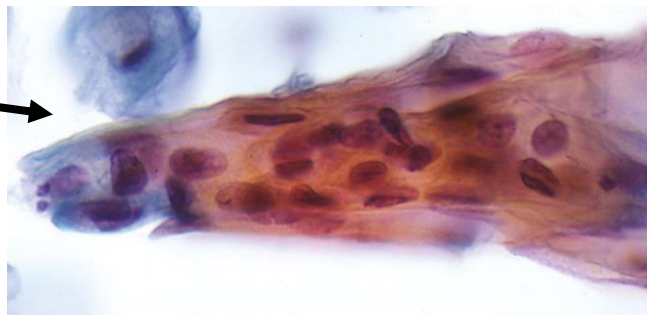


This case has some hyperchromasia and nuclear contour irregularities (but not enough for LSIL), and was called ASC-US



Abnormal keratotic cells?

Be sure to scrutinize parakeratosis, as it can show HPV-related changes too. If there are abnormal nuclei, consider ASC-US (or higher)

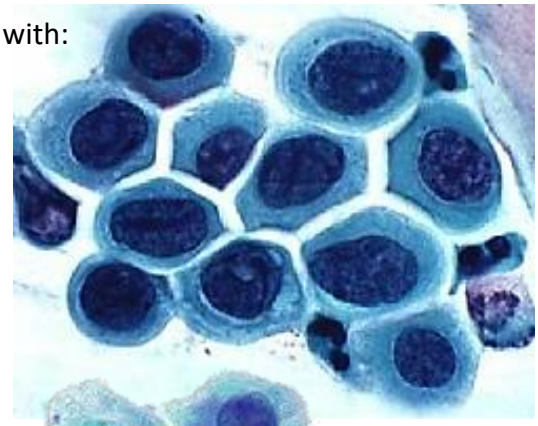


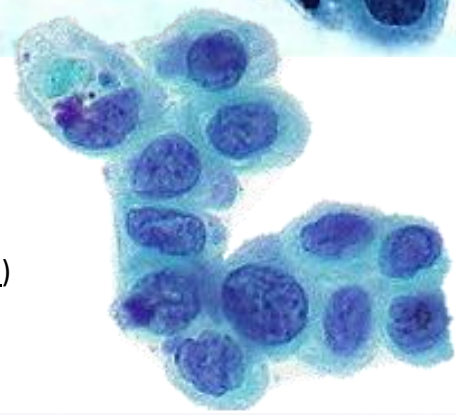
High-Grade Squamous Intraepithelial Lesion (HSIL)

Immature keratinocytes (minimal cytoplasm, **High N/C ratios**) with:

- **Markedly irregular nuclear contours**
  - *(Hint: think in 3-dimensions)*
- **Irregular chromatin**
  Usually, Hyperchromasia (but can be *hypo*chromatic)
- **Pleomorphic** (different than neighbors—few "twins")
- Variable cell size and cytoplasm quality

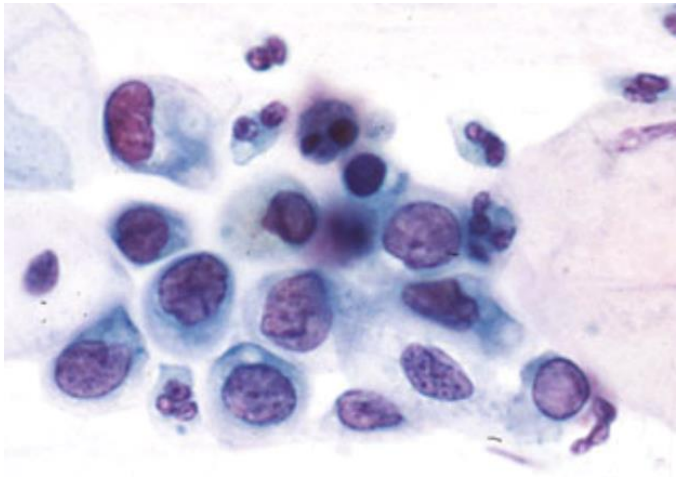
***Think**: **boulders** or **raisins** with all the nuclear irregularities*

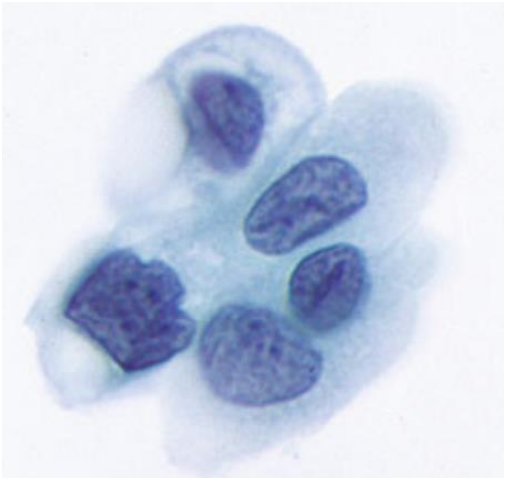




Some findings, but "not enough"?
Consider "Atypical Squamous Cells—Cannot exclude HSIL" (**ASC-H**)

Can be either Quantitative (i.e., only rare atypical cells) or Qualitative (e.g., only moderate atypia)



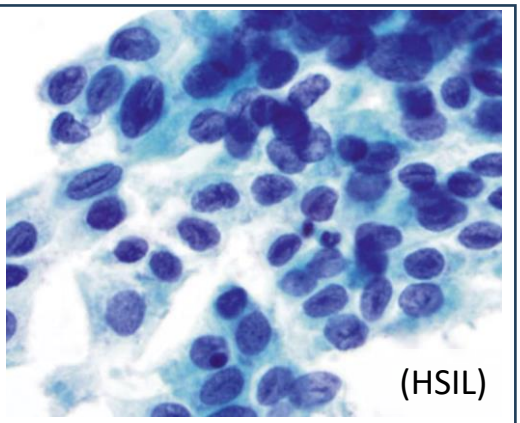


Pro tip: Although it isn't necessary for diagnosis, I often look in the background of possible HSIL cases for LSIL, which supports an HPV-mediated dysplasia (and mentally allows me to relax some).

"Hyperchromatic Crowded Groups" (HCGs)
aka "Crowded sheet pattern" or syncytial aggregates
Names used for high N:C ratio groups with a syncytial appearance.
DDX: HSIL, atrophy, glandular cells, immature metaplasia

Essentially, use same criteria used on single cells: look for nuclear contour and chromatin irregularities (admittedly, this is *harder* though in crowded groups, so try looking at the edges).

