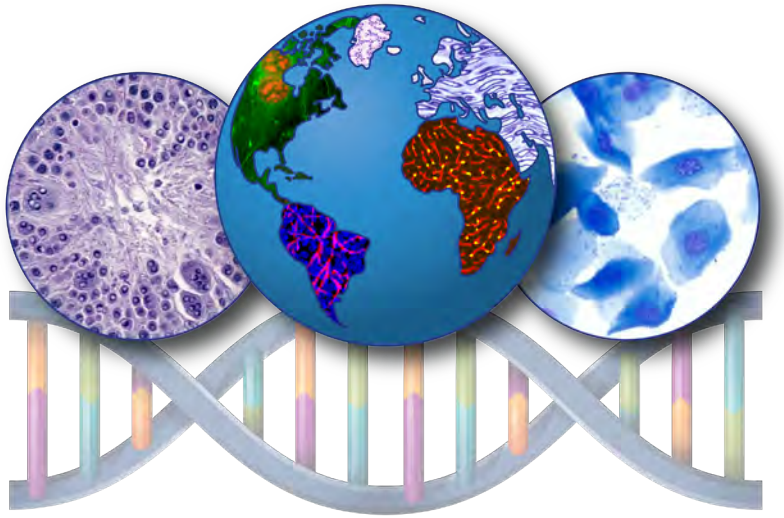
National Institute of
Environmental Health Sciences
Division of Translational Toxicology

Anatomy and Histology of the Normal Rodent Uterus



Division of Translational Toxicology Global Toxicologic Pathology Training Program

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

Overview

- Embryology and Development
- Tissue Trimming
- Microscopic Anatomy
- Reproductive Cycles and Histologic Changes
- Congenital Lesions
- Common Artifacts

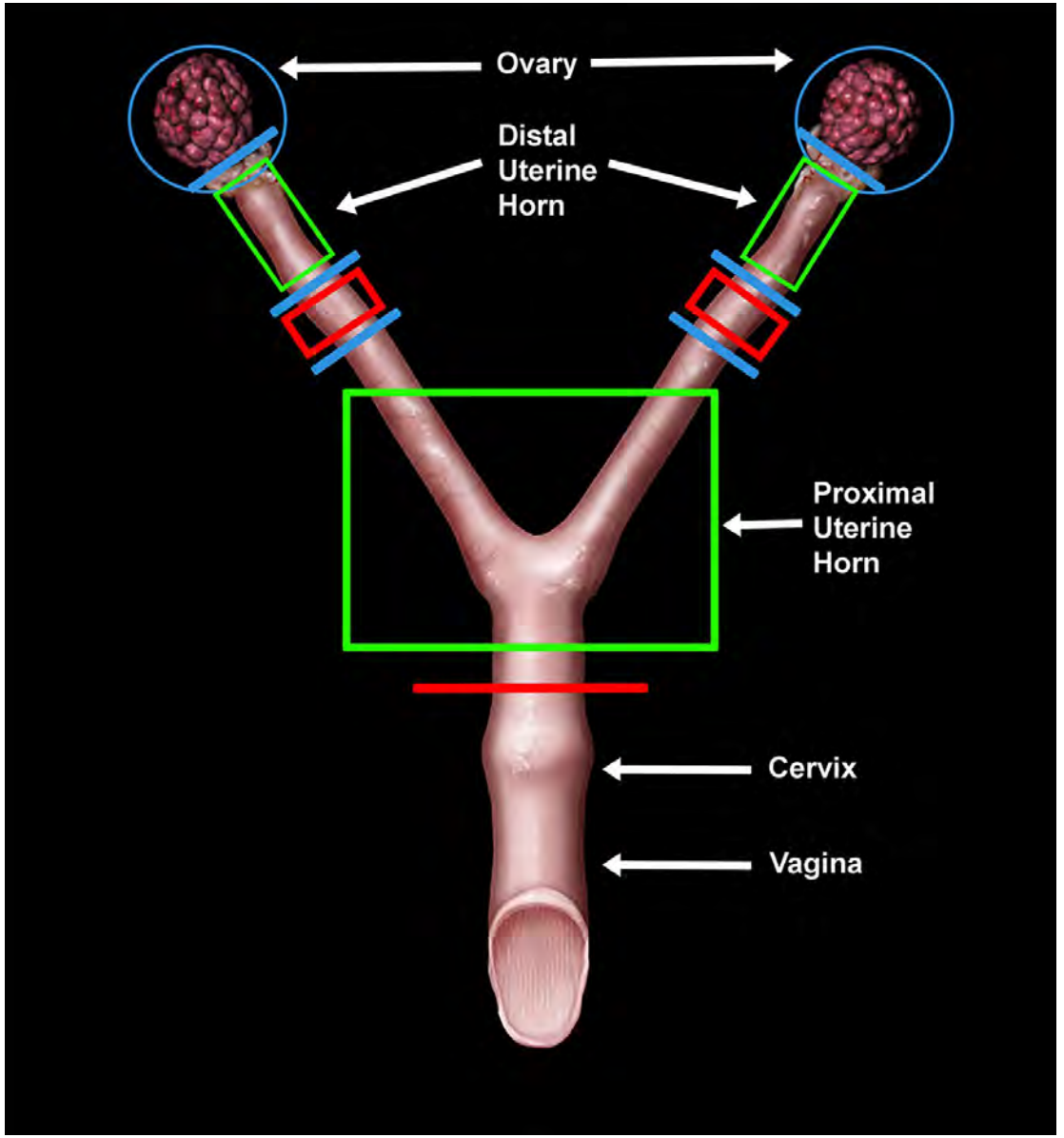
Embryology and Development

- In the early embryo (up to embryonic days [E]13.0-14.5 in mice), the urogenital system is undifferentiated and consists of two paired tubular structures (Wolffian and Müllerian ducts) (Major et al., 2022)
- On E13.0-14.0 in mice, the undifferentiated gonads develop into ovaries (Staak et al., 2003; Major et al., 2022)
- On E15.0-16.0 in mice and E18.0 in rats, the Wolffian ducts, which give rise to the male reproductive tract, regress due to the lack of testosterone and anti-Müllerian hormone production
  - The Müllerian ducts persist and give rise to the oviducts, uterus, cervix and upper vagina (Dixon et al., 2018; Staak et al., 2003; Wilson and Bordoni, 2023)
- Rodents retain two separate tubular structures (two uterine horns, i.e., “bicornuate”)
  - The uterine horns in rats are divided and open into two separate cervical canals, whereas in mice there is one cervical canal shared by both uterine horns

Tissue Trimming

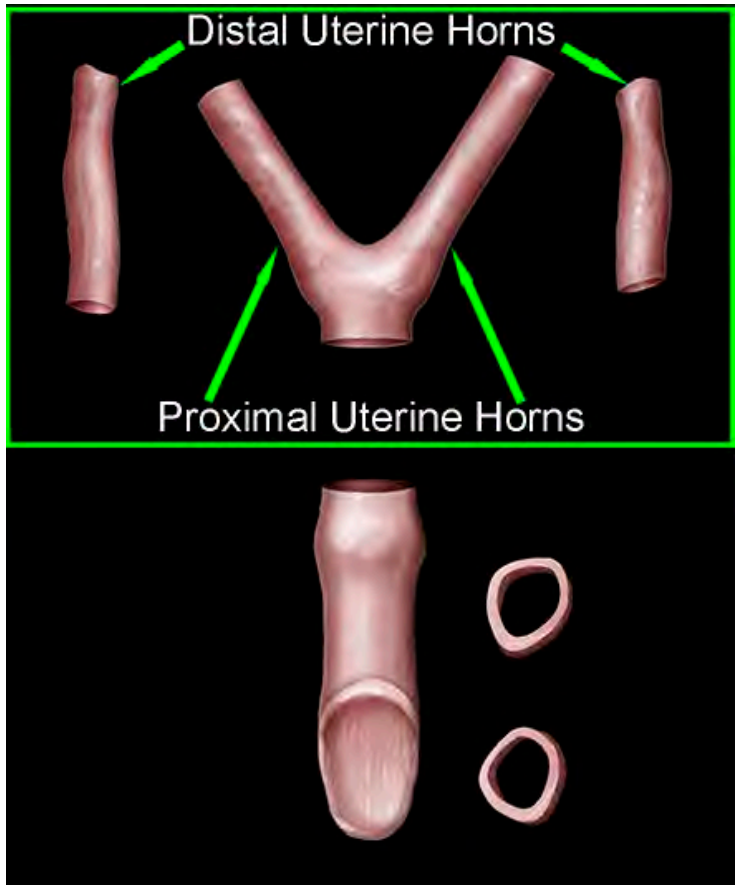
Specifications for trimming and embedding of the uterus from the Division of Translational Toxicology (DTT)

