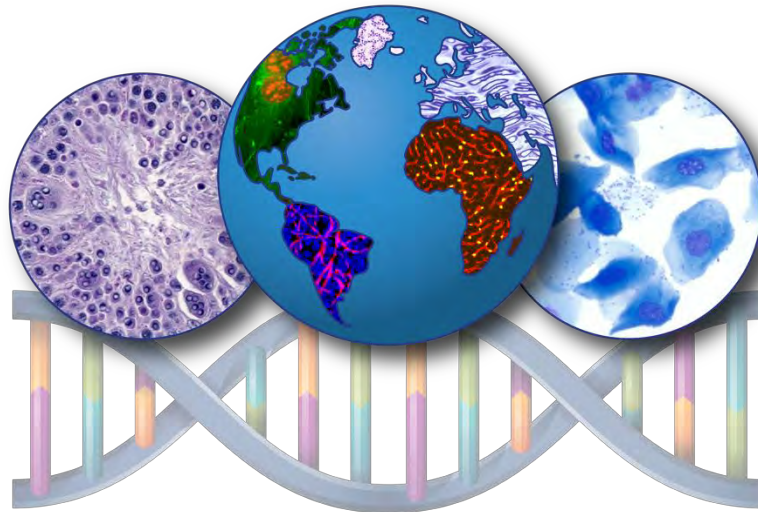




National Institute of
Environmental Health Sciences
Division of Translational Toxicology

Histology and Cytology of the Rodent Estrous Cycle



Division of Translational Toxicology Global Toxicologic Pathology Training Program

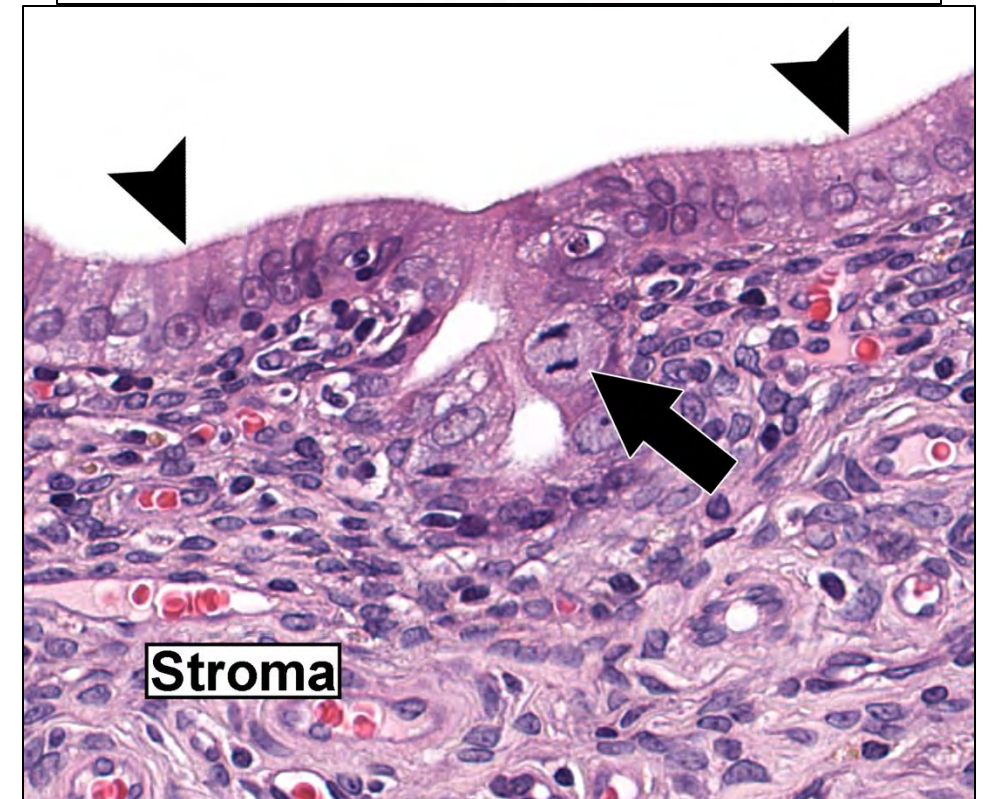
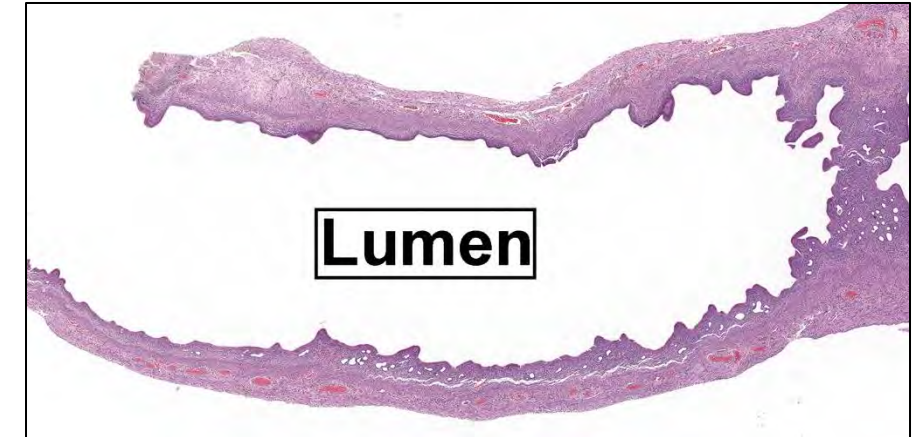
National Institutes of Health • U.S. Department of Health and Human Services

Duration of estrous cycle stages in rats and mice (hours)

Estrous cycle stage	Rats	Mice
Proestrus	≤14	≤ 24
Estrus	24-48	12-48
Metestrus	6-8	≤24
Diestrus	48-72	48-72

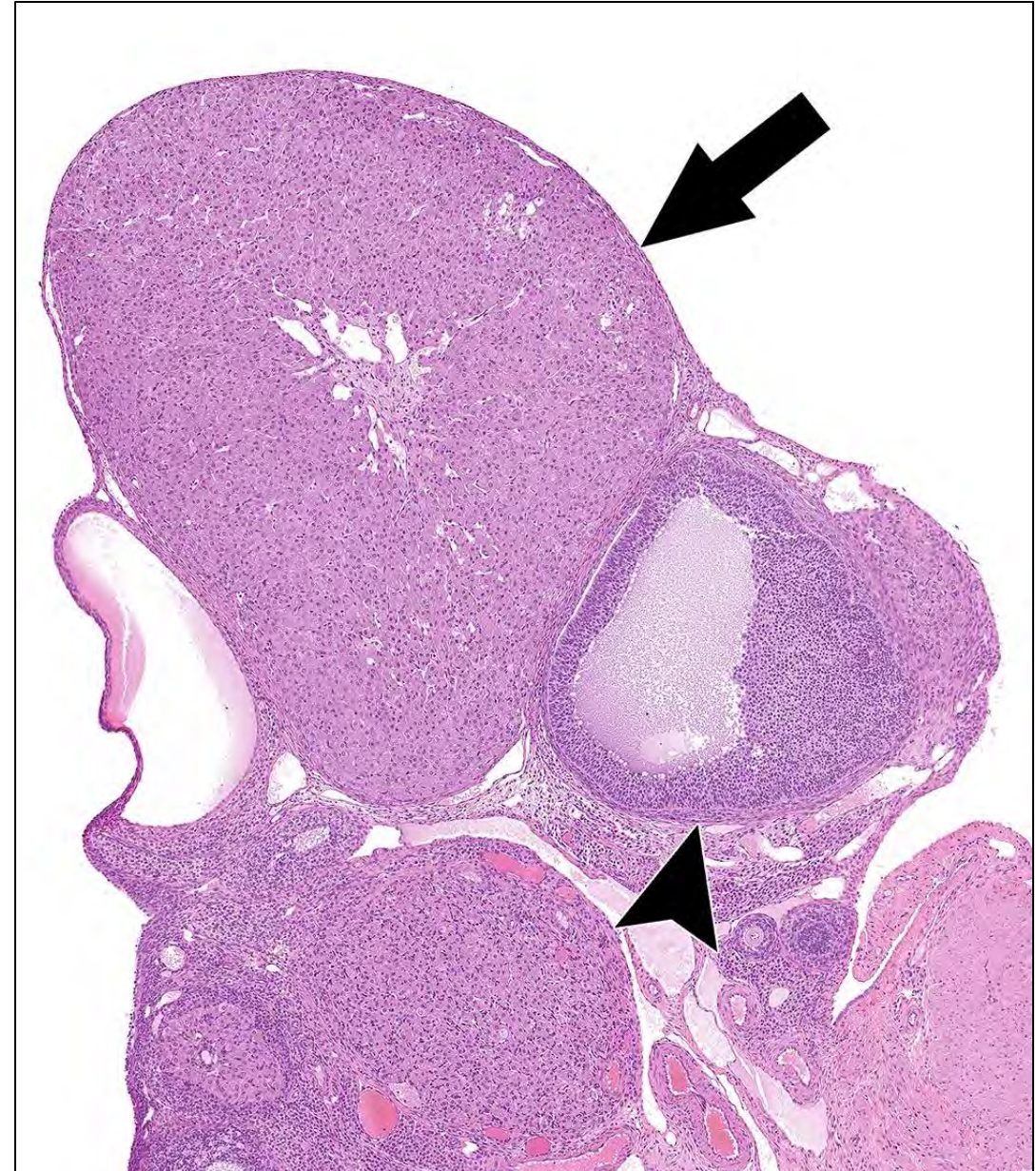
Proestrus: Uterus

- The lumen (labeled) is dilated and filled with clear fluid, especially toward late proestrus (Westwood, 2008; Dixon et al., 2014)
- Under high levels of estradiol, epithelial cells (arrowheads) increase in height (low to tall columnar)
 - Mitotic figures (arrow) are prominent in the epithelium (Dixon et al., 2014)
- The stroma shows prominent vasculature and early edema
 - May have few leukocytes



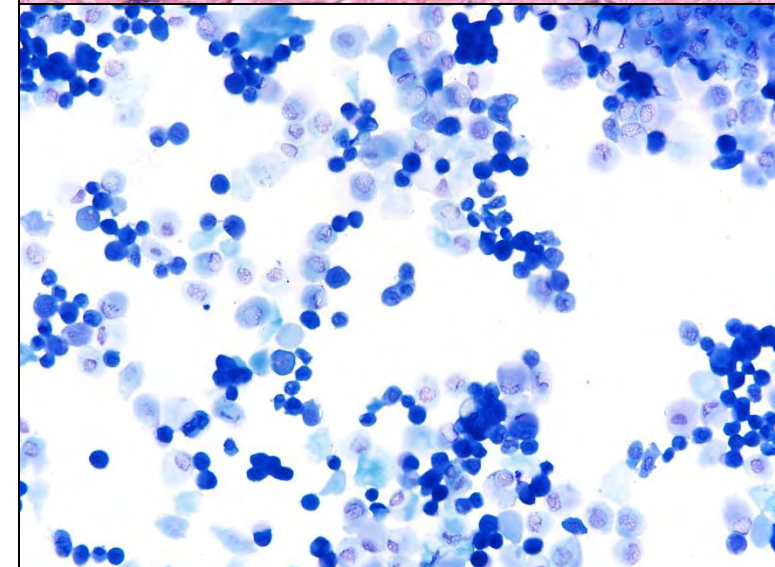
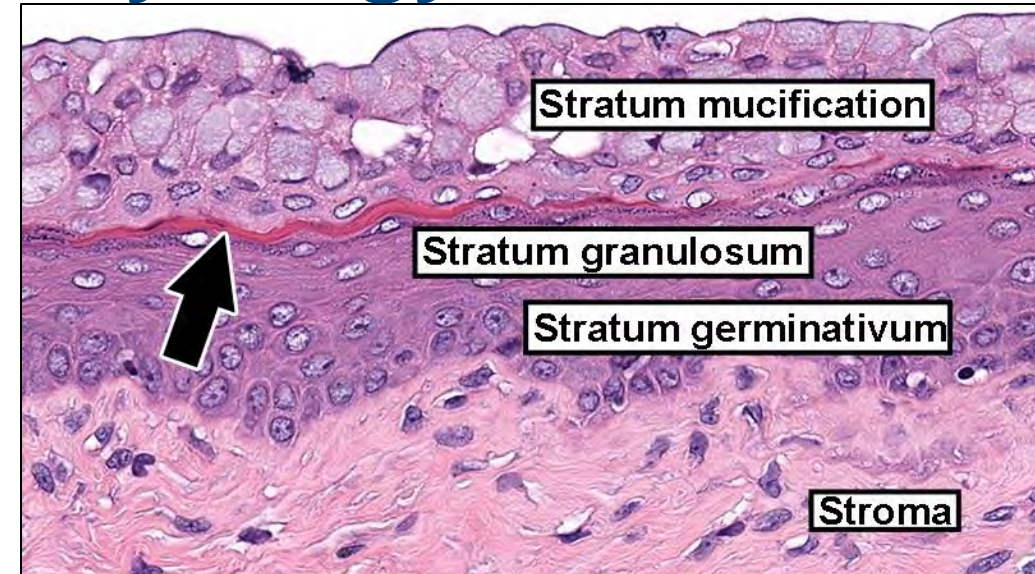
Proestrus: Ovary

- Large tertiary follicles (arrowhead) are prominent along the surface
 - Tertiary follicles are the largest and most developed, and have a pronounced internal-cystic area (antrum)
- Several atretic antral tertiary follicles may be present
 - Atresia is a term describing degeneration of follicles at various stages of development
- Corpora Lutea (CL)
 - A corpus luteum is the progesterone-synthesizing structure that forms from the post-ovulatory follicle
 - Recent CL (arrow) are eosinophilic by the end of proestrus, with cytoplasmic vacuolation or apoptosis of luteal cells \pm necrosis