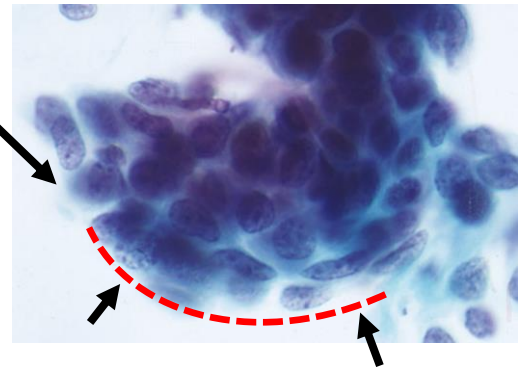
Atrophy, when viewed in a single focal plane, will generally show no nuclear overlapping, while dysplastic lesions will show nuclear overlapping. Mitoses are suggestive of dysplasia.



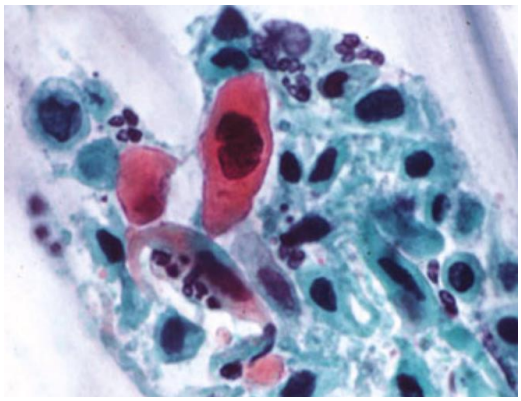
(HSIL)

Flattening at the edges of an HSIL group is suggestive of endocervical gland involvement (and favors squamous over glandular cells).

The same is true of central spindling and whirling.



"HSIL with Features Suspicious for Invasion"
HSIL cells with keratinized cytoplasm
present that are *not* accompanied by the characteristic background features of invasion: necrosis or tumor diathesis
OR
the slide may contain features suggesting tumor diathesis (blood, necrosis, or granular proteinaceous debris in the background), but overtly malignant cells may not be identified



What if there are a few weird high N:C ratio cells, but you're not quite at ASC-H, which usually results in colposcopy (and you don't want to ignore them either)?

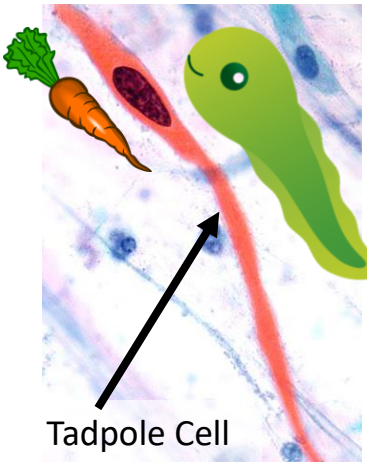
In this scenario, I consider the diagnosis of ASC-US. This allows for "HPV-triage" in many circumstances, such that the patient will be referred for colposcopy only if they test positive for HPV. This can allow for some patients to avoid likely unnecessary procedures if I have a somewhat low index of suspicion.

Squamous Cell Carcinoma

Non-keratinizing SCC: may look like HSIL (similar findings).

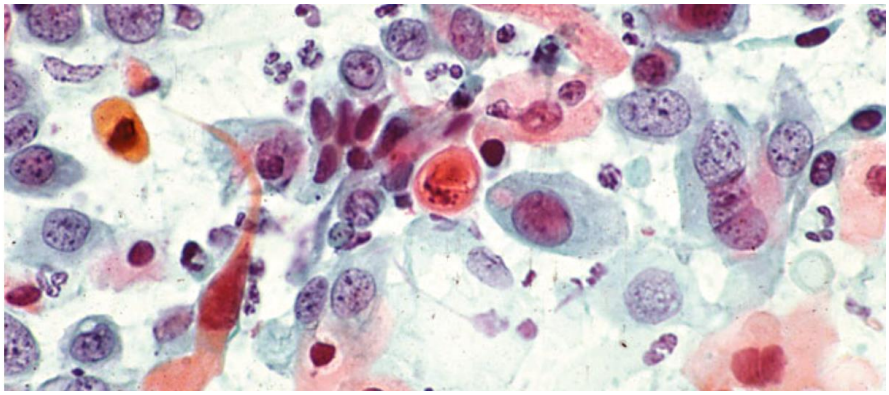
Keratinizing SCC: Pleomorphic cells with hyperchromatic, irregular nuclei, prominent orangeophilic (keratinizing) cytoplasm, and bizarre shapes (like "Tadpoles" or snakes)

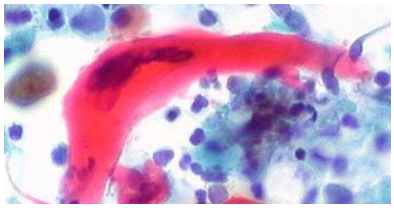
Clues to invasion: "Tumor diathesis" (Necrotic debris)
Prominent nucleoli



Tadpole Cell

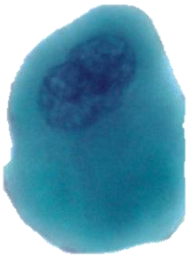
*Sometimes these nuclei are described as looking like **carrots**.*

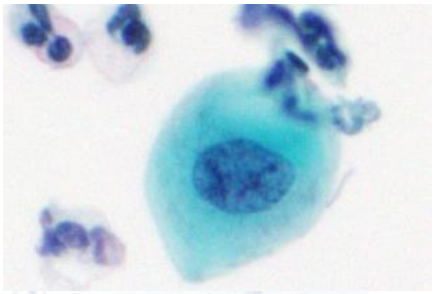




Non-Neoplastic findings

Squamous metaplasia

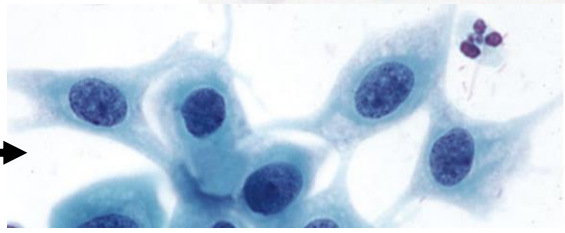




Protective response (to inflammation/trauma) converts endocervical cells to squamous cells.
Count as sampling of transition zone