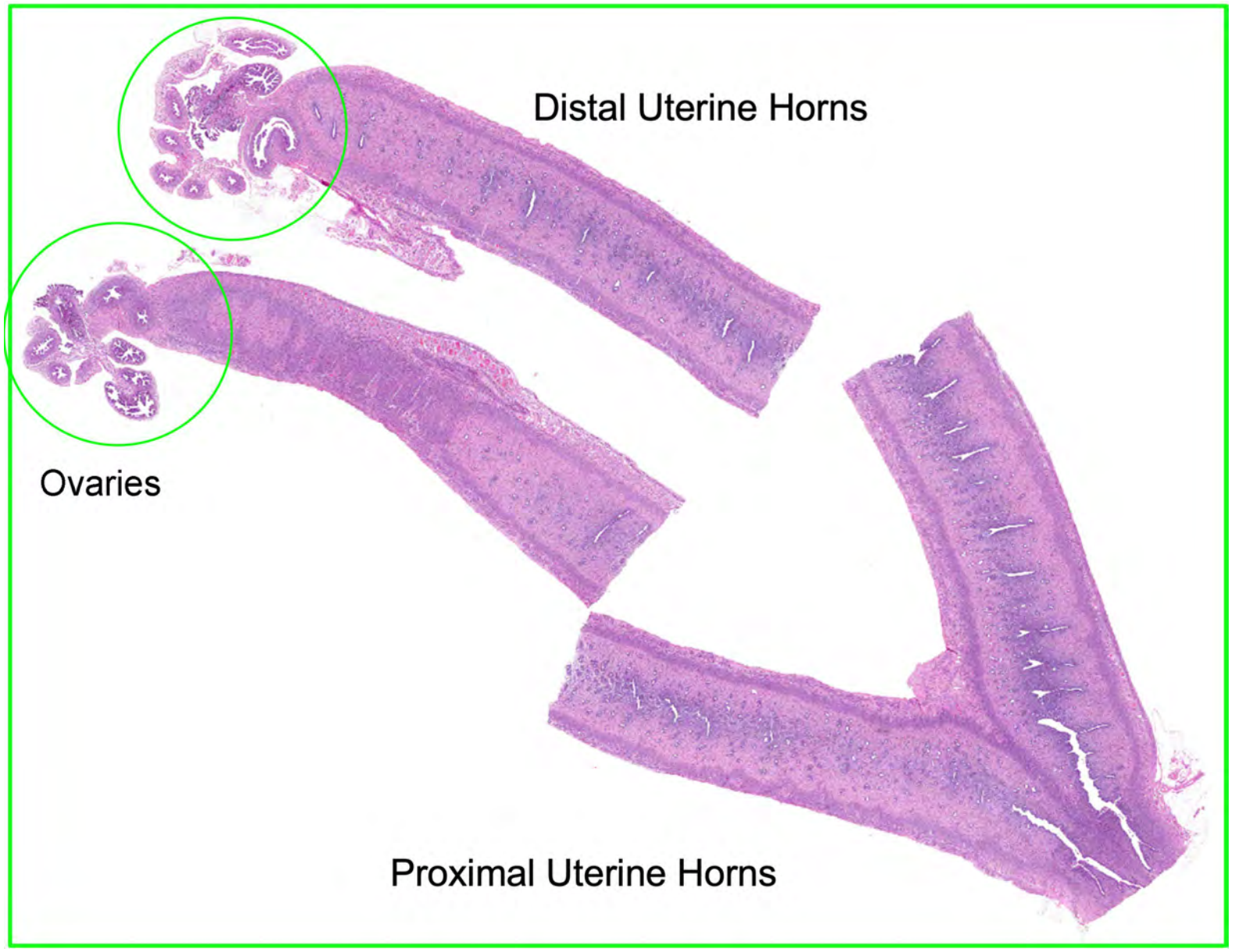
- Remove the ovaries with oviducts attached (blue circles)
- Transect the uterine body to separate the uterine cervix and vagina from the uterine body and horns (red line)
- Transect (red rectangles) the uterine horns at the midpoint, taking a transverse section from each horn
- The uterine body with attached portions of uterine horn are placed in a cassette with the two free portions of uterine horns (green rectangles)



Sectioning - longitudinal

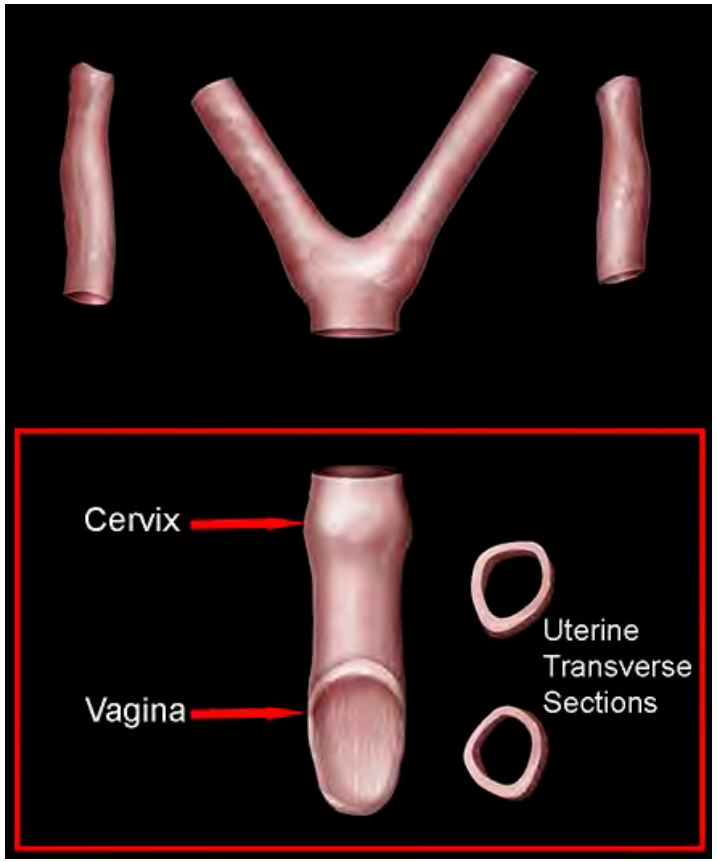
Longitudinal Sections of Uterus

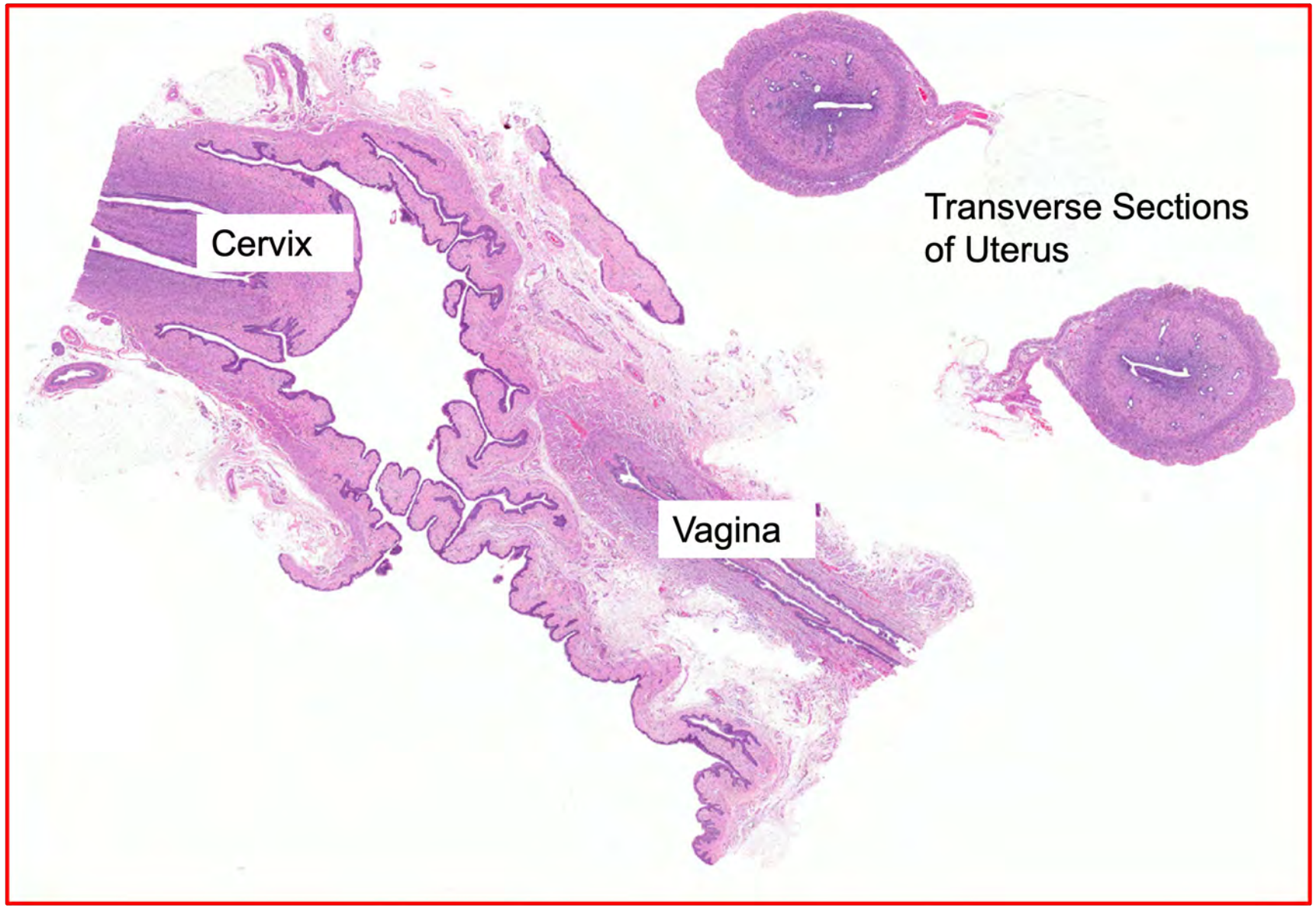




Sectioning - transverse

Transverse Sections of Uterus and Longitudinal Section of Cervix/Vagina





Advantages of Longitudinal Sectioning of Female Reproductive System

- Increased detection of reproductive targets
- Increased detection of neoplastic and nonneoplastic lesions
- Can help identify origin of neoplasms
- Less reliance on small selection of gross lesions for uterus, uterine cervix and vagina
- Improved accuracy in application of severity grades
- Increased detection of preneoplastic or subtle lesions