Proestrus: Vagina Histology and Cytology

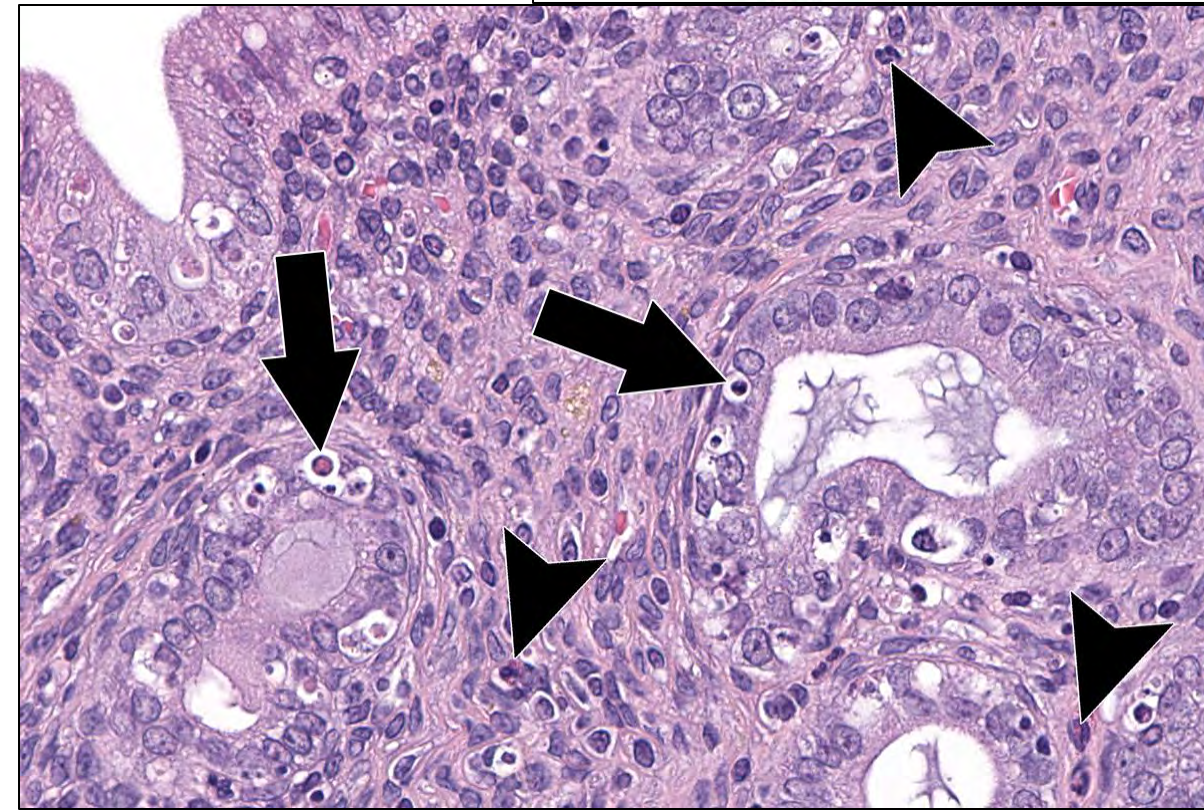
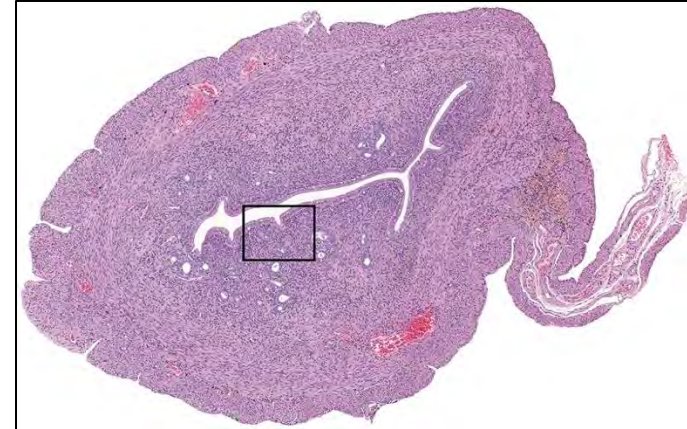
- Histology (Hobbie and Dixon, 2020):
 - The formation of the **stratum granulosum** (a keratohyaline [purple] granule rich layer) defines the start of proestrus. It is followed by formation of the **stratum mucification** (a pale mucinous layer), and with the **stratum germinativum**, gives a 3-layered appearance
 - In mid- to late proestrus, the brightly eosinophilic, amorphous, **stratum corneum** (black arrow) forms between the stratum mucification and stratum granulosum, giving a 4-layered appearance
- Cytology (Cora et al., 2015)
 - Vaginal cytology smears are prepared by vaginal swab or lavage and typically stained with metachromatic stains (e.g., Toluidine blue O, Wright's-Giemsa, etc.)
 - Predominantly small, round, basophilic, nucleated epithelial cells
 - Found individually, and in clusters, sheets or strands
 - Neutrophils are absent or rare



Toluidine blue O
stain

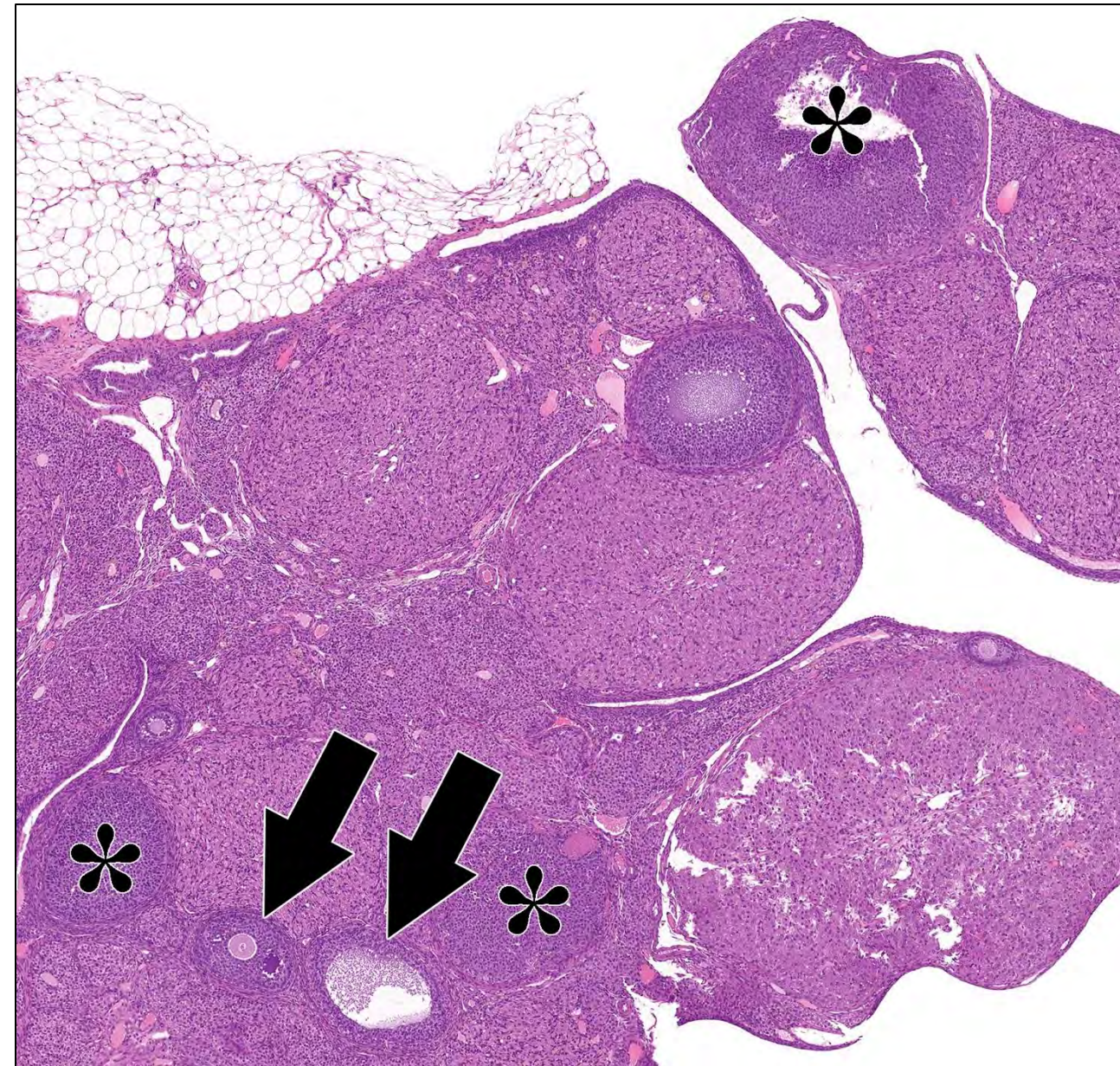
Estrus: Uterus

- Degeneration (seen as apoptotic cells, defining the start of estrus (arrows)) is seen in the glandular epithelium, followed by the luminal epithelium
- Mitotic figures and luminal dilation may be present early on, but decrease later
- The number of infiltrating inflammatory cells (arrowheads) is high in the endometrial stroma



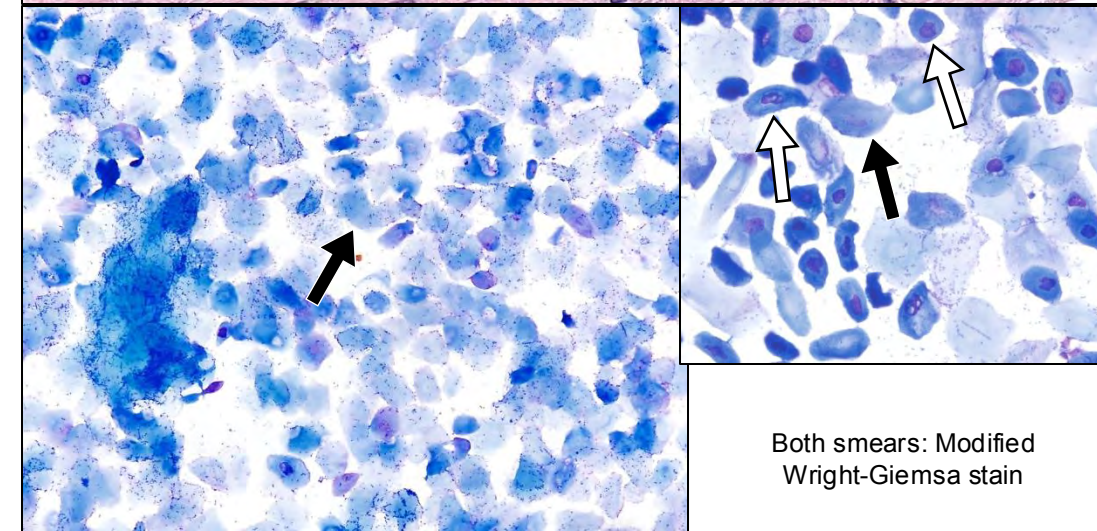
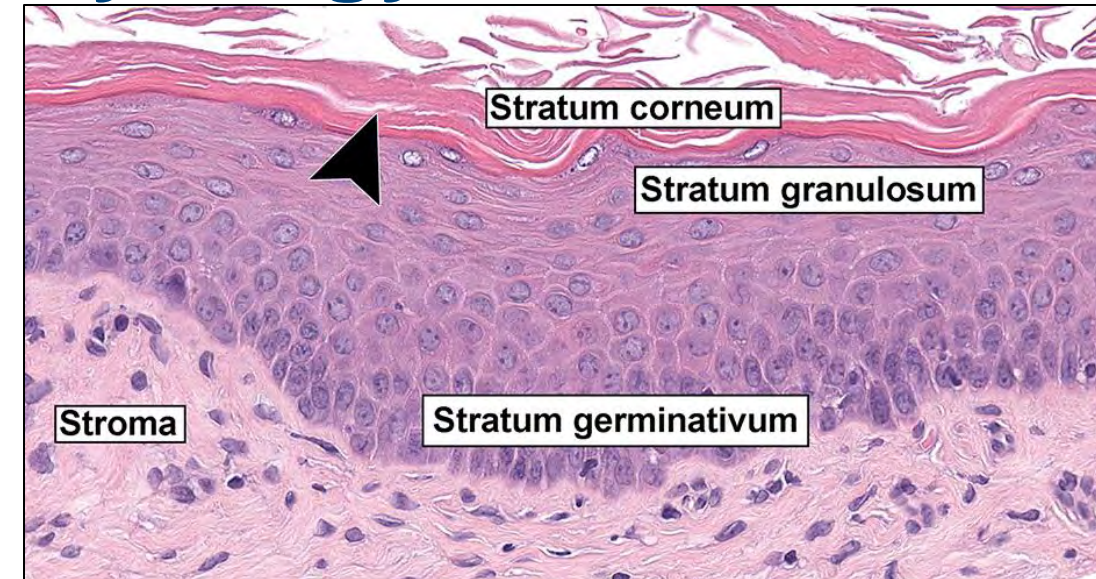
Estrus: Ovary

- Follicles: There are no healthy tertiary follicles, but a few scattered primary \pm secondary follicles are present (arrows)
- Corpus luteum (CL): Newly formed CLs are present and are basophilic, with or without central cavities (*)
 - CLs have newly formed blood vessels, which help differentiate them from large follicles or atretic follicles