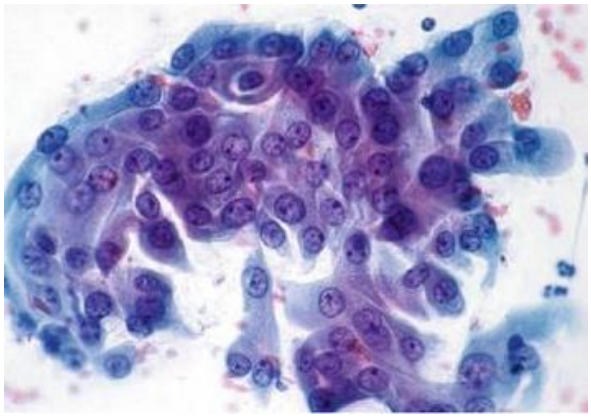
Thick, "Dense" cytoplasm (consistent, dark teal)
Sharply defined cell borders.
Round/oval nuclei, usually central
Normal nuclear size. Even chromatin.

On conventional preparations, can have "spidery" cytoplasmic projections.



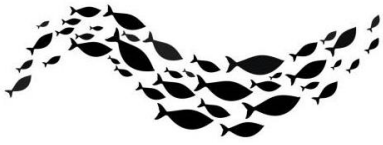
Although we are often somewhat "*forgiving*" of squamous metaplastic cells, they don't have a "get out of jail free card." That is, we might allow them to be high N:C ratio, and maybe a little dark, but they can't have nuclei that are too irregular. If they do, consider HSIL or ASC-H.

Reparative/Inflammatory Changes



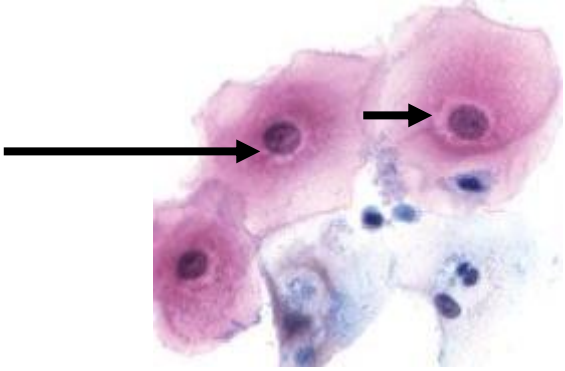
Classic "Repair"

Enlarged nuclei with Prominent Nucleoli.
Round nuclear contours with fine, pale chromatin.
Normal N:C ratios, but variably sized
Cohesive flat sheets of cells with "**streaming**" like pulled taffy or a school of fish.
Background inflammation



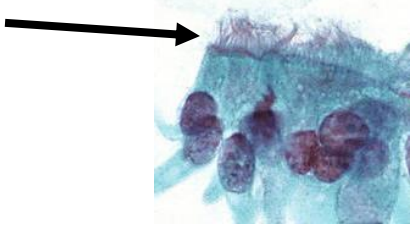
General inflammatory change

Mild nuclear enlargement (<2x size)
Fine, pale chromatin
Often nucleoli
Shouldn't be much nuclear overlapping
Can see **small** perinuclear clearing, but smaller, and more even than koilocytic halos

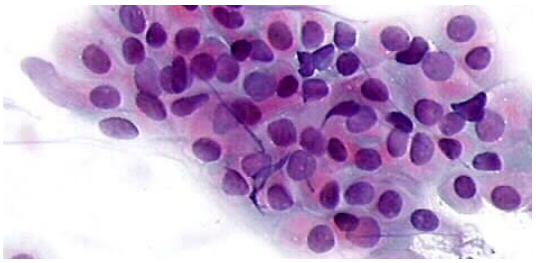


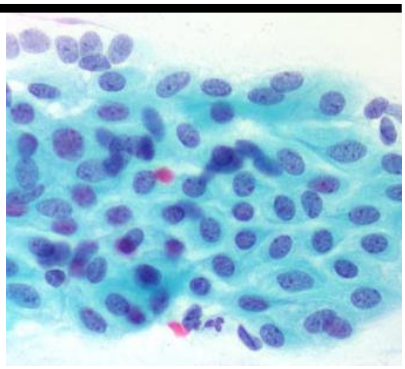
Tubal metaplasia

Normal endocervical cells replaced by **Fallopian tube-like** cells.
Pseudostratified, **ciliated epithelium**.
Nuclei can be enlarged/reactive.
Main importance: *mimic* of neoplasia



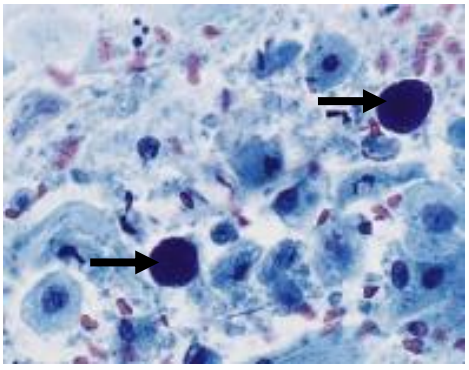
Atrophy





Seen in **LOW estrogen states:**
- Postmenopausal
- Postpartum
- Premenarche
- Turner syndrome

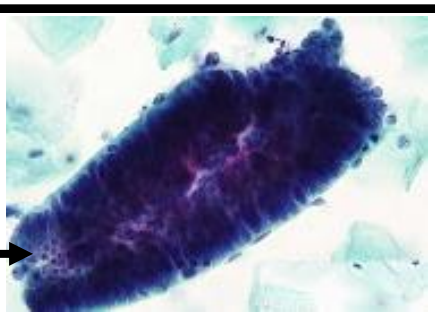
Predominance of **basal and parabasal cells** (High N:C ratios)
Prone to injury→ often inflammation



Immature cells that can *mimic* HSIL, but, unlike HSIL, they should have:
Finely granular chromatin. May be slightly hyperchromatic.
Smooth nuclear contours. May be slightly elongated
Often have nearby "identical twins" (similar cells)

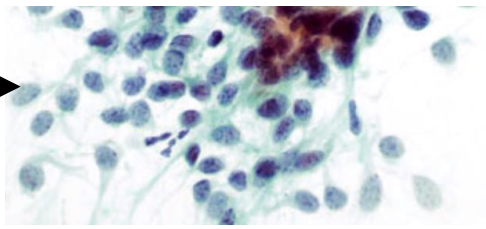
Can see "Blue blobs" (see →) and granular debris ("atrophic vaginitis," mimicking tumor diathesis, but no karyorrhectic nuclear debris)

(Direct) Lower Uterine Segment Sampling



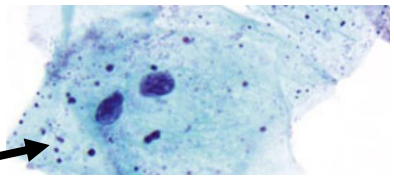
Unlike spontaneously shed endometrial cells, which are seen in rounded "exodus" balls, these are **intact glands *with* stroma**.
Very cellular clusters.