(Elmore et al., 2020)

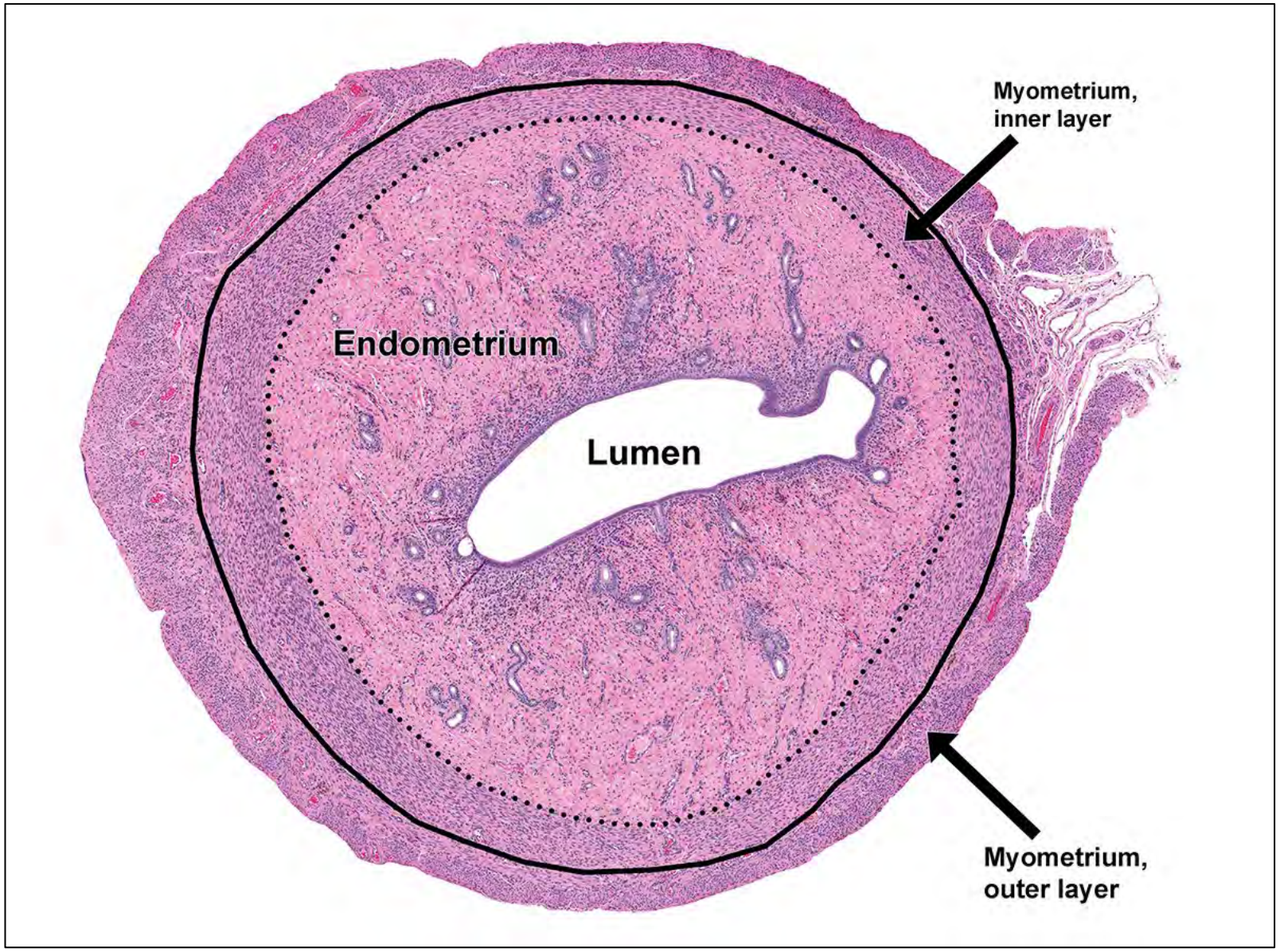
Normal Uterus Histology: Transverse Section

Endometrium

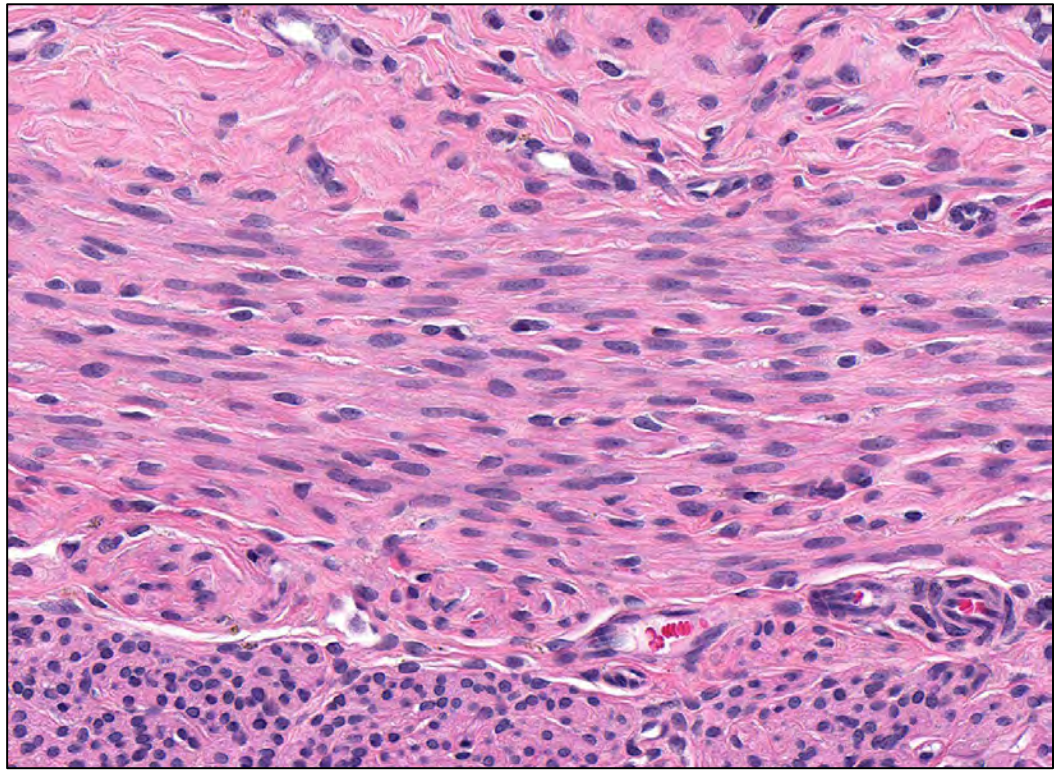
- The glandular, central portion of the uterus
- A dynamic structure composed of tubular glands embedded in a specialized and highly-cellular stroma
- The lumen of the uterus is visible as an elongated and central clear space