Estrus: Vagina Histology and Cytology

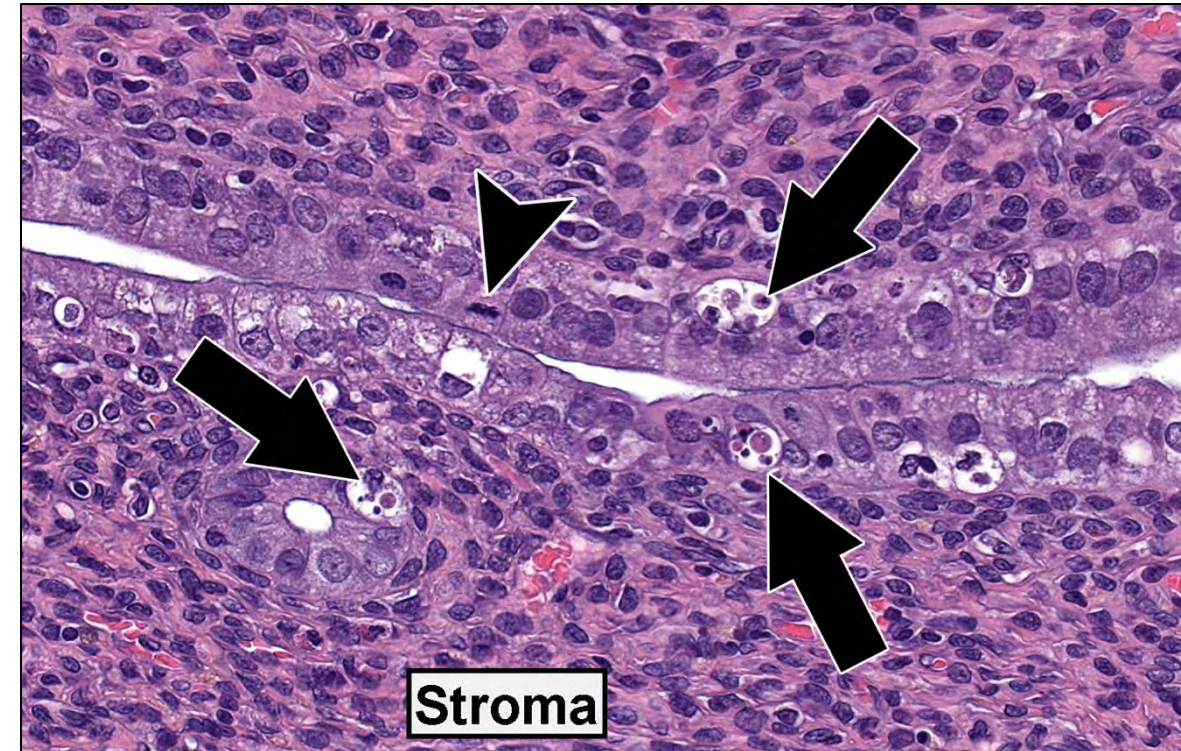
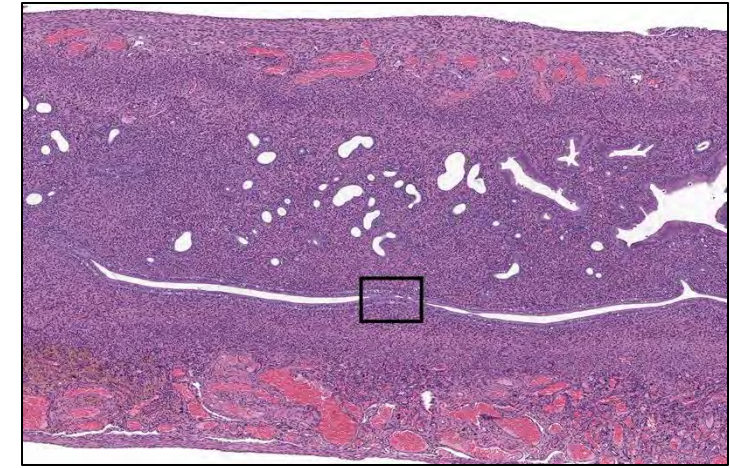
- Histology (Dixon et al., 2014):
 - The outer mucoid layer has sloughed, and the cornified layer/**stratum corneum** (arrowhead) is exposed and thickened, but progressively detaches throughout the stage
 - Mitotic activity diminishes, and variable numbers of neutrophils ± eosinophils infiltrate the epithelium
- Cytology (Cora et al., 2015):
 - Predominately keratinized epithelial cells without nuclei (anucleated; black arrows) with absent or occasional nucleated epithelial cells (white arrows); bacteria may be adhered or in the background; neutrophils absent or rare
 - A moderate number of nucleated epithelial cells can appear in late estrus in rats (pictured on right; this is not seen in mice)



Both smears: Modified
Wright-Giemsa stain

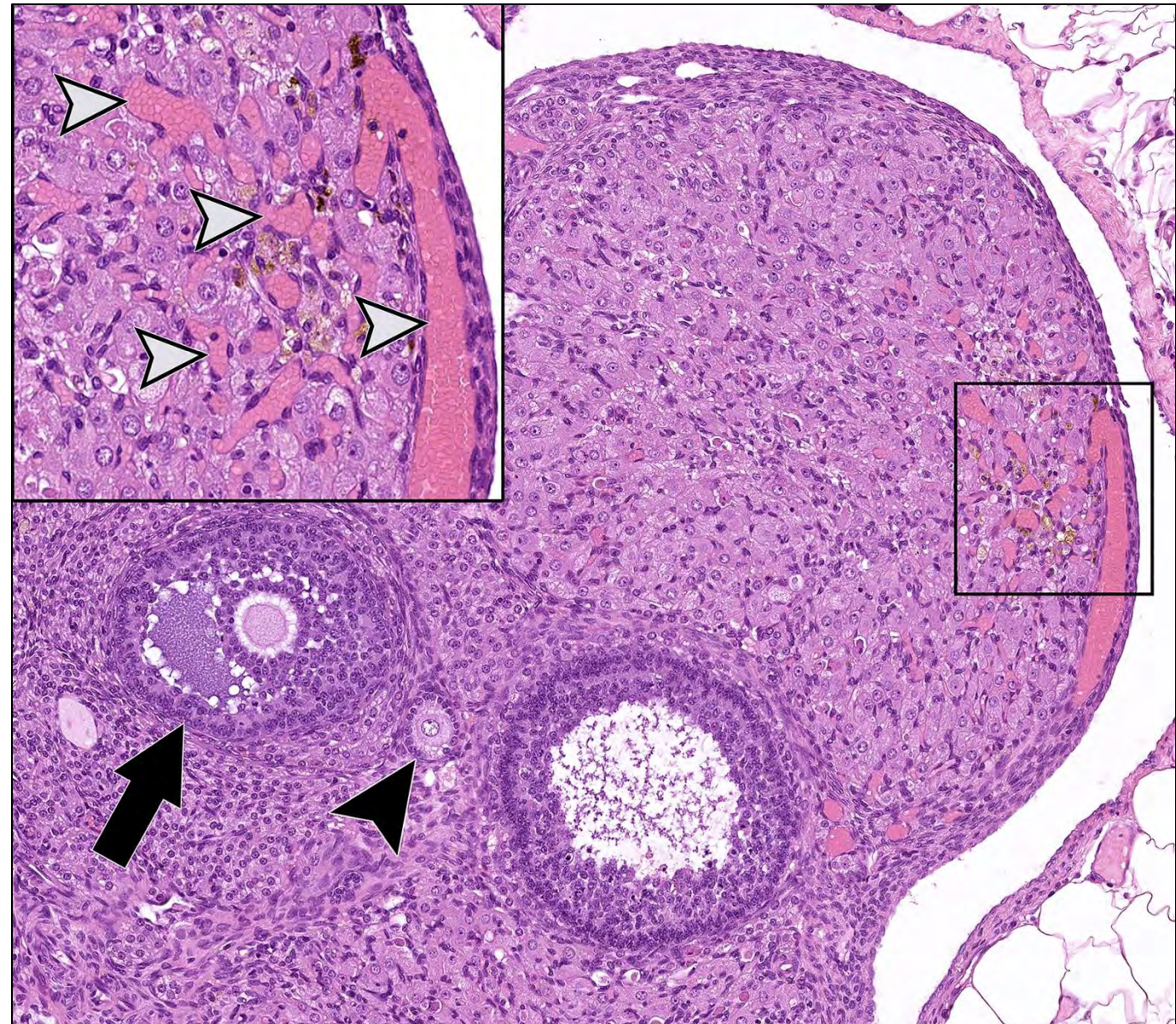
Metestrus: Uterus

- Mitotic activity returns in the epithelium (arrowhead); apoptotic cells (arrows) are present but to a lesser extent than in estrus
- Epithelial cells that survived or are newly formed are low columnar



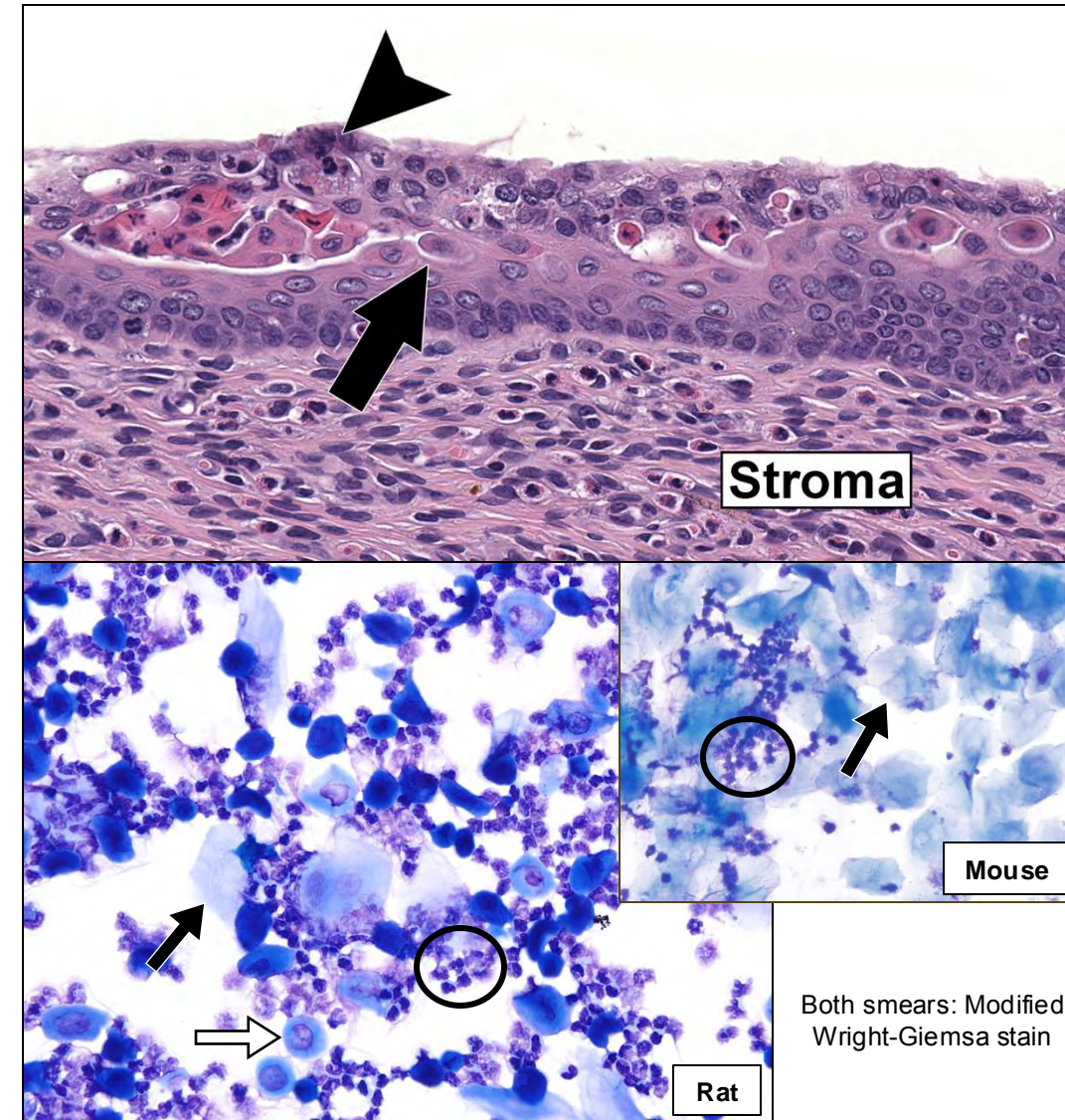
Metestrus: Ovary

- Follicles: Few smaller tertiary and many scattered primary (black arrowhead) and secondary follicles (black arrow) are present
- Primary follicles are the smallest of the developing follicles and are surrounded by only one layer of cuboidal cells
- CL: CLs have increased in size and cells have more prominent, but slightly less basophilic cytoplasm
- Note the small vessels within the CL (white arrowheads in the inset), distinguishing it from a follicle, which has no internal vessels



Metestrus: Vagina Histology and Cytology

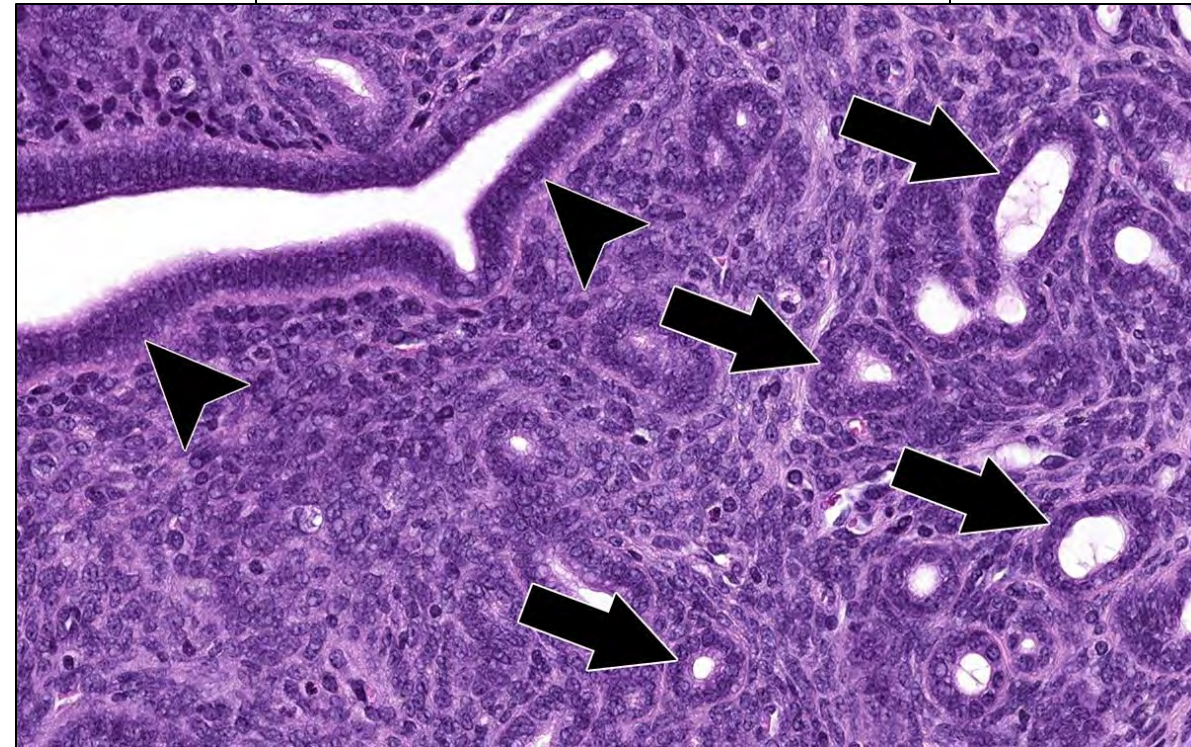
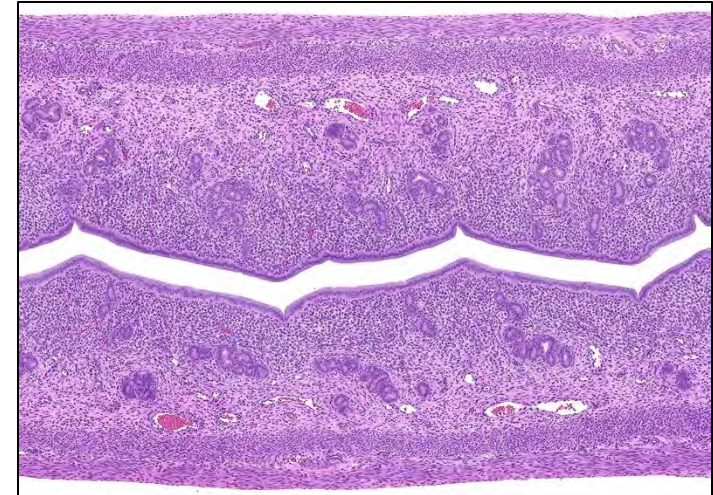
- Histology:
 - The **stratum corneum** is completely detached, defining the start of metestrus (Dixon et al., 2014)
 - There is progressive loss of the cornified epithelium (**stratum granulosum**; black arrowhead) and the superficial cells of the basal layer (**stratum germinativum**; black arrow)
 - Variable granulocyte (neutrophil) infiltration
 - At the end of metestrus, the epithelium is at lowest level of thickness (Westwood, 2008)
- Cytology (Cora et al., 2015):
 - Neutrophils alongside keratinized anucleated epithelial cells (black arrows) +/- nucleated epithelial cells (white arrow); left = rat metestrus, right = mouse metestrus
 - Neutrophils (black circles) increase as the stage progresses, being in very high numbers during peak metestrus