Glands: Branching tubular glands. Columnar cells. Palisaded round to oval nuclei with dark but even chromatin.
Should NOT see prominent nucleoli (otherwise consider atypia)



Stroma: Disorganized spindled cells with scant cytoplasm.
May contain vessels.

Keratotic changes

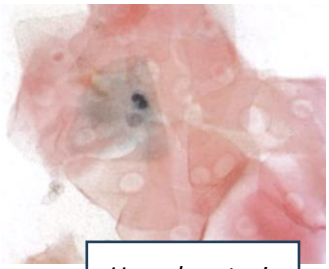


Cervix is usually non-keratinizing, but it can undergo some degree of keratinization as a *reactive/protect phenomenon*

Keratohyaline granules—Small, dark cytoplasmic dots. Precursor to full keratinization

Parakeratosis—Miniature superficial squamous cells with dense orangeophilic/eosinophilic squamous cells with small, dark nuclei.

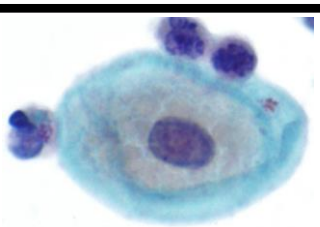
Hyperkeratosis—Anucleate but otherwise unremarkable mature polygonal squamous cells. Empty spaces or "ghost nuclei" may be noted.



Hyperkeratosis

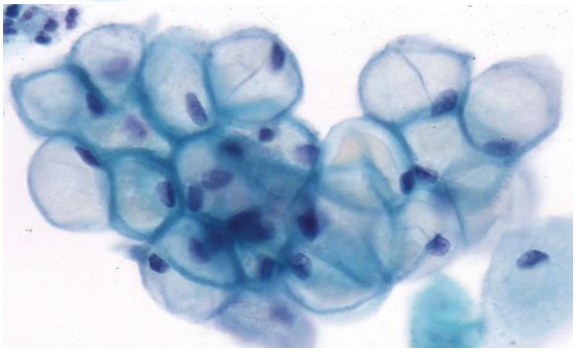
Parakeratosis

Pregnancy-related Changes



Navicular cells—<u>glycogen</u>-laden intermediate cells with flattened "boat-like" contours.

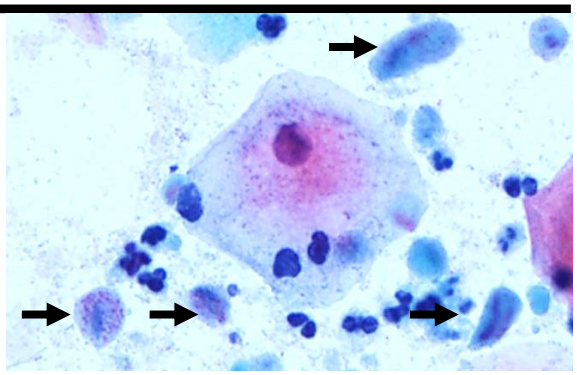
Glycogenation can <u>*mimic*</u> an LSIL halo, however, glycogenation is usually <u>*diffuse*</u> (while SIL is a subset of cells) and there shouldn't be associated nuclear atypia.



(Less likely, may see decidual changes or Arias-Stella reaction, but these are likely hard to diagnose on a Pap!)

Trichomonas Vaginalis

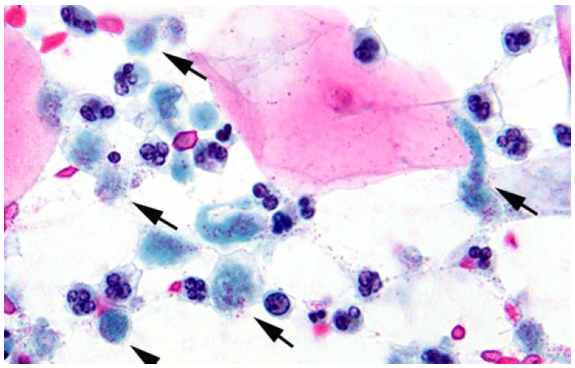
<u>Pear-shaped</u>, oval protozoan STD
Pale, eccentric elongate nucleus
<u>Red cytoplasmic granules</u>
Can see flagella



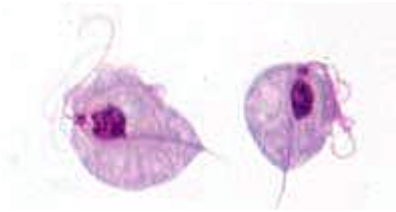
Often associated with Leptothrix

Often acute inflammation (particularly balls of neutrophils) and inflammatory halos.

(When I see lots of neutrophils, I always spend a few seconds on high power looking for Trich)

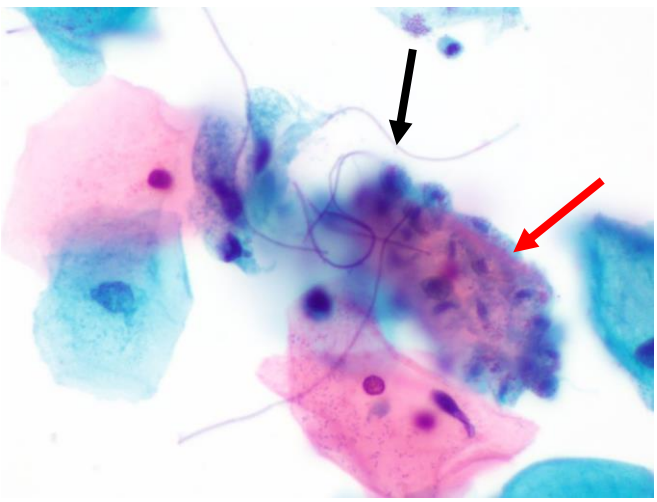


Treat with antibiotics



Leptothrix

Non-pathogenic filamentous bacterium that looks like short thread (→).