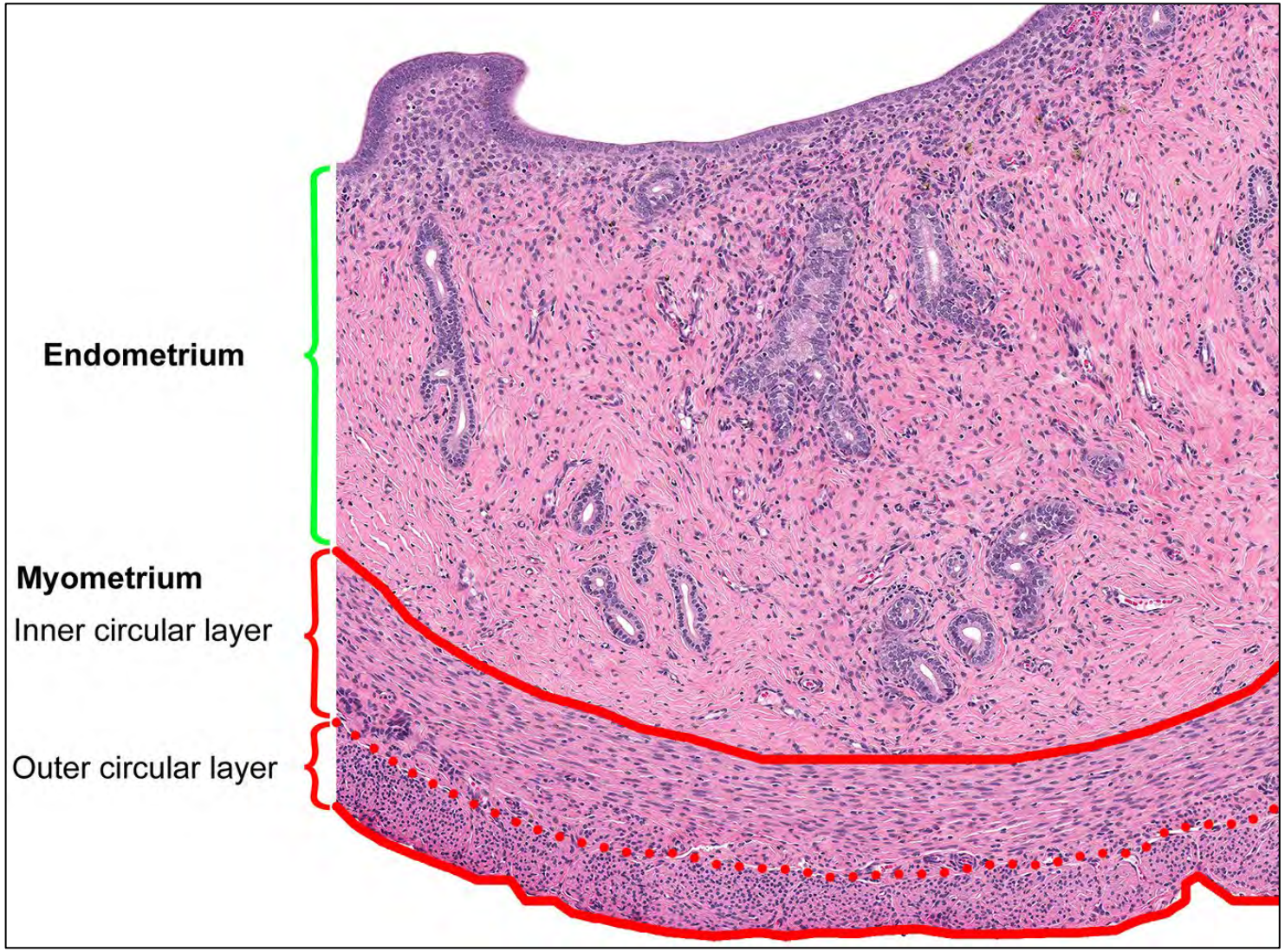
Myometrium

- The smooth muscle layer surrounding the endometrium
- Composed of an inner and outer layer of smooth muscle fibers
  - Inner layer: transversely oriented (“circular”)
  - Outer layer: longitudinally oriented



Normal Uterus: Myometrium

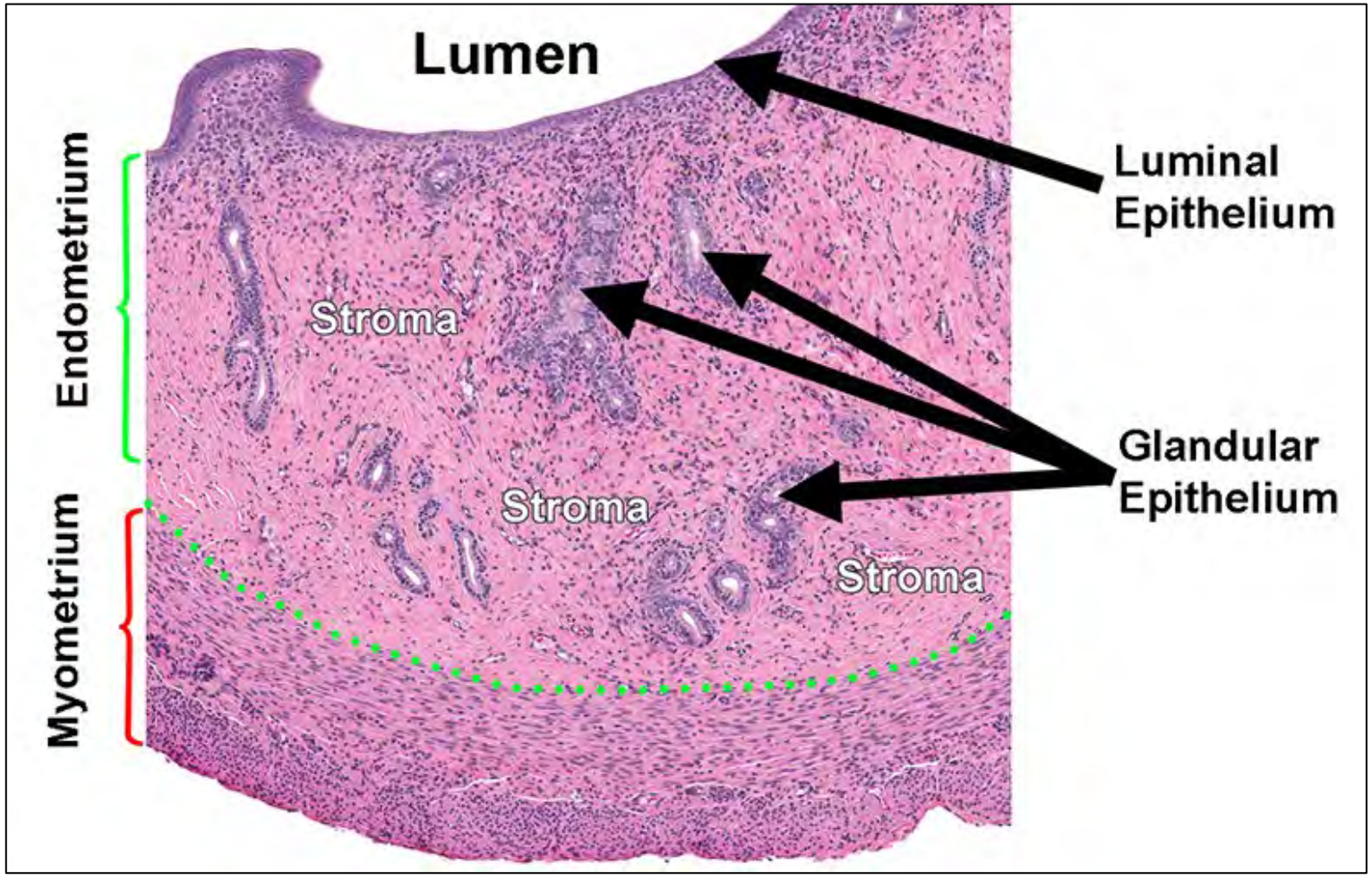




Myometrium

- Smooth muscle cells (myofibers) are elongate with tapered ends and oval (sometimes “cigar-shaped”) nuclei
- Myofibers are bound together in branching bundles that form the contractile units

Normal Uterus: Endometrium



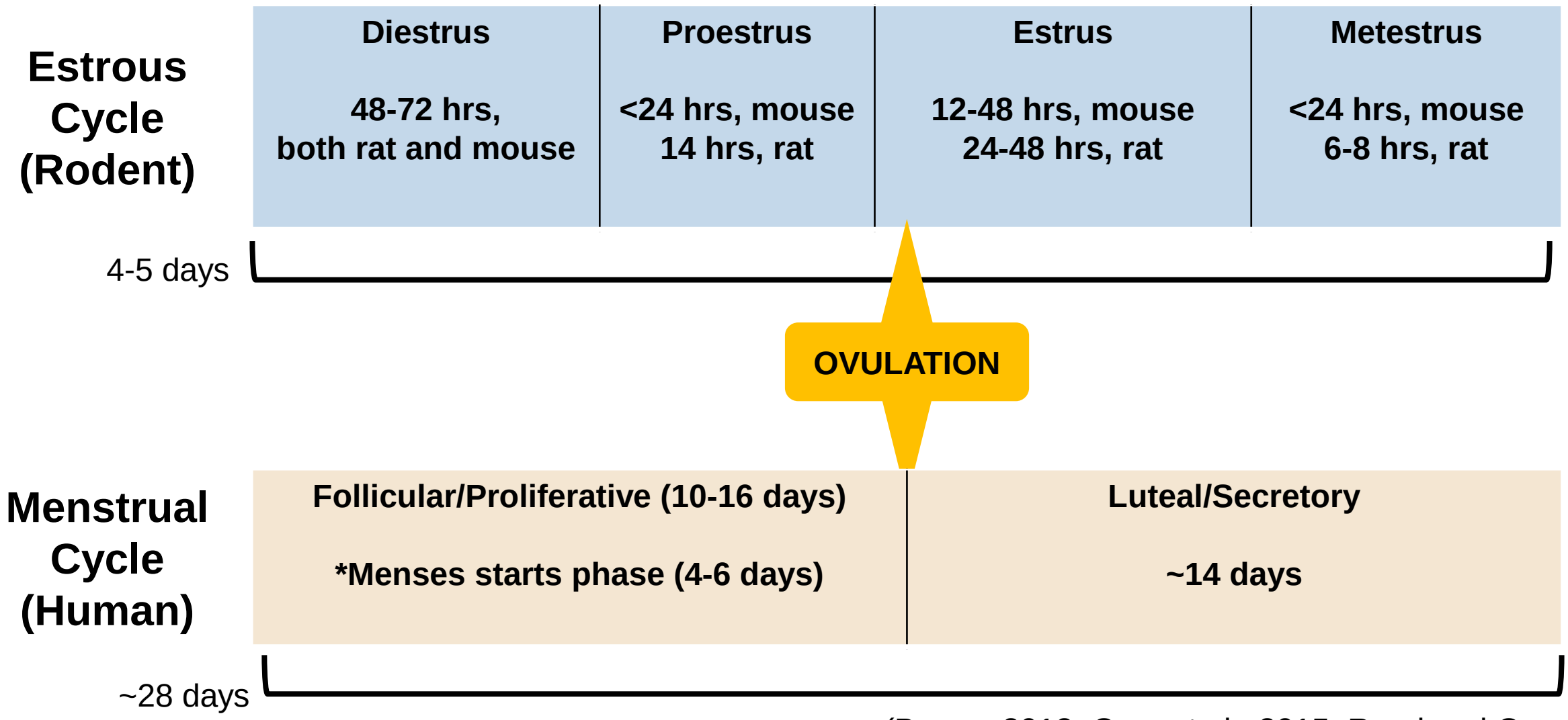
- The endometrial glands and lumen are lined by epithelium, which, like the vaginal epithelium, undergoes hormone-induced (estrogen and progesterone) alterations throughout the estrous cycle
- The underlying endometrial stroma is highly cellular and composed of spindle-shaped mesenchymal cells
- The stroma also contains variable amounts of edema and collagen, depending on the animal's age and estrous cycle stage