Both smears: Modified
Wright-Giemsa stain

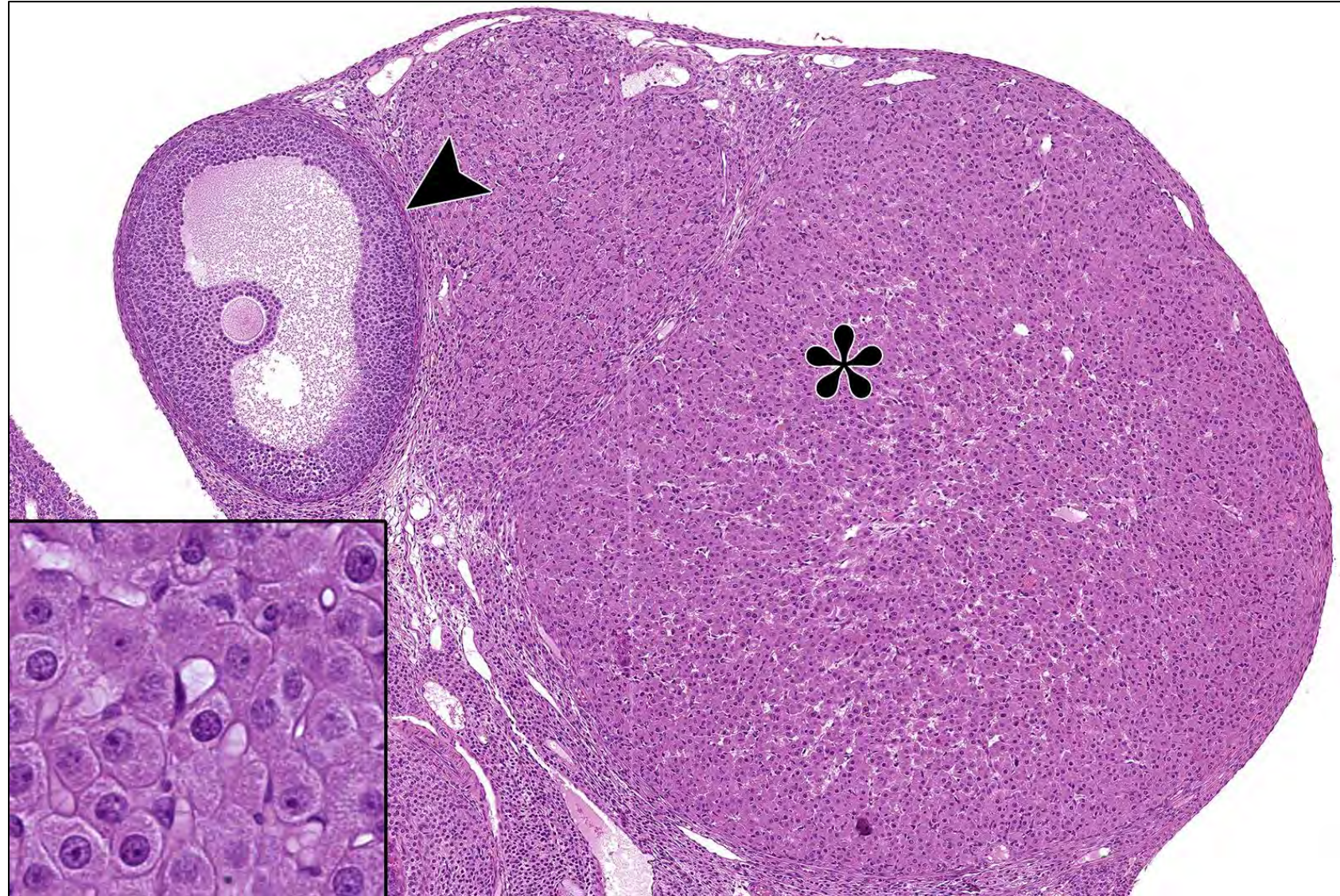
Diestrus: Uterus

- The uterus appears small with a slit-like lumen
- Mitoses and apoptotic epithelial cells are rare
- The luminal (arrowheads) and glandular (arrows) epithelial cells are at their lowest heights (cuboidal or low columnar), and the endometrial stroma is compact but may show slight edema toward end of stage



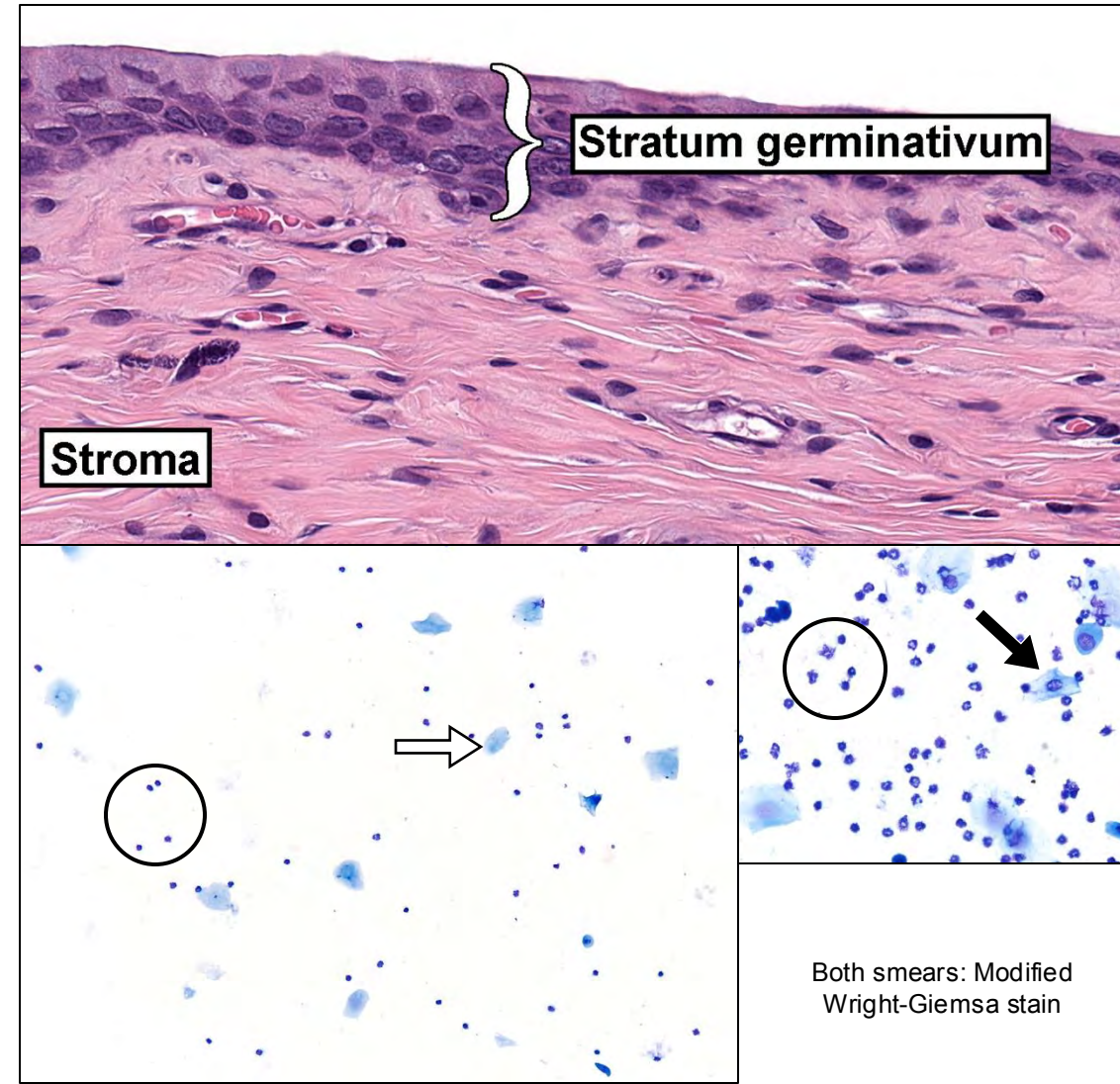
Diestrus: Ovary

- Follicles: Tertiary follicles (arrowhead) are increased in size and number but smaller than those in proestrus
- CL: Newly formed CL (*) have attained their largest size; central cells may start to become vacuolated (inset); may see central fibrosis
 - No degeneration (“luteolysis”) yet



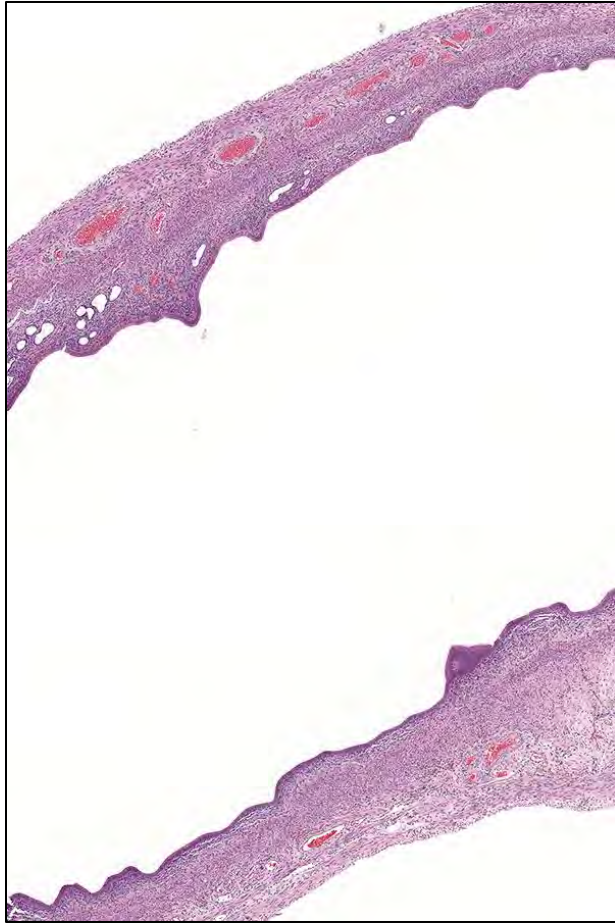
Diestrus: Vagina Histology and Cytology

- Histology (Dixon et al., 2014):
 - The epithelium is at its thinnest (3-7 cell layers) in early diestrus, but increases in thickness towards the end (8-10 cell layers) and lacks a stratum granulosum
 - Variable numbers of stromal inflammatory cells initially, decline as stage progresses
- Cytology (Cora et al., 2015):
 - Substantial decrease in cellularity (vs. metestrus) consisting of variable numbers of neutrophils (circles), nucleated epithelial cells (black arrow) and low numbers of anucleated keratinized cells (white arrow)
 - Sometimes only consists of neutrophils (i.e., no epithelial cells)
 - Clumps of small nucleated epithelial cells may appear in late diestrus (i.e., while in transition to proestrus)



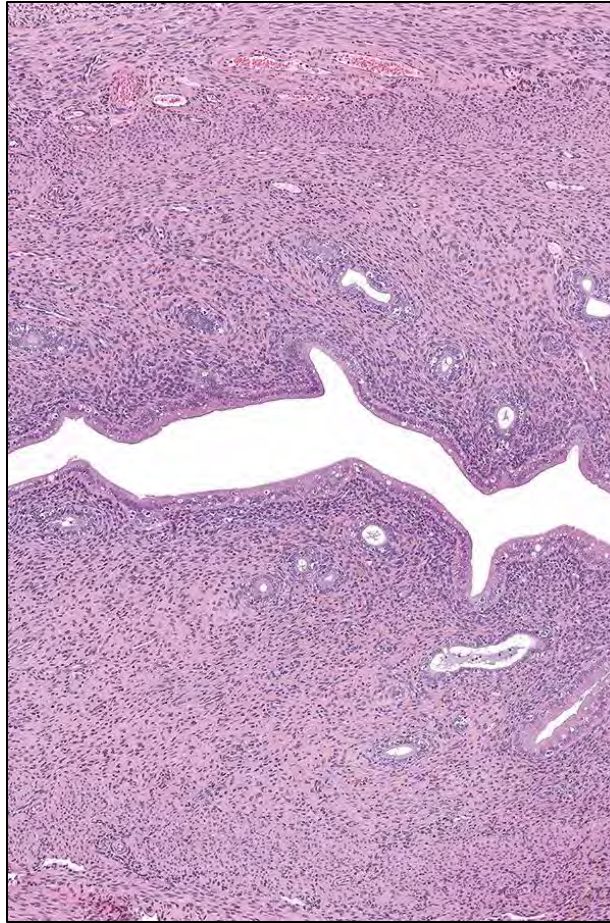
Both smears: Modified
Wright-Giemsa stain

Estrous Cycle Changes: Uterus (Low Magnification)