Associated with trichomonas (→)

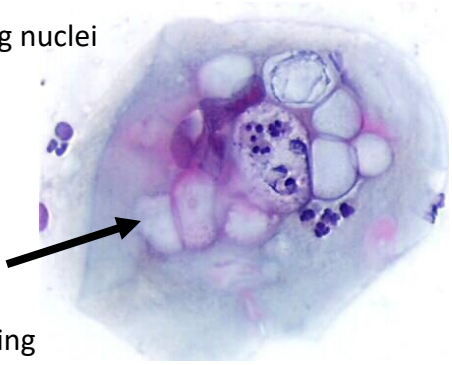
"When you see spaghetti (Leptothrix →), look for meatballs (Trichomonas →)"

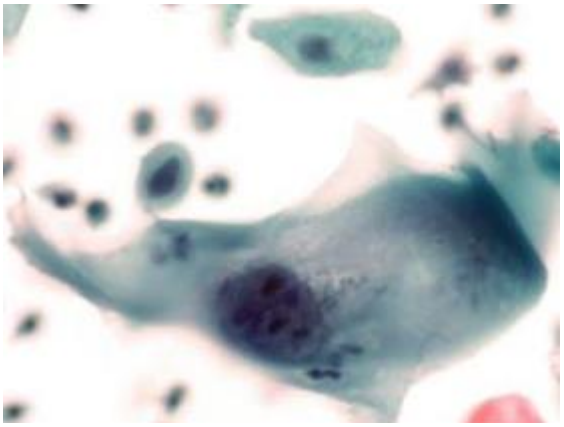
Radiation Changes

Cytomegaly: Big cells with Big nuclei (proportion N:C maintained)

Large, **bizarre cells**

Cytoplasmic **vacuolation** and polychromasia





Multinucleation
Degenerative changes including nuclear pallor, wrinkling or smudging of the chromatin

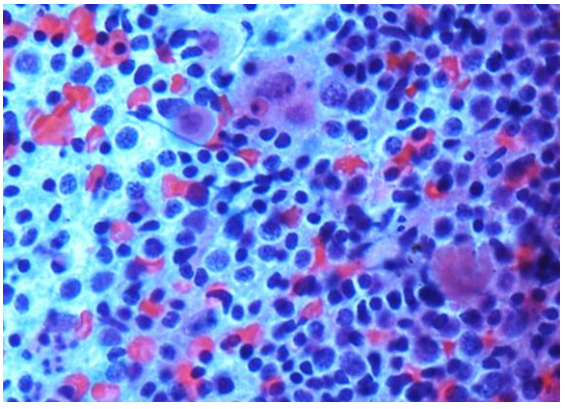
Follicular Cervicitis

Chronic cervicitis that results in the formation of mature lymphoid follicles beneath the epithelium.

Abundant polymorphous **lymphocytes** (small rim of cytoplasm around round nucleus, unlike HSIL, which is irregular)

Numerous tingible body macrophages

Variably sized lymphocytes with plasma cells.



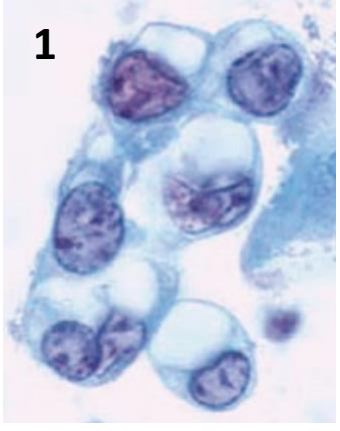
IUD-Effect

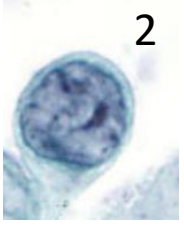
Two characteristic findings:

1) *Clusters* of cells with **abundant vacuolated cytoplasm** (mimicking Adenocarcinoma)

2) *Single cells* with **small, dark nuclei** and scant cytoplasm (mimicking HSIL)

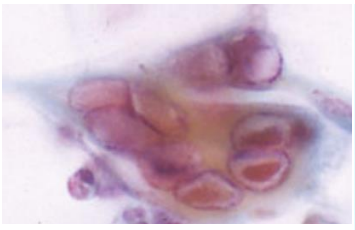
Always know history!
Frequent Actinomyces-like organisms.

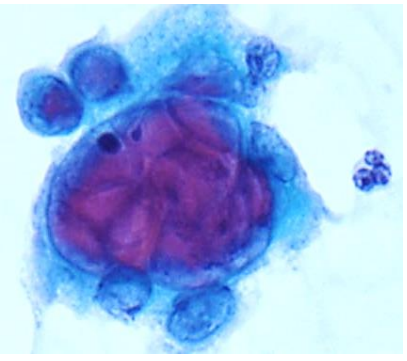




Herpes

3 M's
Molding of nuclei
Multinucleation
Margination of chromatin

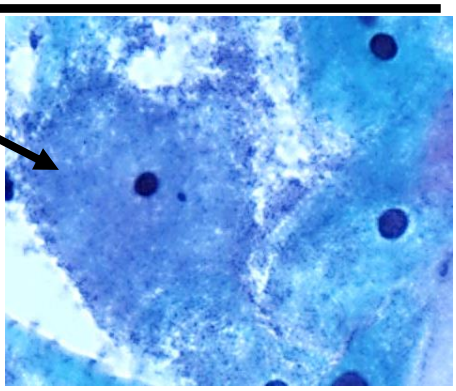




"Ground glass" chromatin with eosinophilic nuclear inclusions

Can treat with acyclovir

"Shift" in flora suggestive of Bacterial Vaginosis

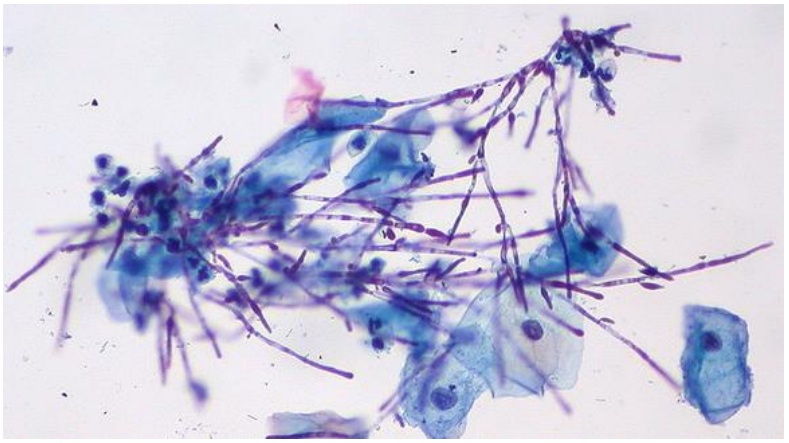


"Clue Cells" = squamous cells <u>covered with shaggy coccobacilli</u> and mixed bacteria rather than the normal lactobacilli

Thick, milky vaginal discharge with a foul "fishy" odor
(positive "whiff" test after adding KOH)

Shift in vaginal flora from lactobacilli to a polymicrobial process involving several types of anaerobic bacteria, like *Gardnerella vaginalis*
Treat if symptomatic.