Female Reproductive Estrous Cycle

- Rats and mice, like most mammals, have an estrous cycle
  - The endometrial epithelium is not shed
- In contrast, humans and Old World nonhuman primates have menstrual cycles, as do four species of bat, the elephant shrew, and the spiny mouse (Bellofiore et al., 2017)
  - In menstrual cycles, the superficial layer of the endometrium (stratum functionalis) is shed with each cycle (Yamaguchi et al., 2021)
- Understanding the normal cyclic changes in the ovary, uterus, and vagina is important for the recognition of uterine pathology in rodents
  - Examination of the hormonally-responsive organs (ovary, uterus, and vagina) and hypothalamic-pituitary-gonad axis together allows for the recognition of hormonal imbalances

(See Dixon et al., 2014 and Westwood, 2008 for further information on the rodent estrous cycle)

Female Reproductive Cycles: Estrous vs. Menstrual

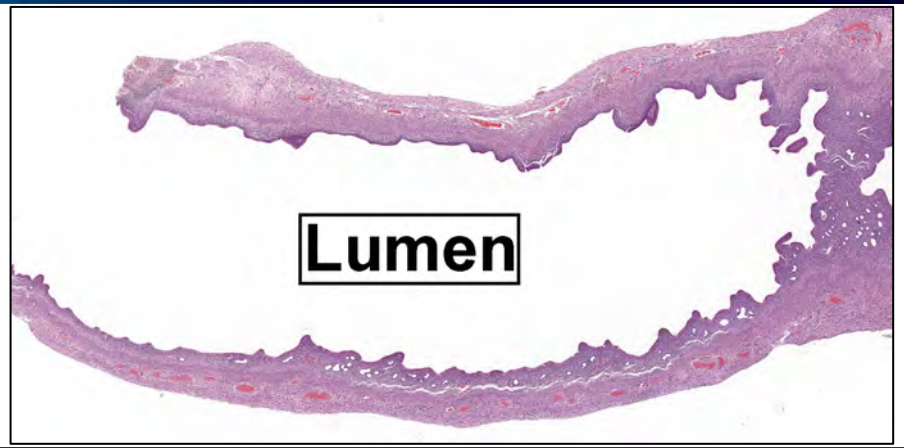


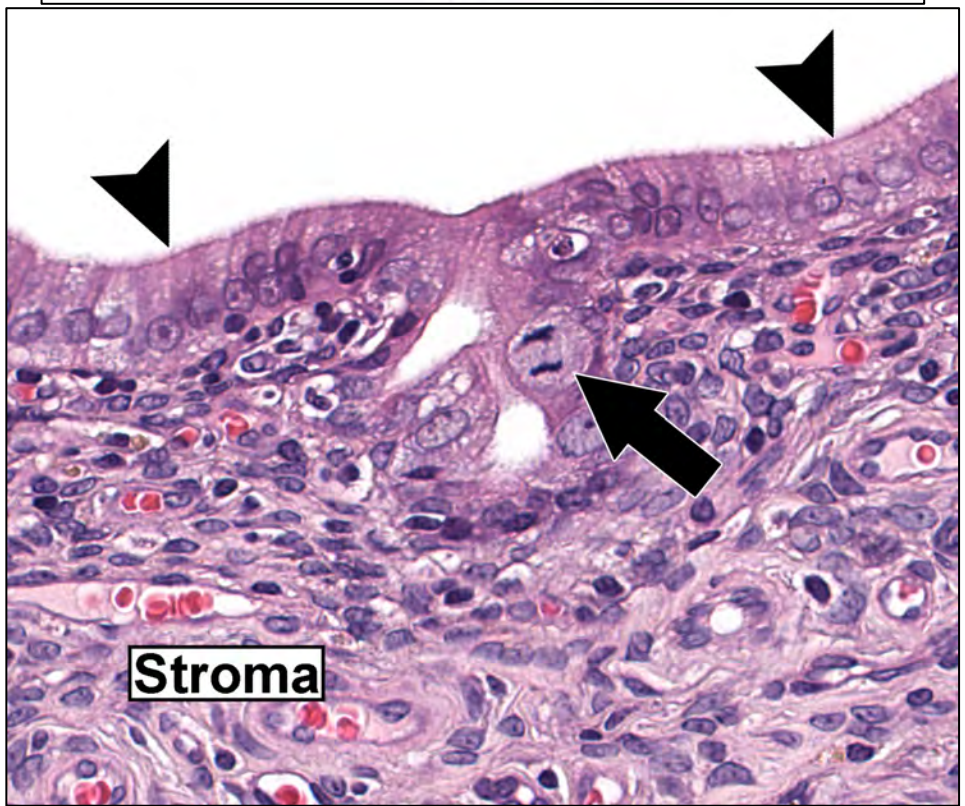
(Bower, 2018; Cora et al., 2015; Reed and Carr, 2018)

Estrous Cycle: Histologic Changes

Proestrus: Uterus

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

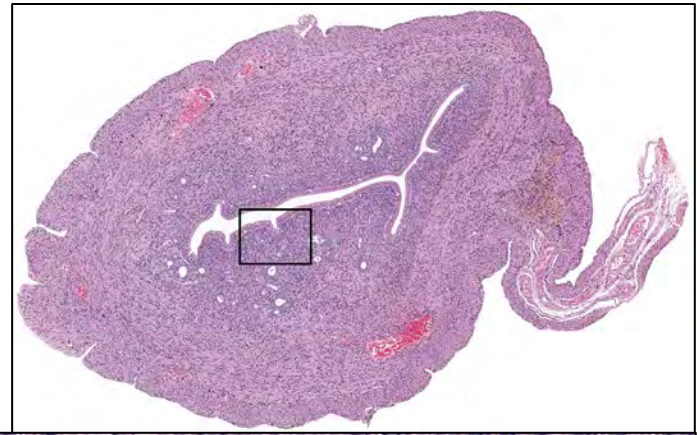


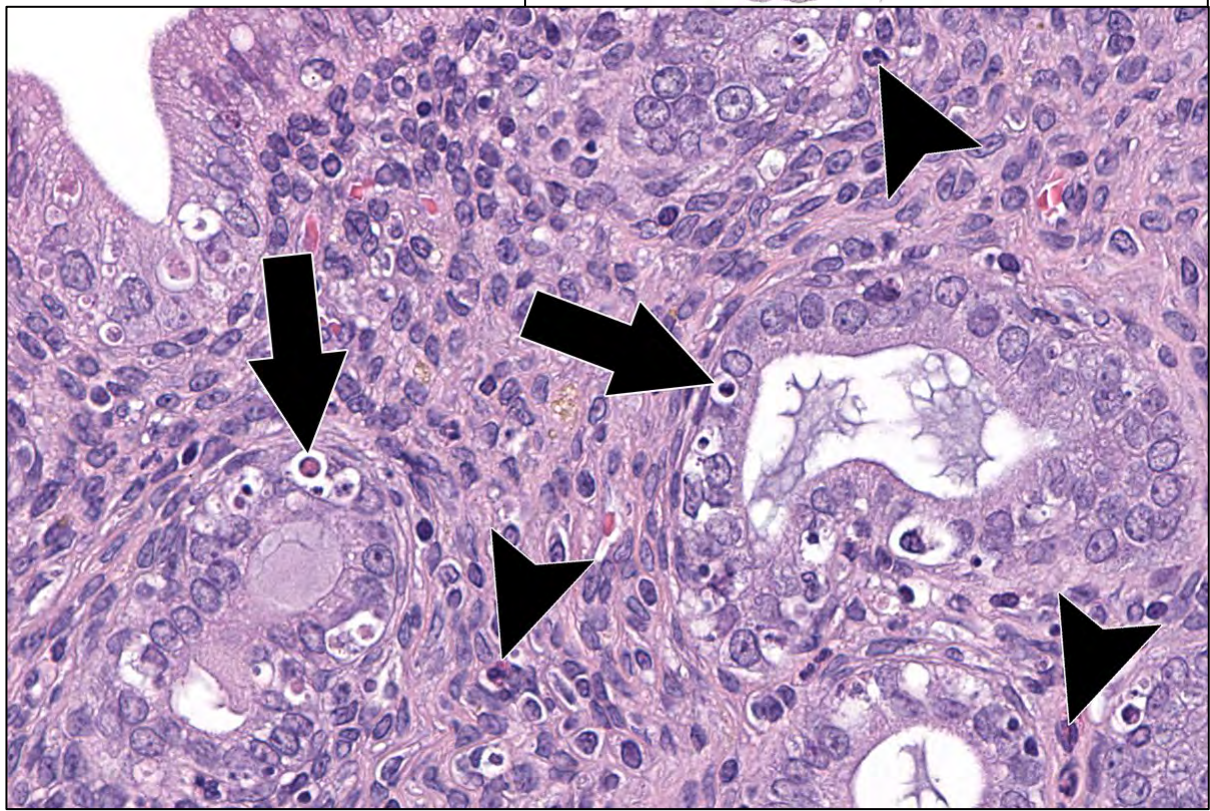


Estrous Cycle: Histologic Changes

Estrus: Uterus

- Degeneration (seen as apoptotic cells, which defines 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 stroma