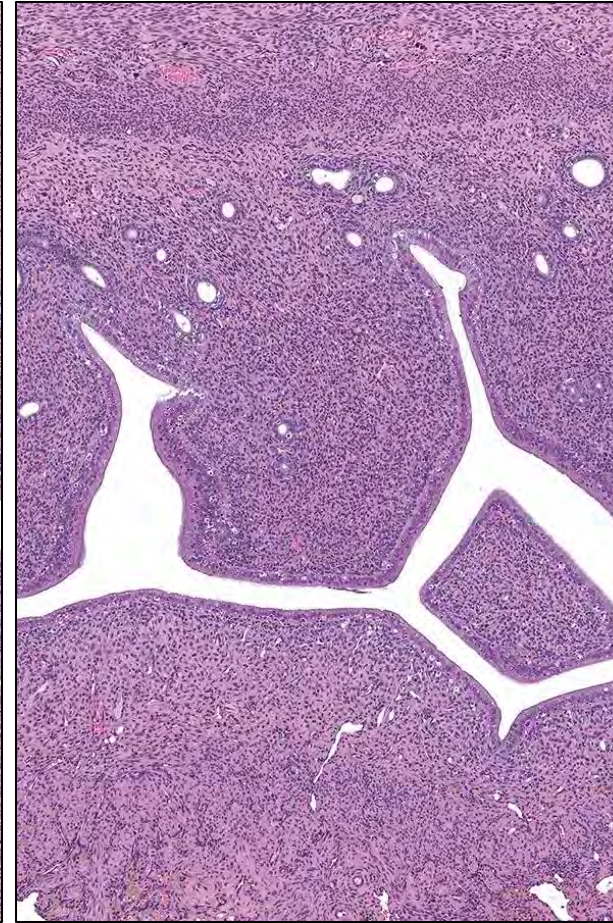
Proestrus:

- The lumen is dilated with clear fluid
- Epithelium is low to tall columnar



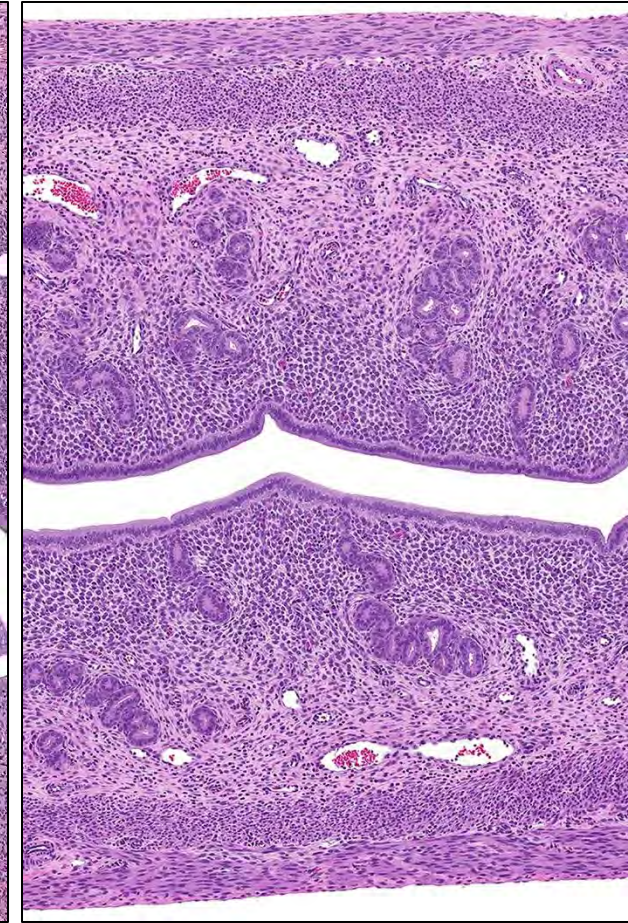
Estrus:

- Lumen may still be slightly dilated
- Glands may show slight dilation
- Epithelium is tall columnar



Metestrus:

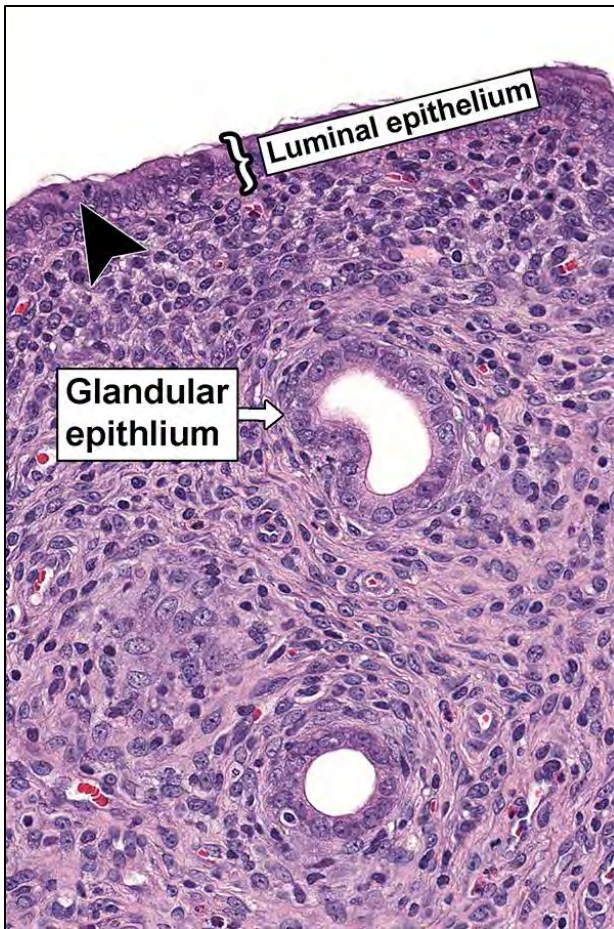
- The epithelium is reduced in height in comparison to estrus



Diestrus:

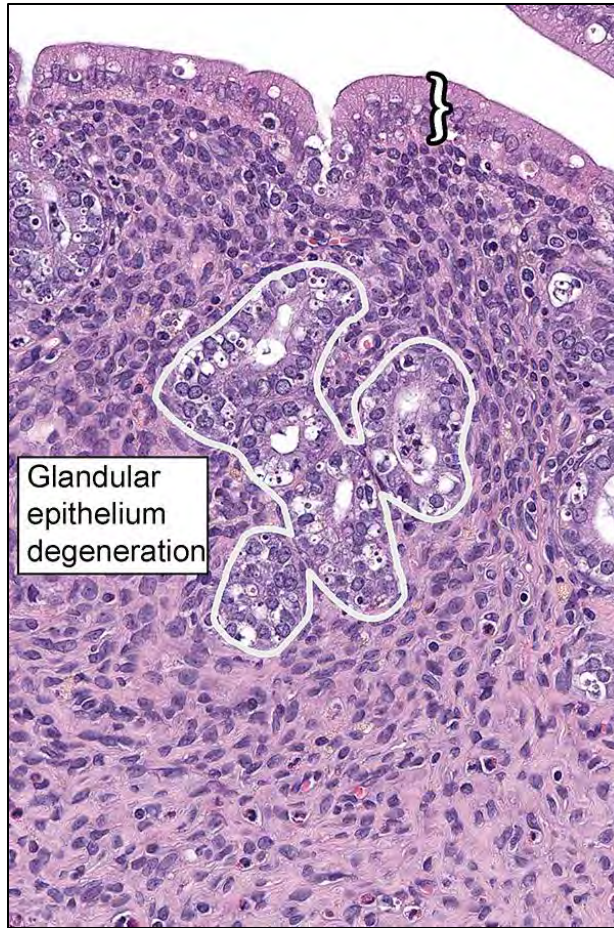
- The uterus appears small and inactive, with a slit-like lumen
- Epithelium at lowest height

Estrous Cycle Changes: Uterus (High Magnification)



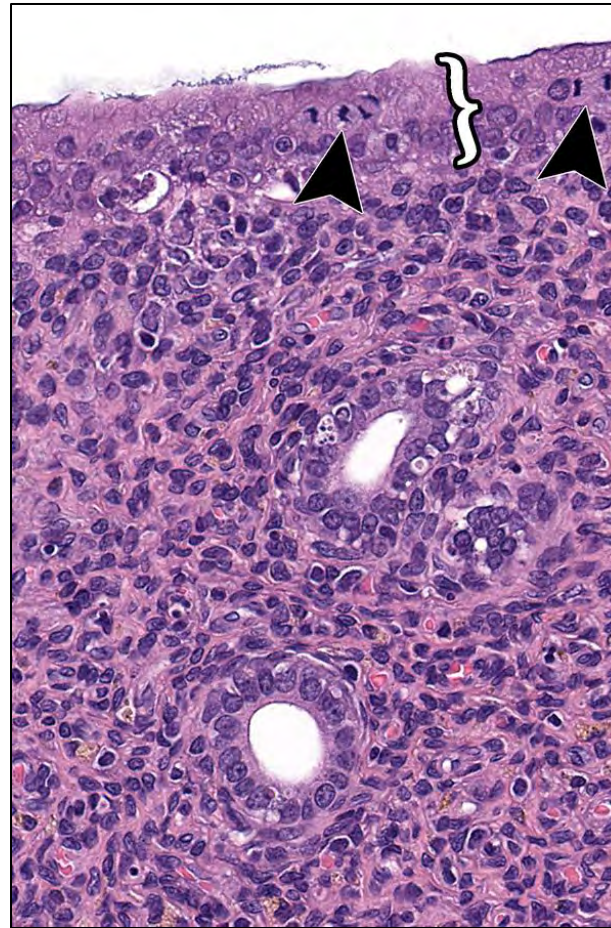
Proestrus:

- Low to tall columnar epithelium (bracket)
- Mitoses are frequent (arrowhead)
- No epithelial degeneration



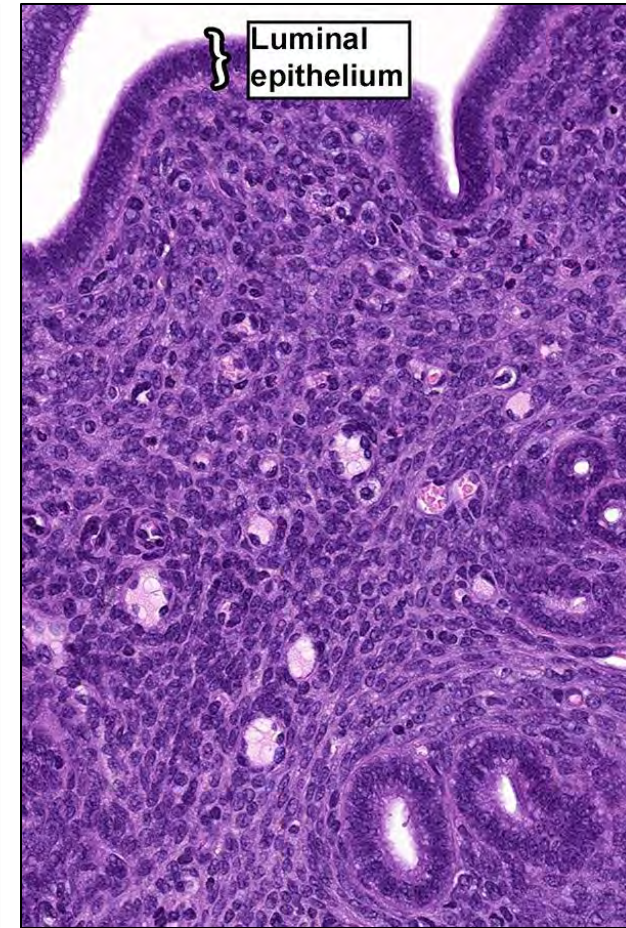
Estrus:

- Glandular, followed by luminal, epithelium degeneration (apoptosis)
- Mitoses decrease, leukocytes increase



Metestrus:

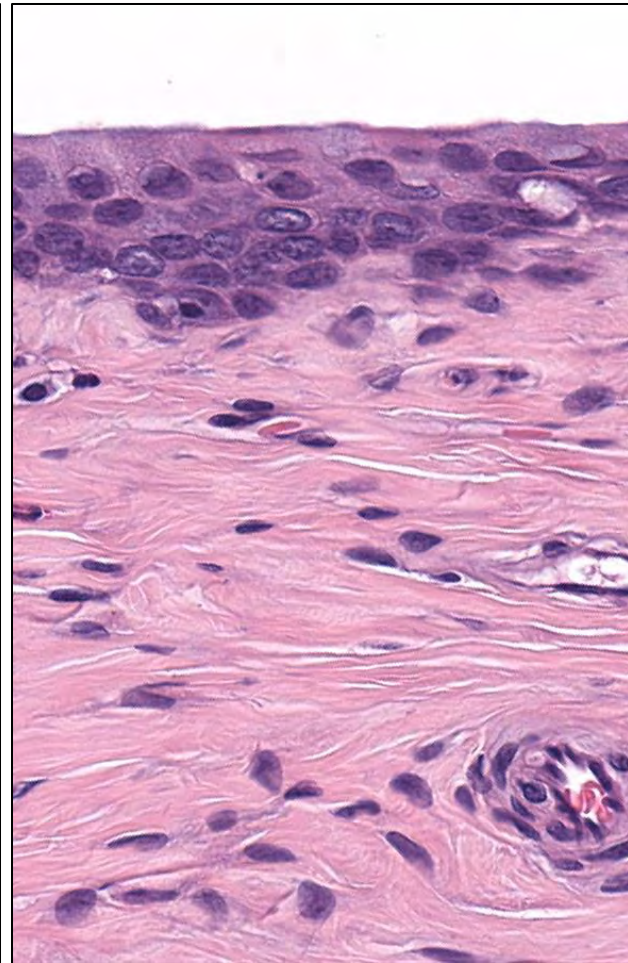
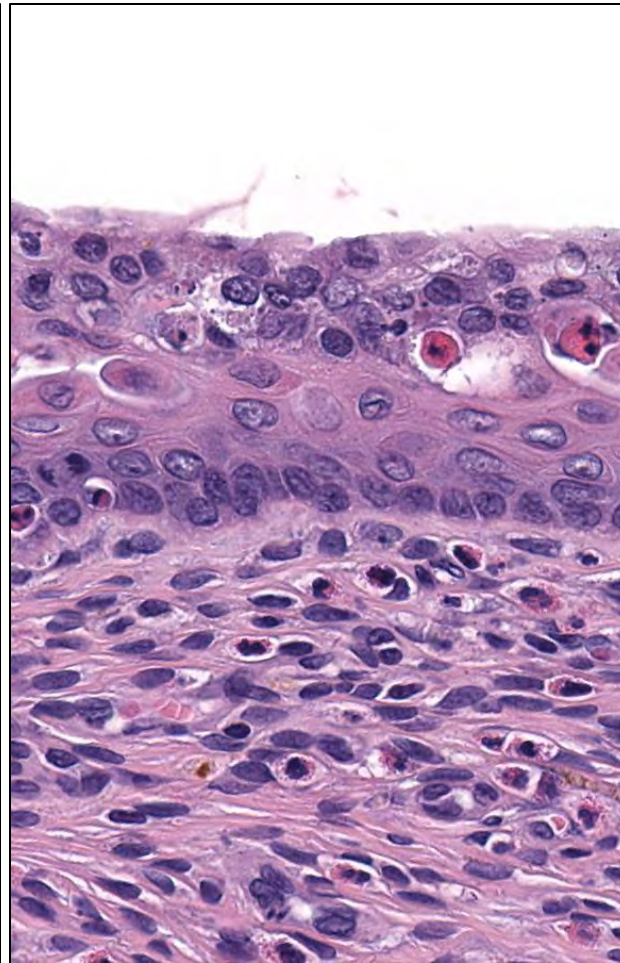
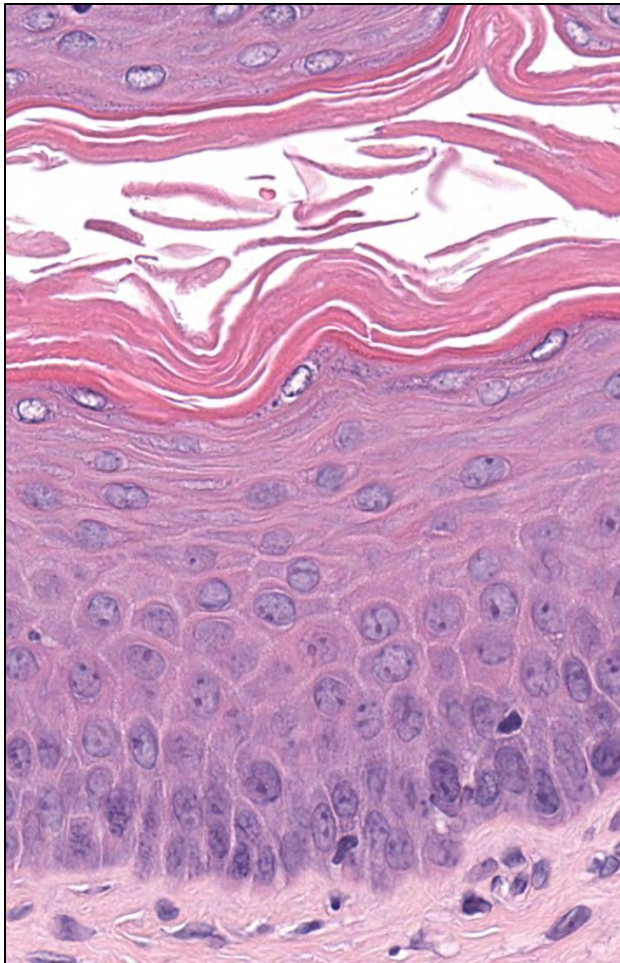
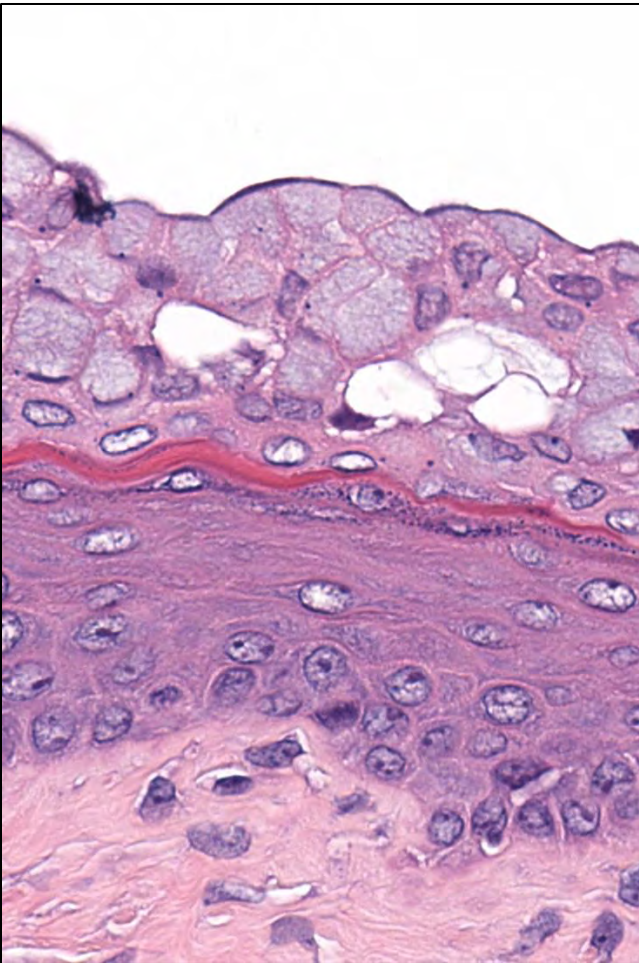
- Continued degeneration of the luminal or glandular epithelium
- Mitoses return (arrowheads)
- Moderate leukocytes, decreasing



Diestrus:

- Epithelial height is low
- No epithelial degeneration
- Stroma is compact (slight edema toward end)

Estrous Cycle Changes: Vagina



Proestrus:

- By mid to late, the epithelium arranges into 4 layers (stratum mucification, corneum, granulosum, and germinativum)

Estrus:

- Outer mucoid layer is sloughed
- Stratum corneum is exposed and thick; cells in lumen
- No stratum mucification

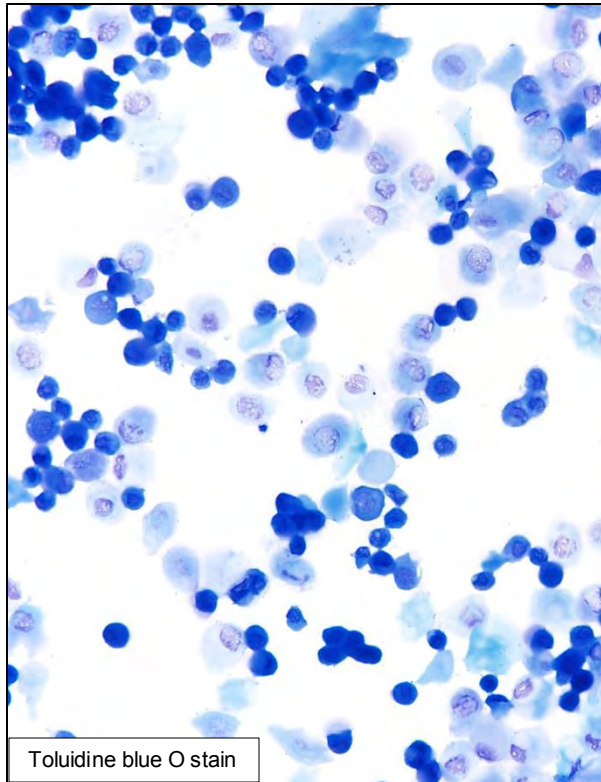
Metestrus:

- No stratum mucification, corneum, or granulosum
- Marked epithelium leukocyte infiltration at start, then decreases

Diestrus:

- Epithelium is thin
- Composed only of stratum germinativum
- At end, early mucification

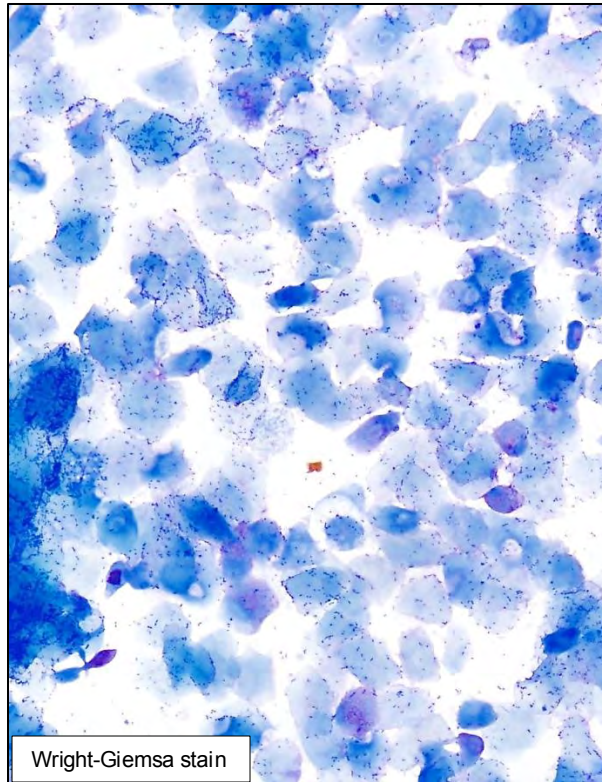
Estrous Cycle Changes: Vaginal Cytology



Toluidine blue O stain

Proestrus:

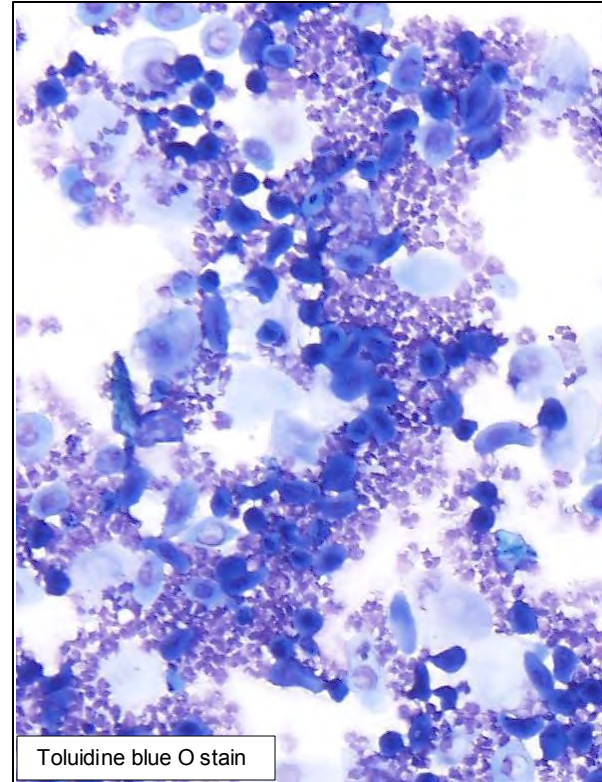
- Predominantly small nucleated epithelial cells
- Neutrophils are absent to rare



Wright-Giemsa stain

Estrus:

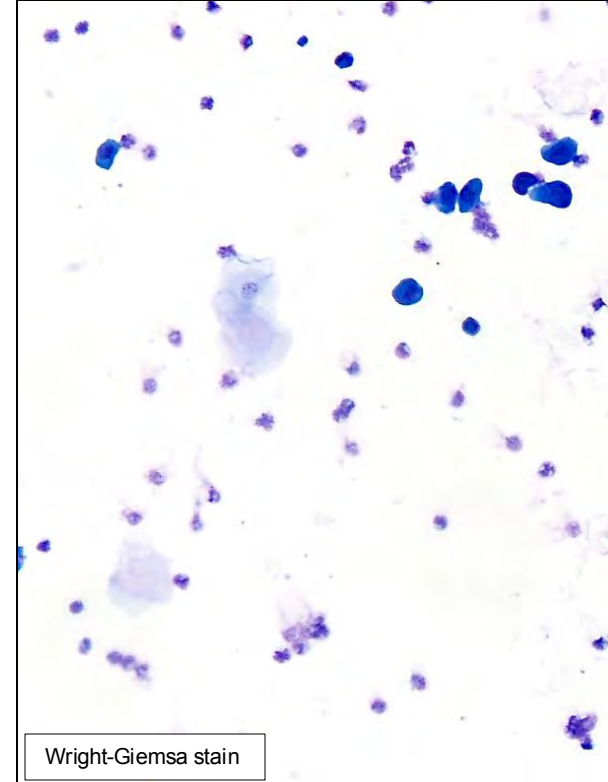
- Mainly anucleated, keratinized epithelial cells; increased number of nucleated epithelial cells in late *rat* estrus
- Numerous bacteria may be adhered or free in background



Toluidine blue O stain

Metestrus:

- Neutrophils present alongside anucleated epithelial cells +/- nucleated epithelial cells (species dependent)
- Neutrophils increase as stage progresses, being in very high numbers at peak metestrus