Candida

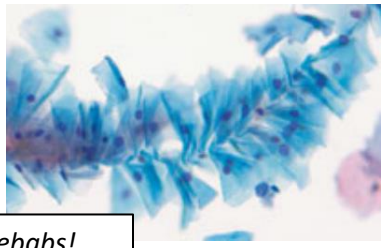


Fungal species that can cause infections throughout the GYN tract (and other areas).
Thick, "cottage cheese" discharge

Eosinophilic yeast forms and **<u>pseudohyphae</u>**
("Spaghetti and meatballs")
Often <u>tangled or skewering squamous cells</u>

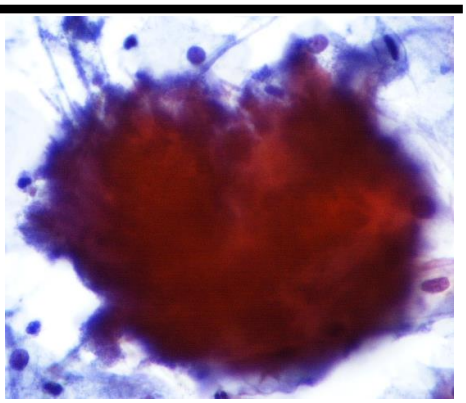
Can have variable associated inflammation or inflammatory halos

Usually only treat if symptomatic



Look like shish kebabs!

Actinomyces

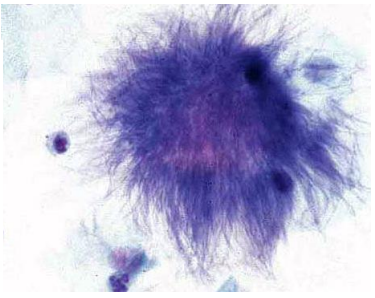


Gram-positive **anaerobic bacteria**
Commonly associated with **<u>IUD</u>** (or other foreign body)

<u>Clumps of Long, filamentous organisms</u>
Tangled clumps of bacteria that look like "cotton balls" or "<u>dust bunnies</u>". Radially arranged.

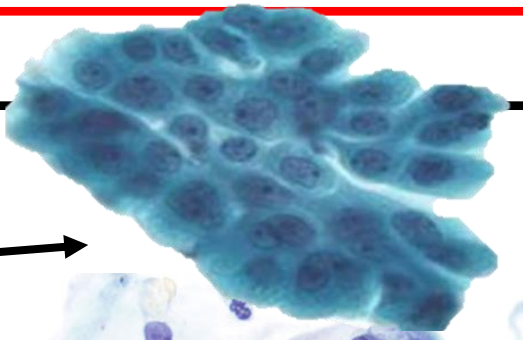
Much thinner than candida.

No need to remove IUD or treat if asymptomatic



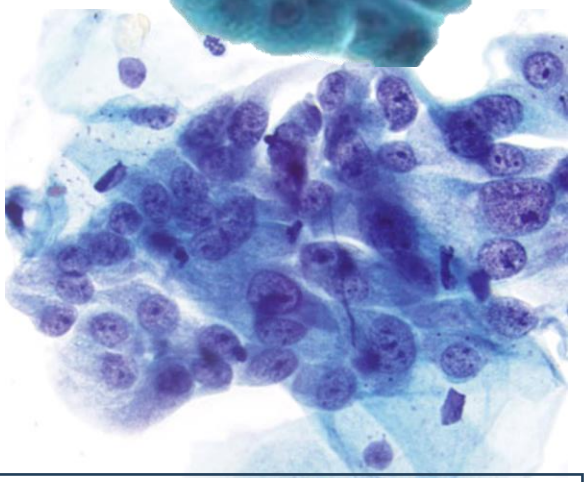
Glandular Abnormalities

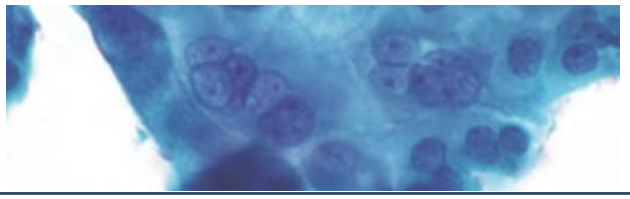
Reactive Endocervical Cells



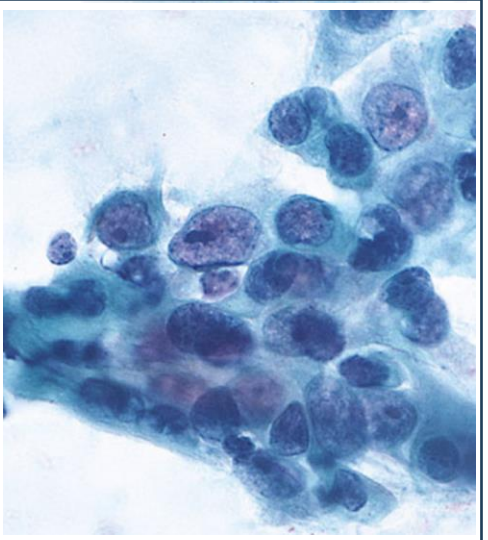
Nuclear enlargement (4-5x),
Uniform, even, finely granular chromatin
Round with smooth contours
Hypo/Hyperchromasia, allowed to a limited extent
Prominent nucleoli common.
Not too crowded. Mitoses, but no apoptosis.
Can be bi/multinucleated.

Can see tubal metaplasia→ look for cilia!
Often mucin-depleted.