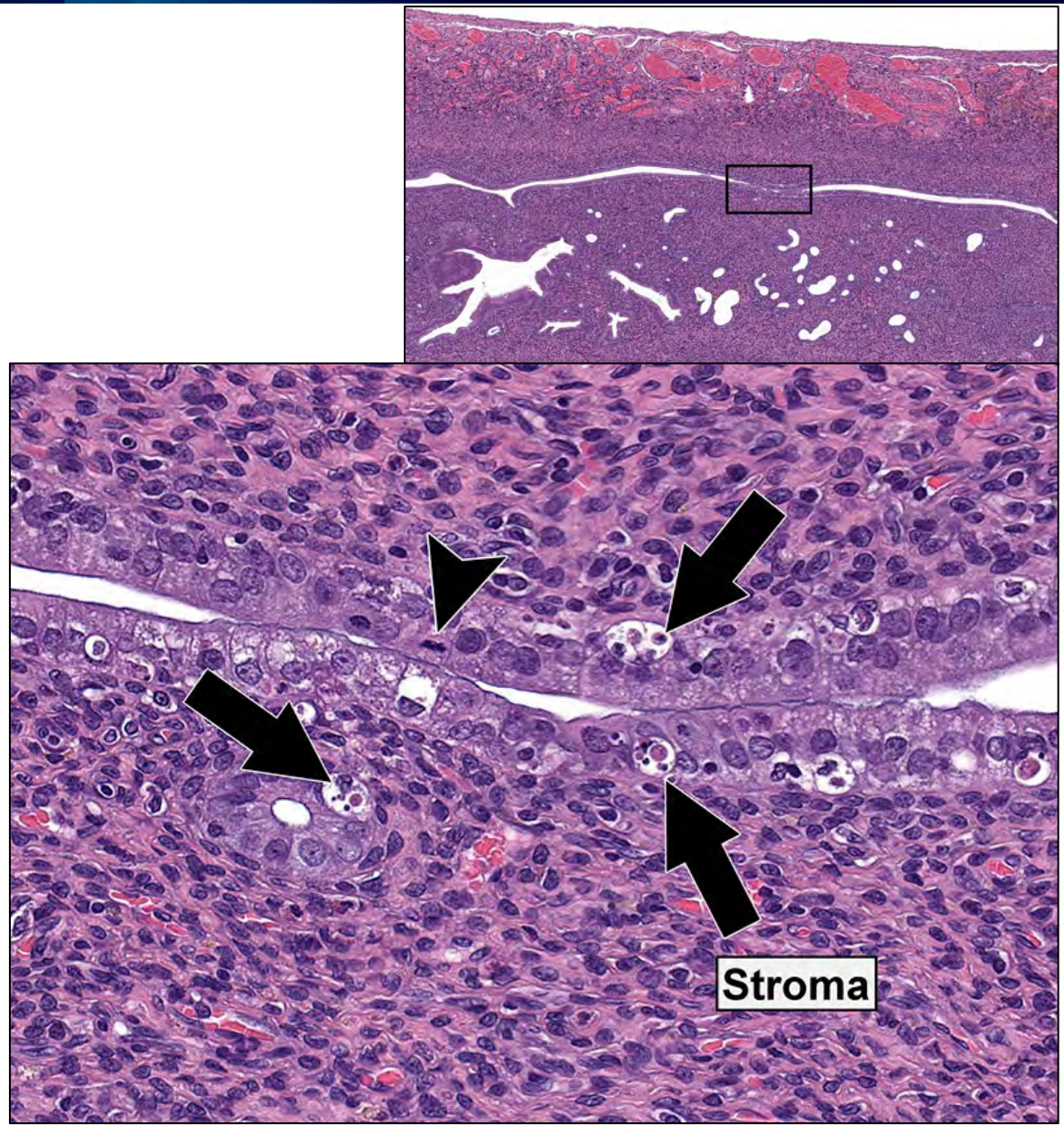


(Hobbie and Dixon, 2020; Westwood, 2008)

Estrous Cycle: Histologic Changes

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

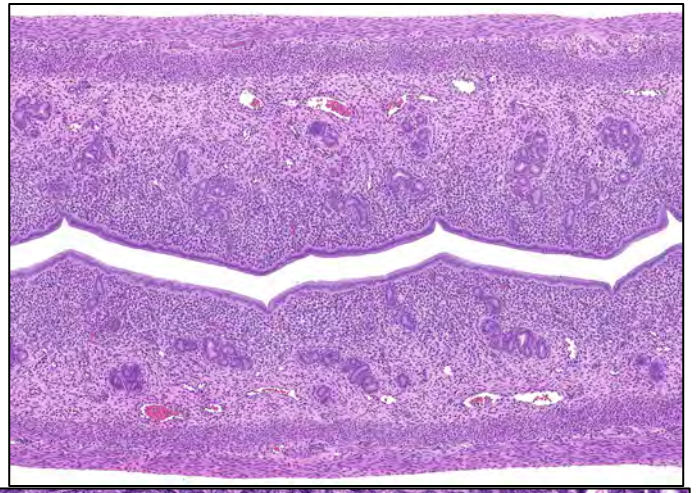


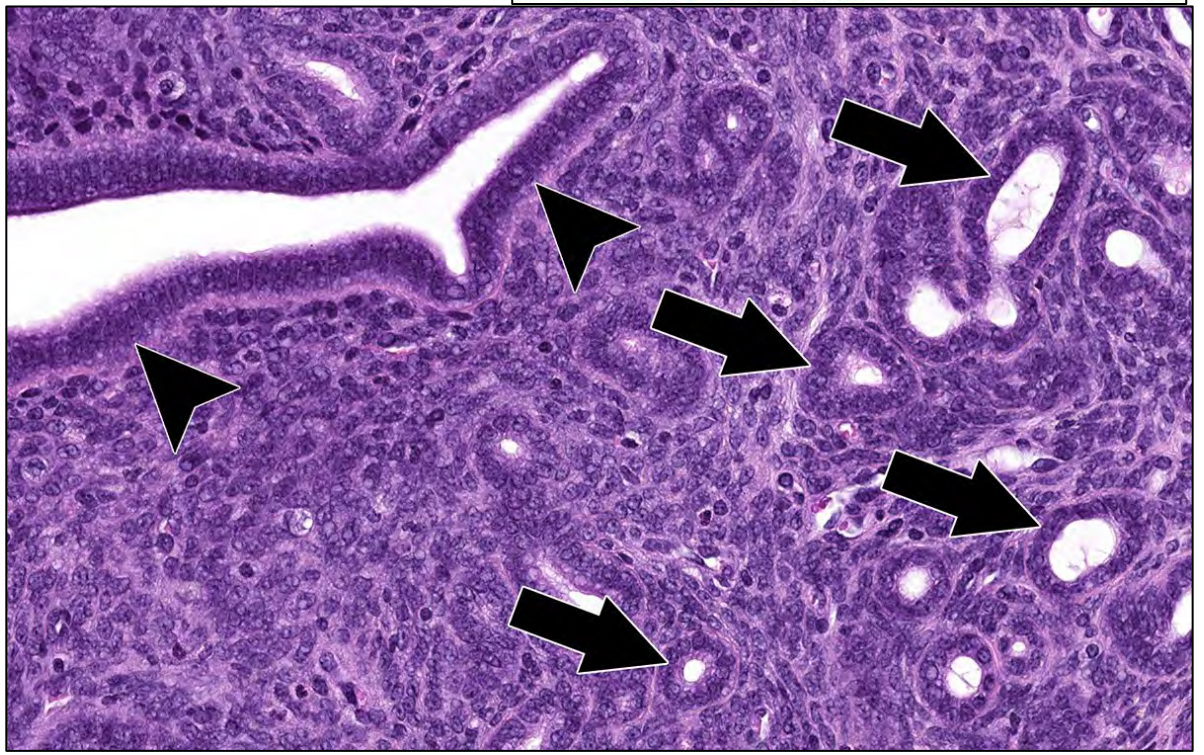
(Dixon et al., 2014)

Estrous Cycle: Histologic Changes

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 (labeled in bottom image) is compact but may show slight edema toward end of stage