Wright-Giemsa stain

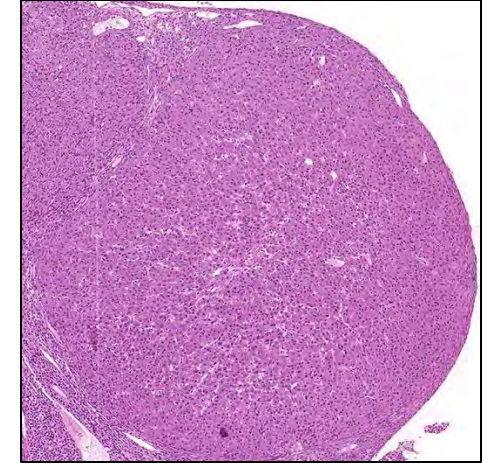
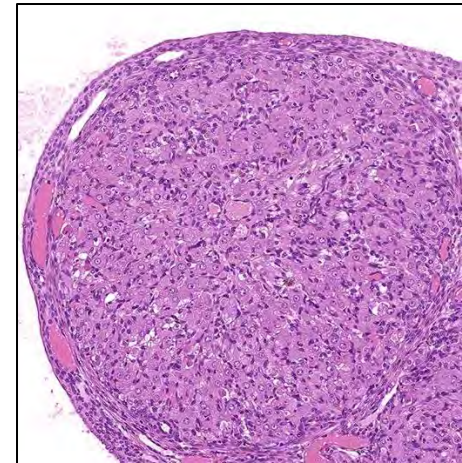
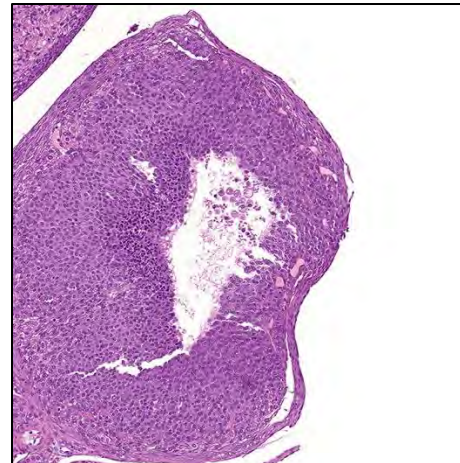
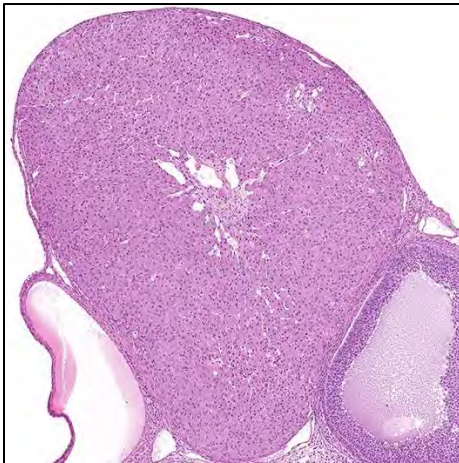
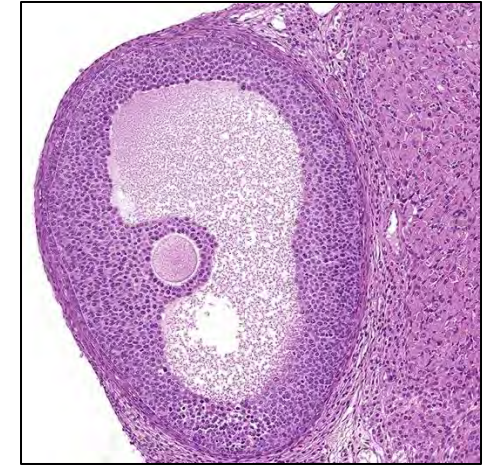
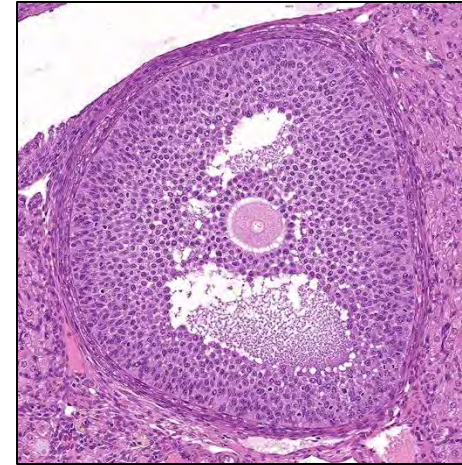
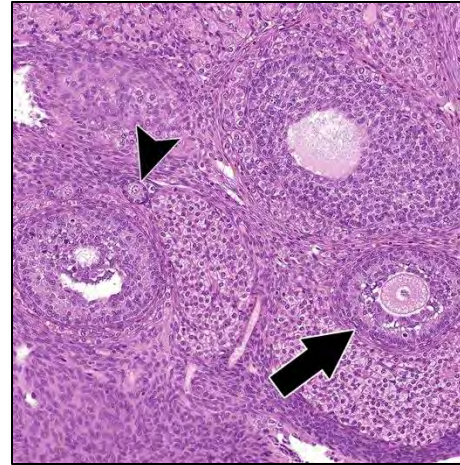
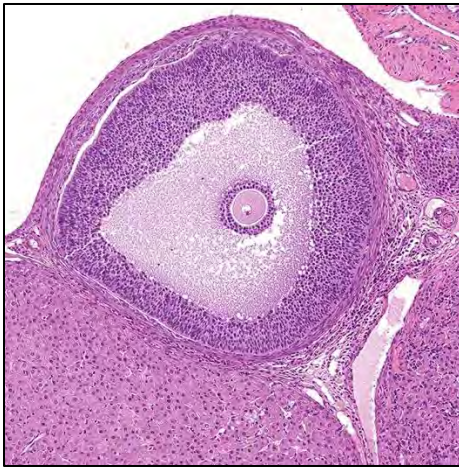
Diestrus:

- Substantial decrease in cellularity (vs. metestrus)
- Nucleated epithelial cells alongside neutrophils and occasional keratinized epithelial cells
- Sometimes may only consist of neutrophils

Estrous Cycle Changes: Ovary (Follicles and Corpora Lutea)

Follicles

Corpora Lutea



Proestrus:

- Large tertiary follicles are present
- CL are eosinophilic and vacuolated

Estrus:

- Small follicles (primary [arrow-head] and secondary [arrow])
- New CL are small and basophilic, \pm central cavity

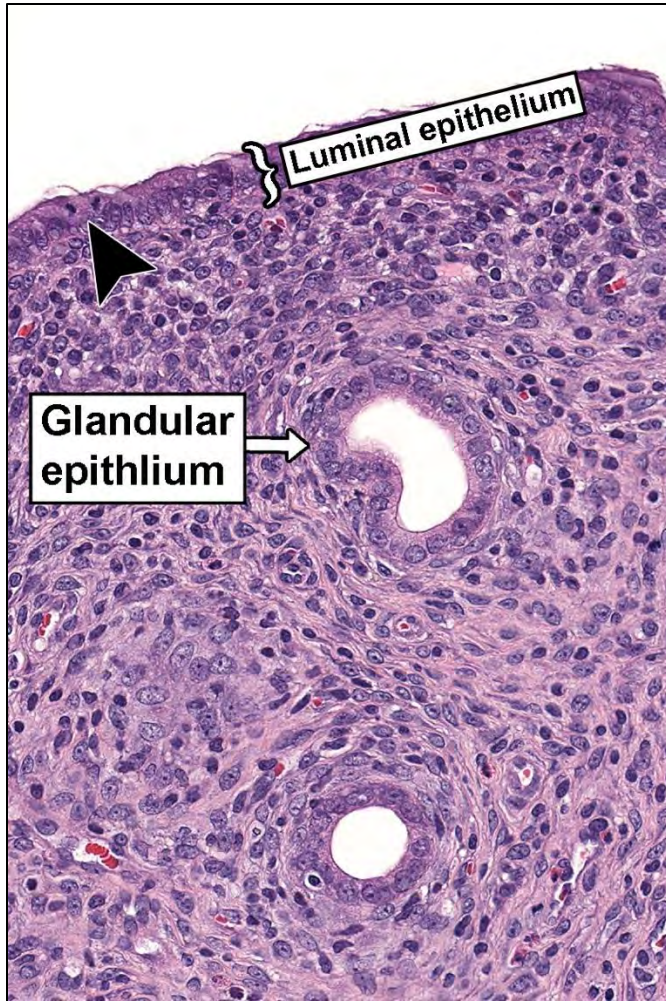
Metestrus:

- Few small tertiary follicles are present
- CL are increased in size, foamy, and eosinophilic

Diestrus:

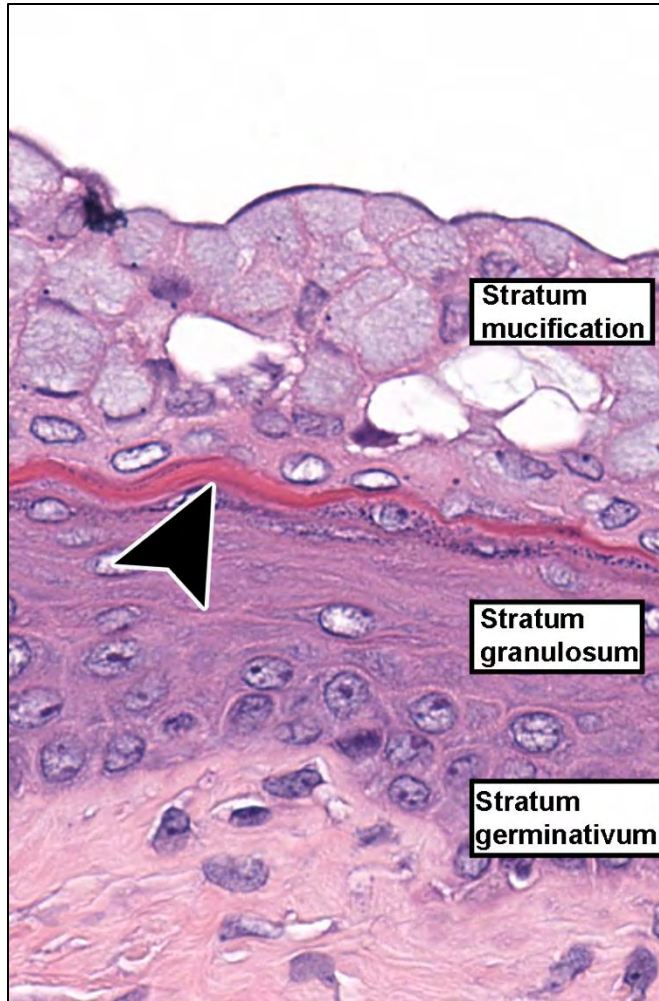
- Tertiary follicles increased in size
- CL attains largest size with vacuolation, fibrosis begins

Estrous Cycle Changes: Proestrus



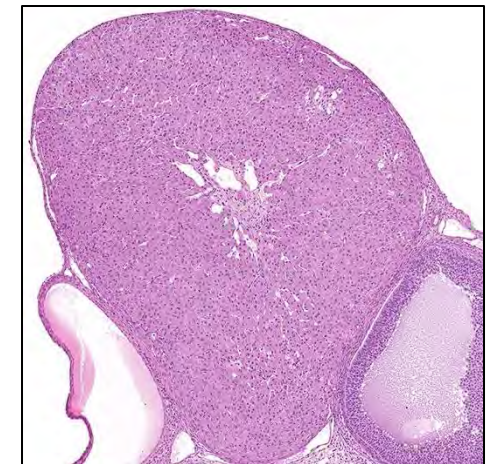
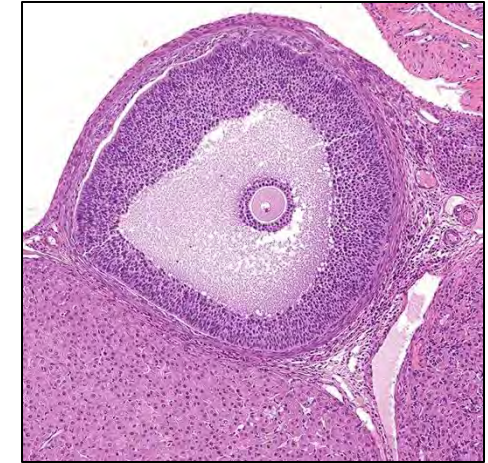
Uterus:

- Low to tall columnar epithelium
- Mitoses are frequent
- No epithelial degeneration



Vagina:

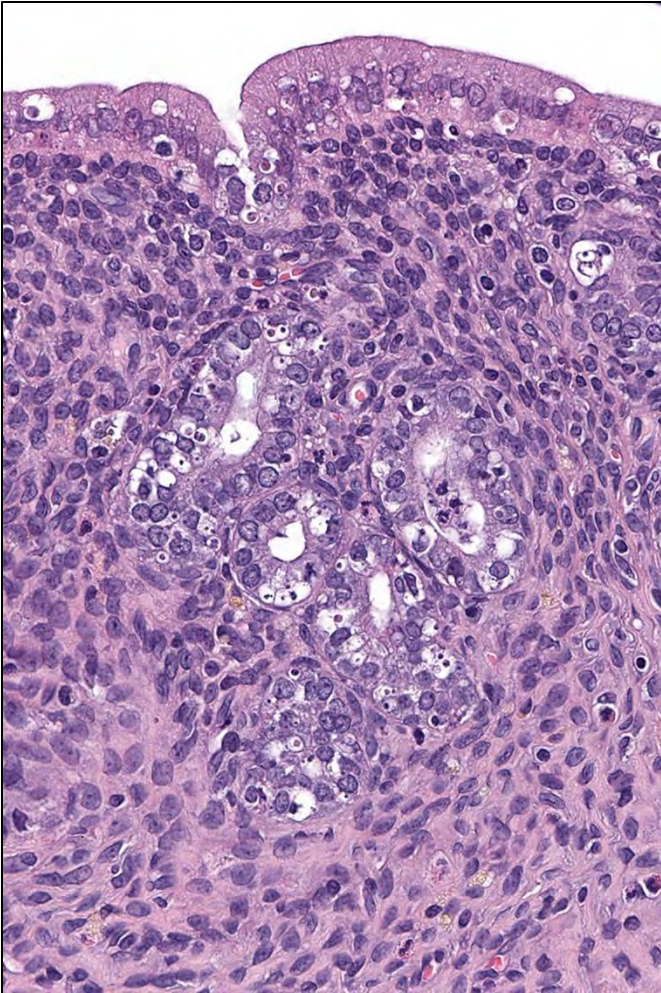
- By mid to late, the epithelium arranges into 4 layers (stratum mucification, corneum (arrowhead), granulosum, and germinativum)



Ovary:

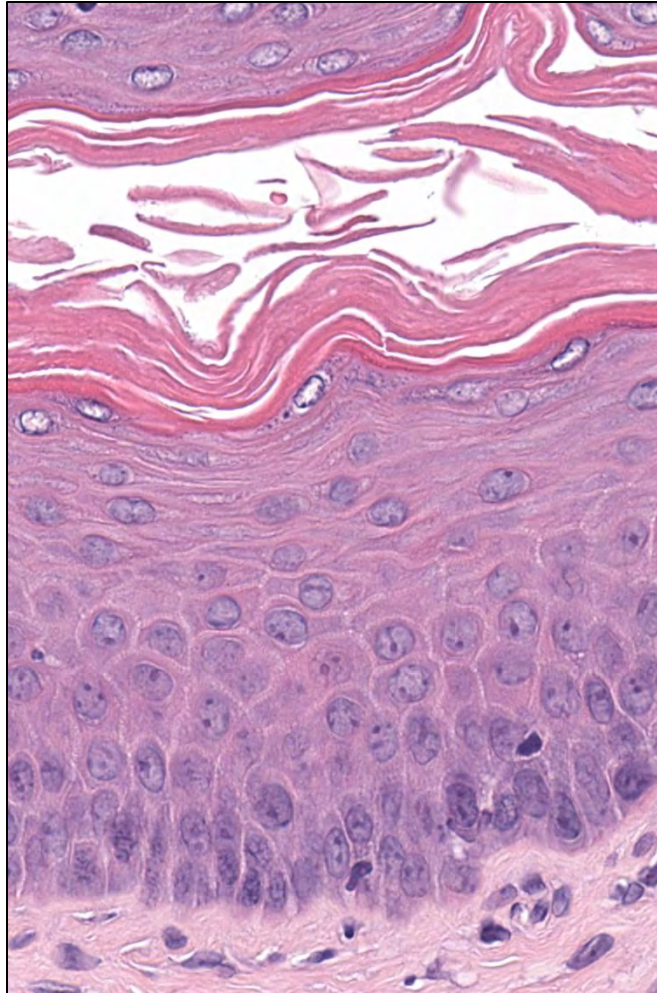
- Large tertiary follicles are present
- CL are eosinophilic and vacuolated