Atypical Glandular Cells (AGC)



Used for glandular cells with nuclear atypia that exceeds obvious reactive or reparative changes but lack unequivocal features of adenocarcinoma.

You are supposed specify as to if you believe them to be endocervical or endometrial, but don't have to (and I generally don't personally as it can often be hard to tell).

They don't include "of Undetermined Significance" as they were concerned AGUS would be confused with ASC-US (that said, many people use the term AGUS, in conversation at least)

You can, "*Favor Neoplastic*," but *not* "*Favor reactive*" (as it turns out, we aren't good at favoring reactive).

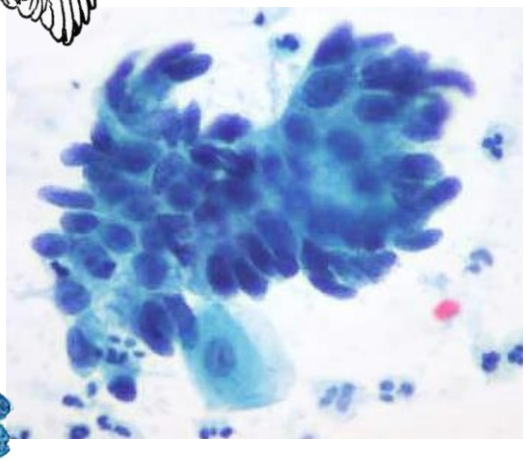
Cytologic criteria	AIS	HSIL	Repair	Tubal metaplasia	Directly sampled endometrium/ endometriosis
Cellularity	Cellular	Usually cellular	Rare fragments	Rare event	Few groups/variable
HCGs	Many	Can be many	Absent	Rare	Present/can be numerous
Sheets/strips	Many with feathering/3D	Syncytia	Flat sheets	Absent/rare	Present, 3D
Nuclear crowding/overlap	Present	Present	Absent	Present but mild	Present
Perpendicular nuclear polarization	Present	Absent	Absent	Present	Can be present
Hyperchromasia	Present	Present	Absent	Mild	Mild
Nuclear shape	Oval/elongate	Round/irregular	Round	Oval/cigar shaped	Oval/cigar shaped
Feathering	Present	Absent/focal	Absent	Rare	Absent/rare
Strips	Present	Absent	Absent	Present	Present
Rosettes	Present	Absent	Absent	Absent	May be present/gland openings/ tubules
Terminal bars/cilia	Absent	Absent	Absent	Present/diagnostic	Rare/may be present
Spindled stroma	Absent	Absent	Absent	Absent	Present
Mitosis/apoptosis	Present	May be seen	Rare	Rare	May be present
p16 pattern	Block positive	Block positive	Negative	Patchy positive	Patchy, focal to rare glandular cells

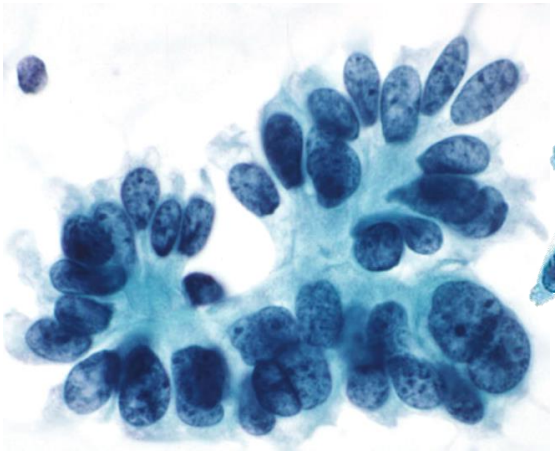
From: The Bethesda System for Reporting Cervical Cytology. 2014.

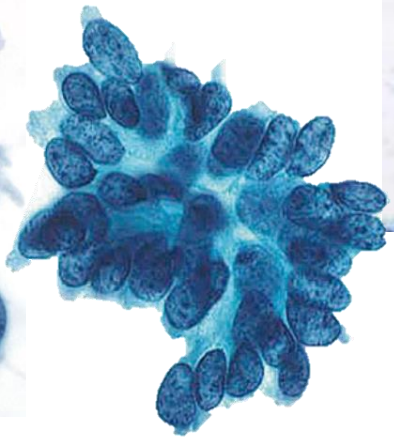
Endocervical Adenocarcinoma In Situ (AIS)

*For AIS, think "**Feathery**," like a bird's wing.*

High N:C ratios with coarse, dark chromatin.
Nuclei enlargement, elongation, pseudostratification and overlap (cigar-like, think GI adenoma)
Cellular **crowding** with rosettes and "**feathery edges**"
Mitoses and **apoptosis**. No nucleoli.
Most strongly associated with HPV18 subtype
Be sure to look for SIL in the background!



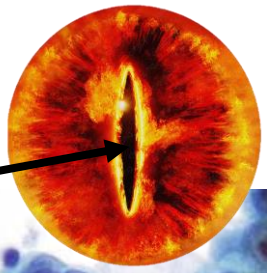


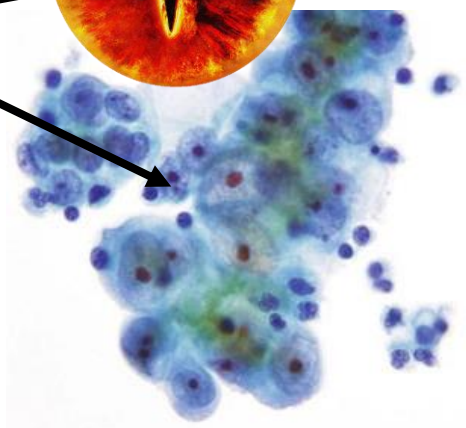


Adenocarcinoma

Morphology variable, depending on site of origin/type.
Generally, more pleomorphic/irregular.

Features suggesting invasion/adenocarcinoma:
1) **Macronucleoli**, *(think: eye of Sauron)*
2)**Tumor diathesis**,
3) **irregular chromatin**, and
4) increased single cells

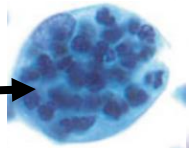


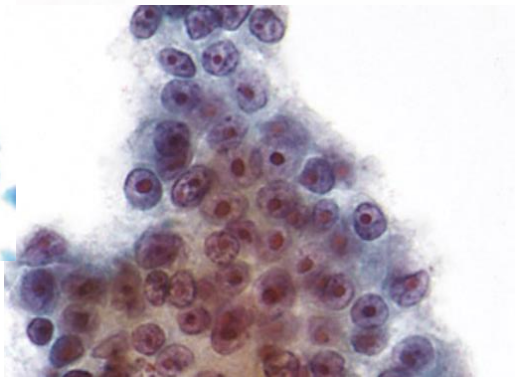


Endocervical adenocarcinoma
Resembles AIS (above), but with additional invasive features.