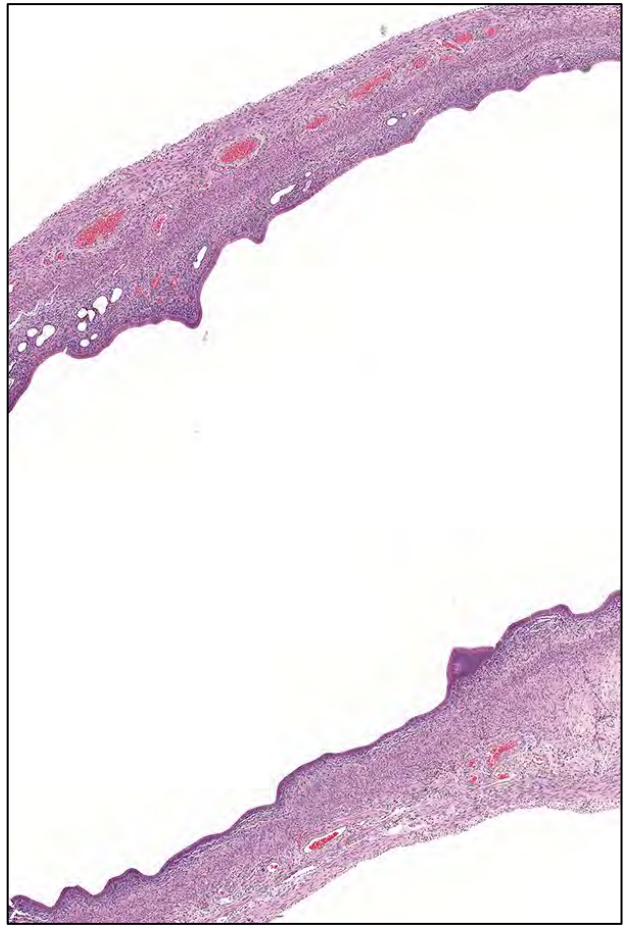




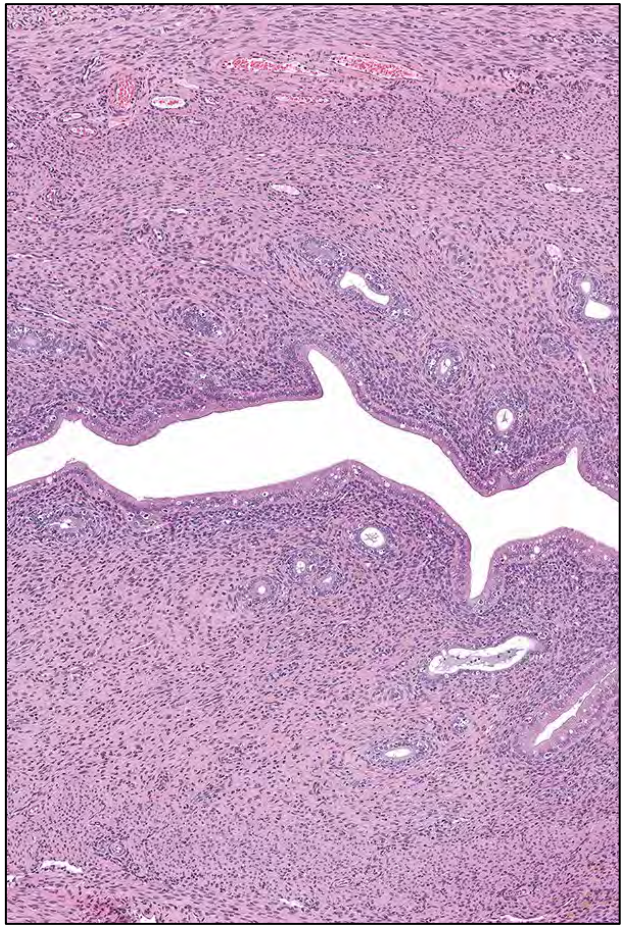
(Dixon et al., 2014; Westwood, 2008)

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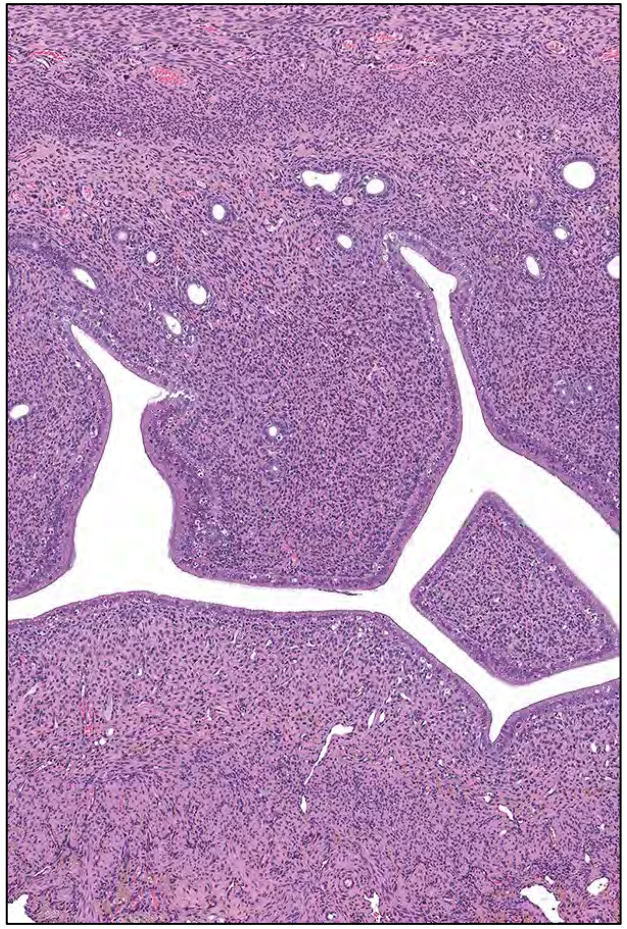
Estrous Cycle Changes: Uterus (Low Magnification)



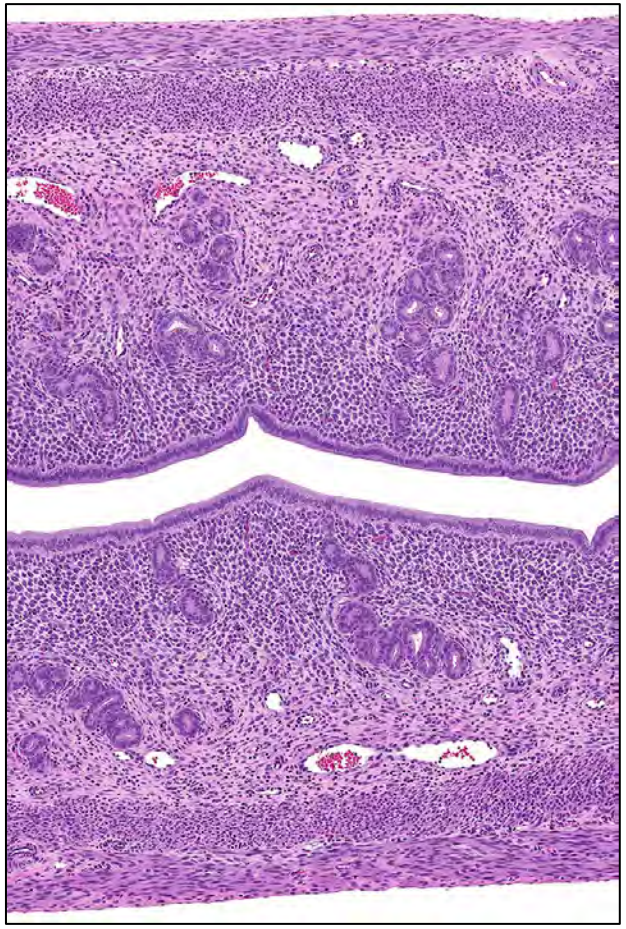
Proestrus:
- The lumen is dilated with clear fluid
- Epithelium is low to tall columnar



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



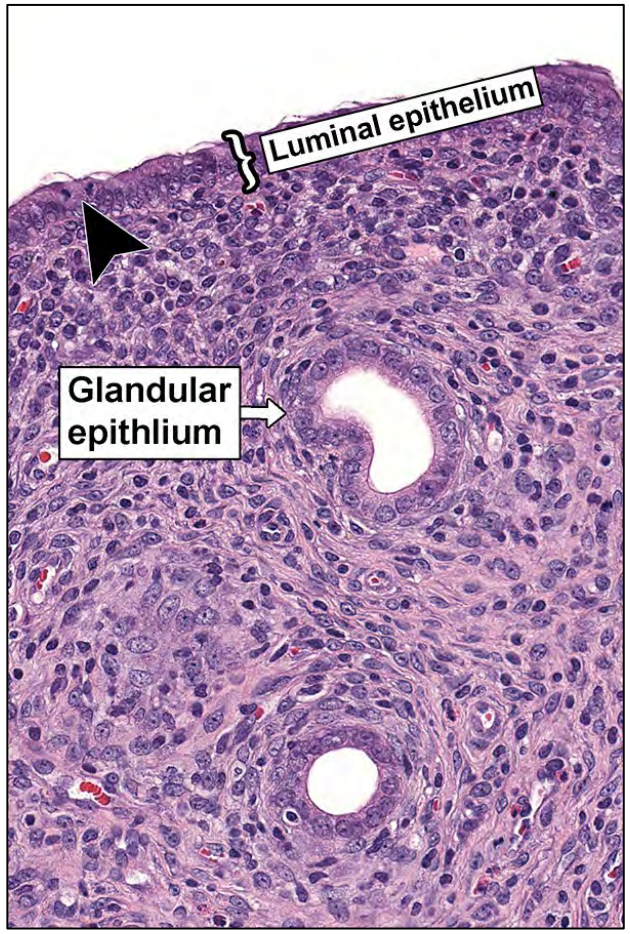
Metestrus:
- The epithelium is reduced in height in comparison to estrus