Estrous Cycle Changes: Estrus



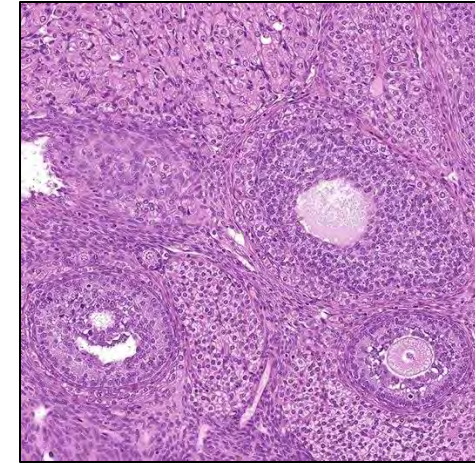
Uterus:

- Glandular, followed by luminal, epithelium degeneration (apoptosis)
- Mitoses decrease, leukocytes increase



Vagina:

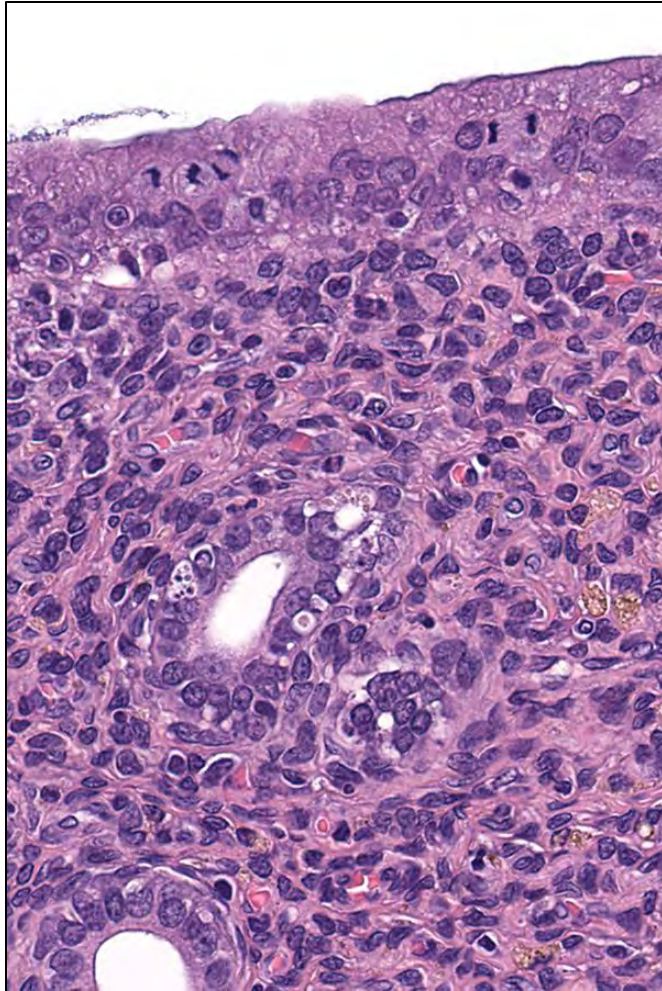
- Outer mucoid layer is sloughed
- Stratum corneum is exposed and thick; cells in lumen
- No stratum mucification



Ovary:

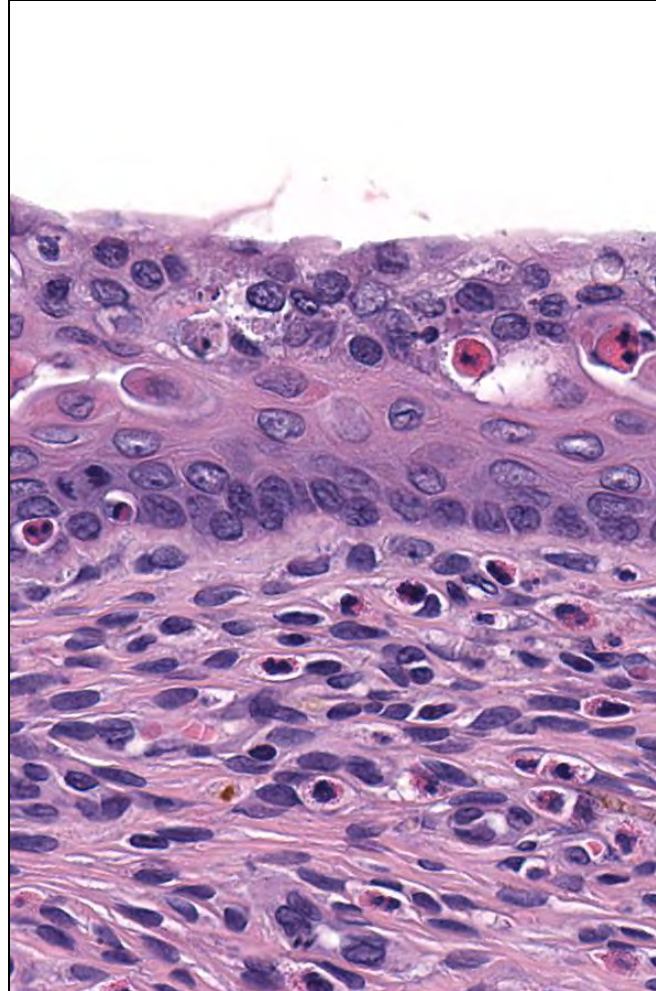
- Follicles are small (primary and secondary)
- New CL are small and basophilic, \pm central cavity

Estrous Cycle Changes: Metestrus



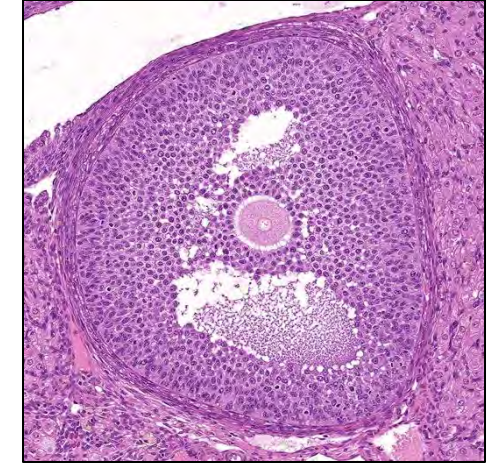
Uterus:

- Continued degeneration of the luminal or glandular epithelium
- Mitoses return
- Moderate leukocytes, decreasing



Vagina:

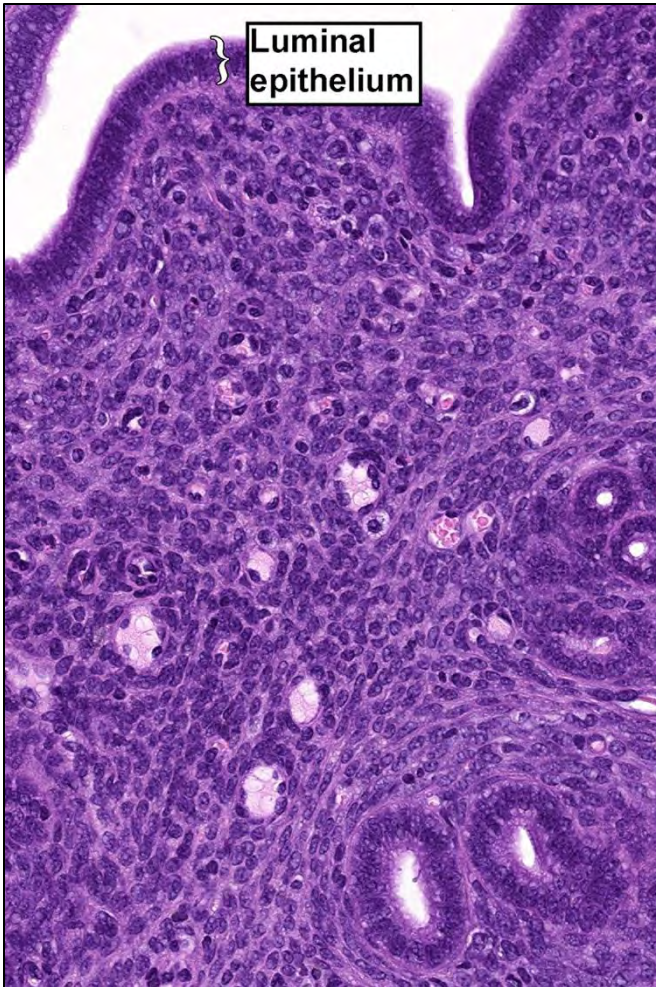
- No stratum mucification, corneum, or granulosum
- Marked epithelium leukocyte infiltration at start, then decreases



Ovary:

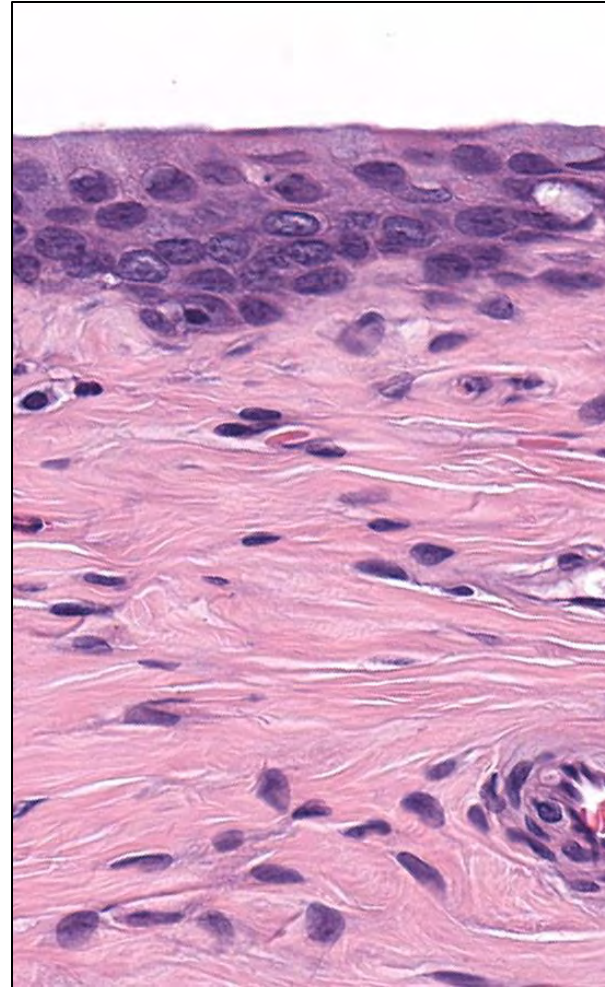
- Few small tertiary follicles are present
- CL are increased in size, foamy, and eosinophilic

Estrous Cycle Changes: Diestrus



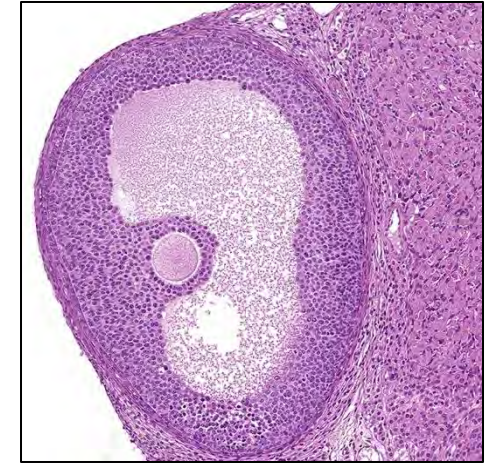
Uterus:

- Epithelial height is low
- No epithelial degeneration
- Stroma is compact (slight edema towards end)



Vagina:

- Epithelium is thin
- Composed only of stratum germinativum
- At end, early mucification



Ovary:

- Tertiary follicles increased in size
- CL attains largest size and vacuolation, fibrosis begins

References

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Environmental Health Sciences
Division of Translational Toxicology

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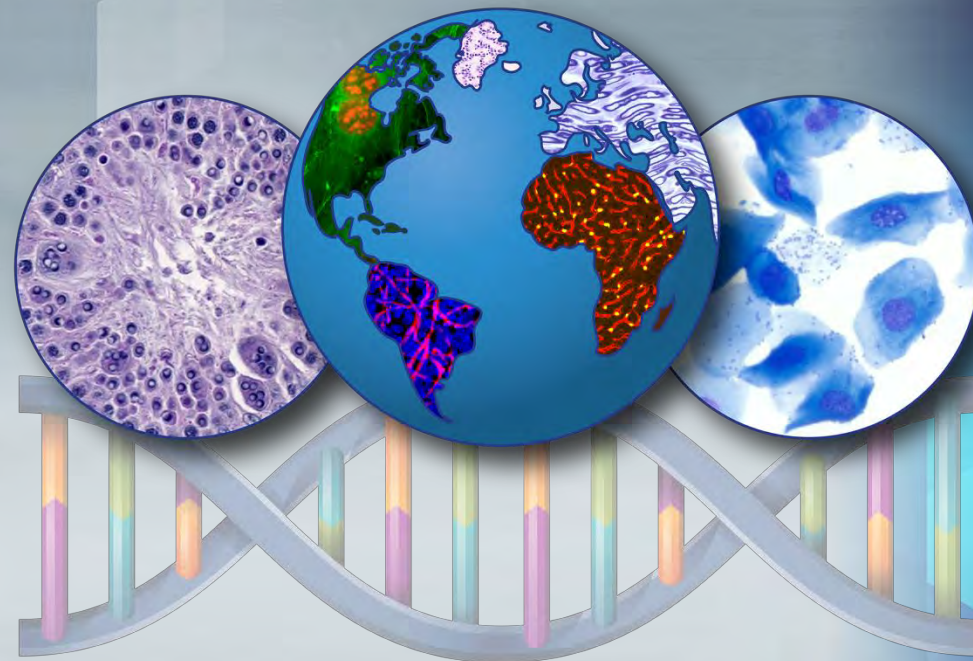
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Global Toxicologic Pathology Training Program