Endometrial adenocarcinoma
Nuclei larger than intermediate cell.
(The bigger the nucleus/nucleolus→ likely the higher grade)

Factors favoring endometrial adenocarcinoma (vs endocervix): prominent cytoplasmic vacuoles full of neutrophils ("bag of polys")





Pro Tip: If there isn't a biopsy, consider trying to make a cell block from the Pap for IHC to further type.

Human Papilloma Virus (HPV)

Sexually **T**ransmitted **D**isease (STD). Circular double stranded DNA virus

Infects <u>transformation zone</u>→ establishes itself and replicates in basal cells.

<u>Detected in virtually all cervical cancers</u>. However, many infections are transient and naturally cleared, so only a minority persist and lead to cancer.

Viral genes responsible for transforming host cells by integrating into the host DNA and disrupting tumor suppressor genes.
E6→ inactivates p53 (blocks apoptosis)
E7→ inactivates Rb (gets rid of cell cycle arrest→ uncontrolled growth)

<u>HPV subtypes</u>
High-risk (associated with cervical cancer and HSIL, but can also cause LSIL)
16, 18, 31, 33
<u>Type 16 is most commonly detected in cervical cancers</u>
Type 18 is associated with Endocervical Adenocarcinoma

Low-risk (Not associated with HSIL. Instead associated with LSIL/condylomas)
6, 11, 42, 43

Anal Cytology

Since it is also part of the "Lower Anogenital Squamous Tract," anal SCC is also usually HPV-mediated and can be detected by Pap smear. Highest risk individuals are those that are HPV-positive, immunosuppressed, and/or participate in anal-receptive sexual intercourse.

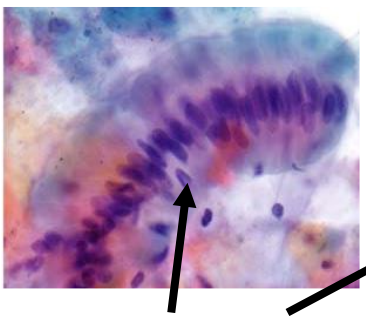
In general, we're **looking for the same findings as in the cervix**, so we use the same terms and criteria.

***<u>Differences</u>* <u>from Cervical Pap:</u>**
Adequacy: 2,000-3,00 nucleated squamous cells
(average of **1–2 cells per HPF** for ThinPrep)

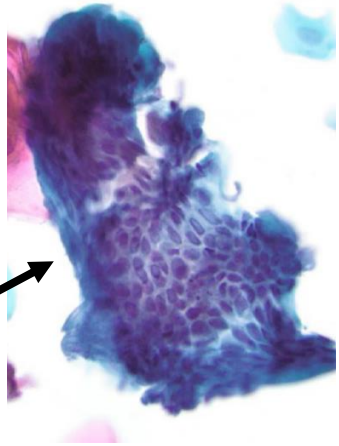
Transformation zone (a "quality indicator") consists of ***<u>rectal</u>*** columnar cells or squamous metaplastic cells (similar to the cervix).

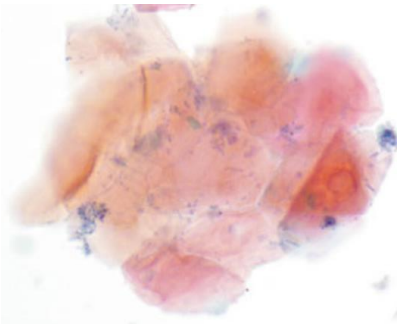
In general, anal Paps are "*dirtier*" with more debris, bacteria, fecal material, and anucleate cells.

Management is generally less aggressive with anoscopy, biopsy, and fulguration, which have relatively low morbidity (things are treated much less aggressively than in the cervix often).

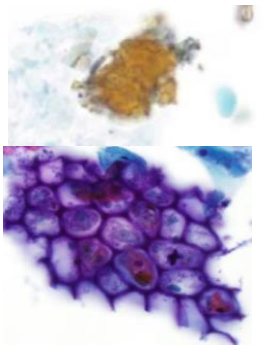


Rectal columnar glandular cells





Anucleate squamous cells with bacteria



Fecal debris (above) and vegetable material (below)

General Management

Remember, this is a *screening test* (primarily for squamous cell carcinoma and its precursors)

So, when interpreting Paps, I often consider what I think the next best step in management is (i.e., do they need colposcopy? How concerned am I?)

This is a very generalized and abbreviated (but hopefully still helpful) summarization!
For a full review of current management guidelines, please refer to the ASCCP website:
http://www.asccp.org/guidelines

Pap result → Next step

Unsatisfactory → Repeat Pap in 2-4 months; But if HPV positive→ colpo

NILM, but HPV positive (> 30 yrs) → Repeat co-testing at 1 yr, OR, HPV subtyping, if high-risk→ colpo

ASCUS → HPV testing ("if you *Ask Us*, you should get an HPV test"), if positive → colpo; Neg→ routine

LSIL → Colposcopy, unless HPV negative, then can do repeat co-testing at 1 yr
21-24 yo with ASCUS or LSIL → follow-up in 12 months (likely to clear spontaneously)

ASC-H → Colposcopy

HSIL→ Colposcopy or LEEP

Atypical glandular cells → Colposcopy with endocervical sampling and endometrial sampling

Screening Recommendations (according to the USPSTF):

Women 21-65 Cytology alone every **3** years
OR Women 30-65 Co-testing (cytology + HPV testing) every **5** yrs
(Don't do HPV testing if less than 30 as high rate of positivity, but also clearance)
(Don't do any testing before age 21, after 65 if they have had good prior screening, or after a hysterectomy for benign reasons)

However, this is currently under revision with a shift toward primary HPV-screening for some individuals. The ACS already has made this shift and recommends the following:

Aged <25: No screening

Aged 25-65: Starting at age 25 y, primary HPV test alone every 5 y (preferred)
OR Co-testing every 5 yrs or cytology alone every 3 yrs are acceptable options

Aged >65: Discontinue screening if adequate negative prior screening