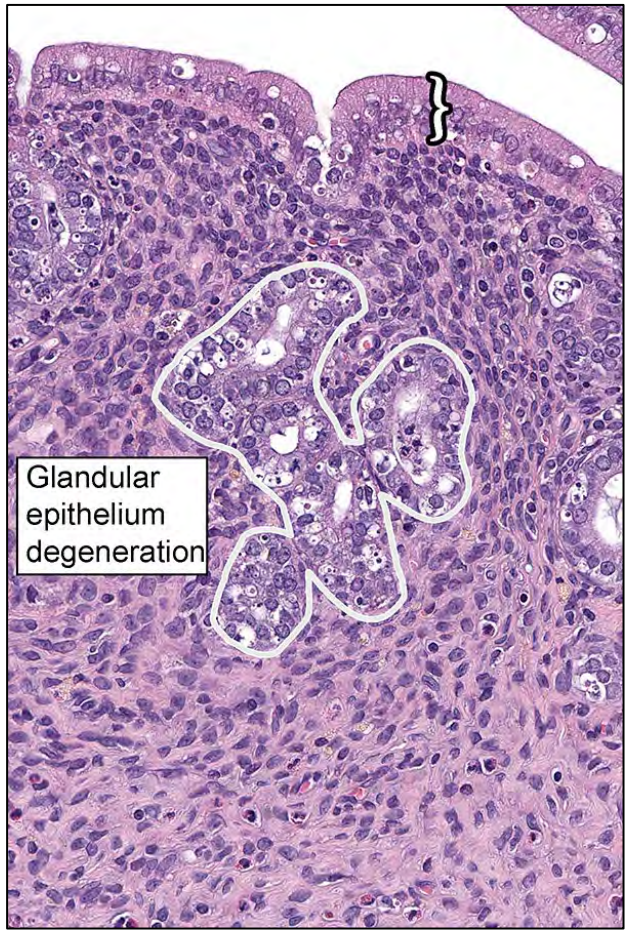


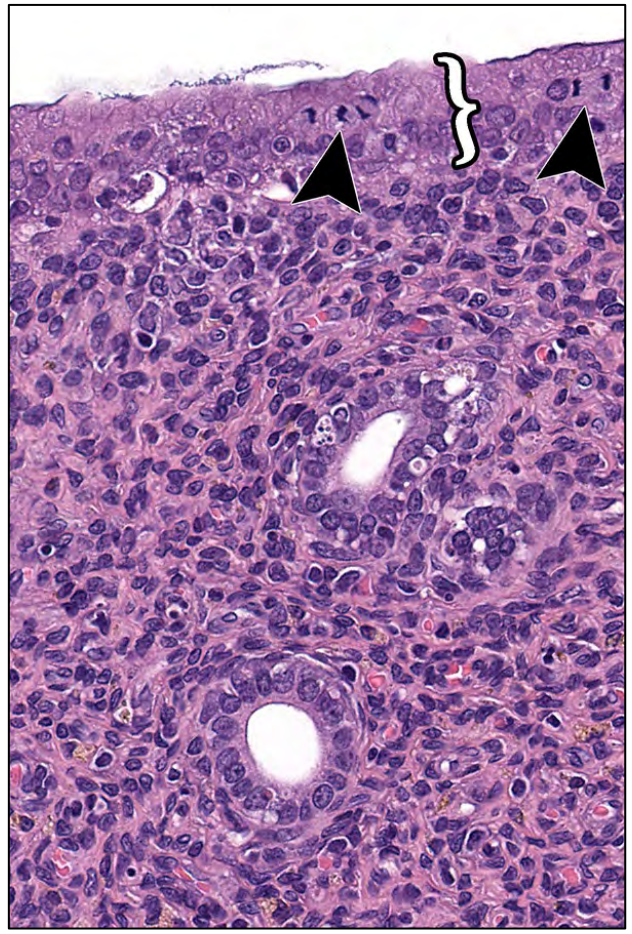
Diestrus:
- The uterus appears small and inactive, with a slit-like lumen
- Epithelium at lowest height

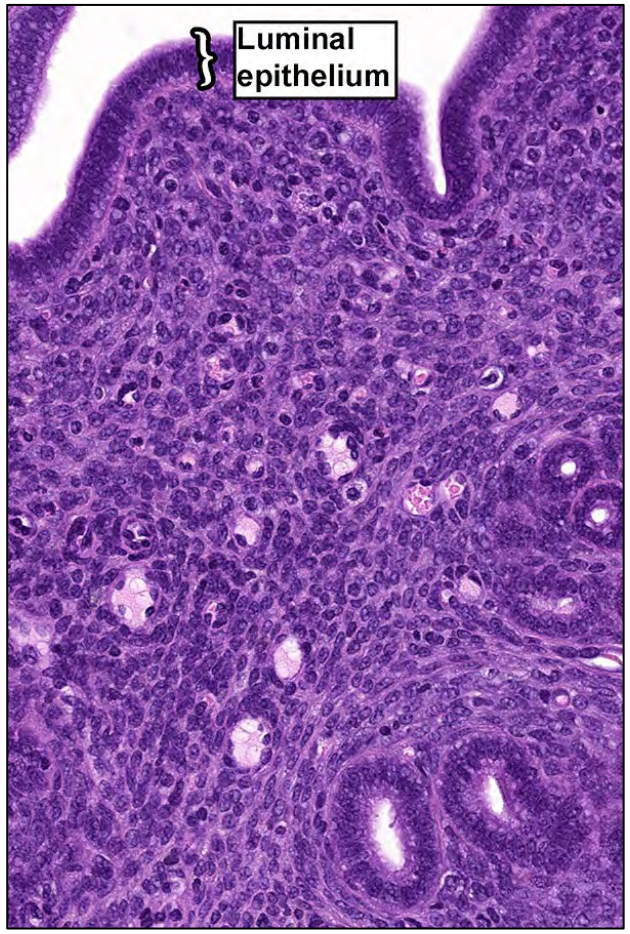
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Estrous Cycle Changes: Uterus (High Magnification)









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)

Congenital Lesions

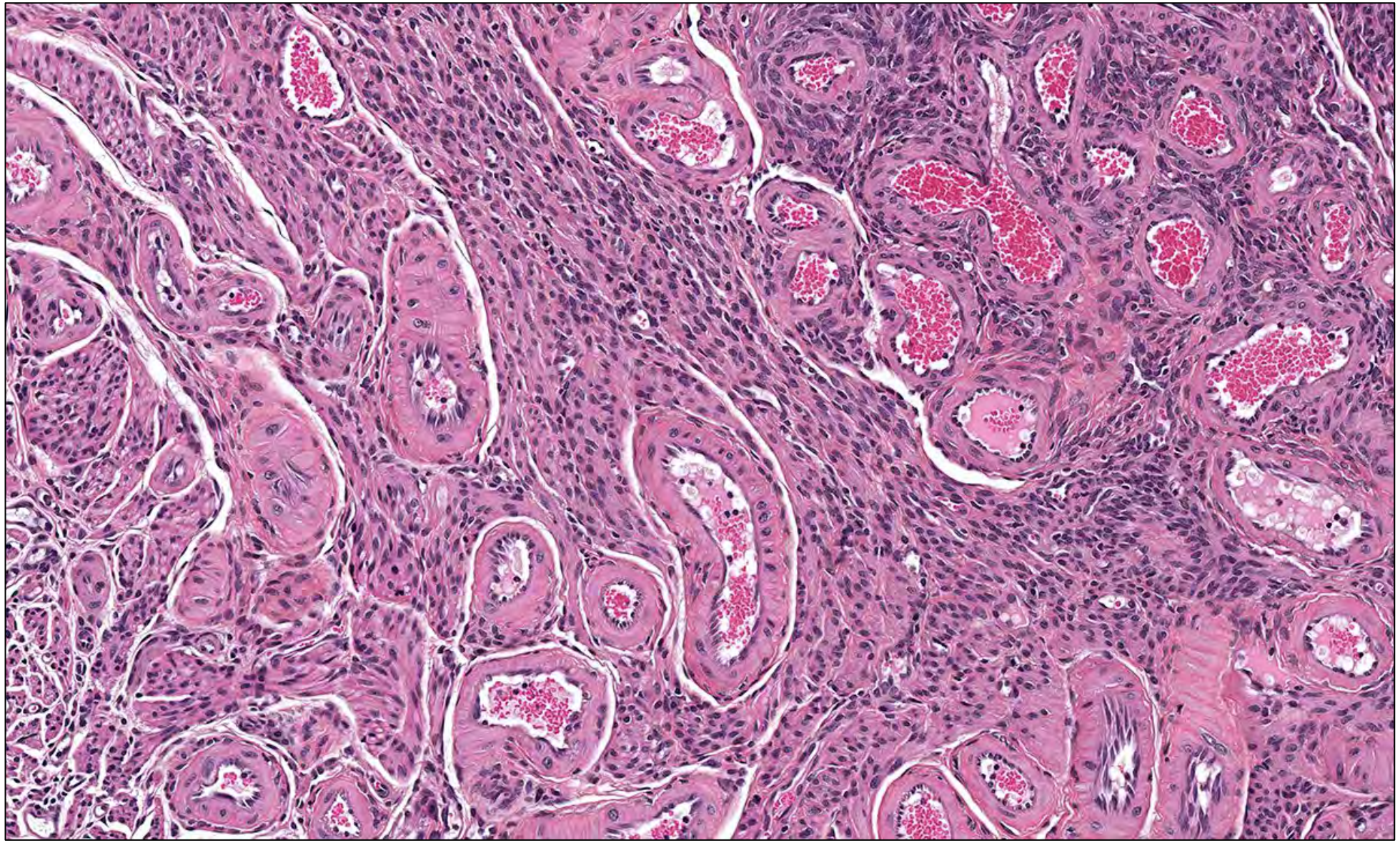
- Agenesis/Hypoplasia
  - Agenesis and hypoplasia are terms describing the absence of or reduced development of an organ, respectively
  - Both conditions in the mouse uterus are rare but described with a higher prevalence in ACI rat strains (Shoji and Harata, 1977)
- Congenital mucometra
  - Congenital mucometra is a condition defined by the distension of the uterus by mucus at birth
  - Caused by lower reproductive tract-anomalies like imperforate vaginal septum
  - Imperforate vaginal septum is rare, but also described in humans, and is due to an incomplete fusion of the vaginal components of the Müllerian duct and the urogenital sinus (Jain et al., 2013)
  - Leads to distension of the caudal abdomen due to mucus accumulation in the uterus

Congenital Lesions

- Vascular Hamartoma
  - Focal area of mature and redundant myometrial vessels
  - Occasionally observed in the B6C3F1 mouse and has been described in the uterus of a Sprague-Dawley rat (Dixon et al., 2018)
  - What feature is present in the vessels which could help distinguish from neoplastic endothelial proliferation, such as hemangioma or hemangiosarcoma?
    - **Advance to next slide**



Congenital Lesions

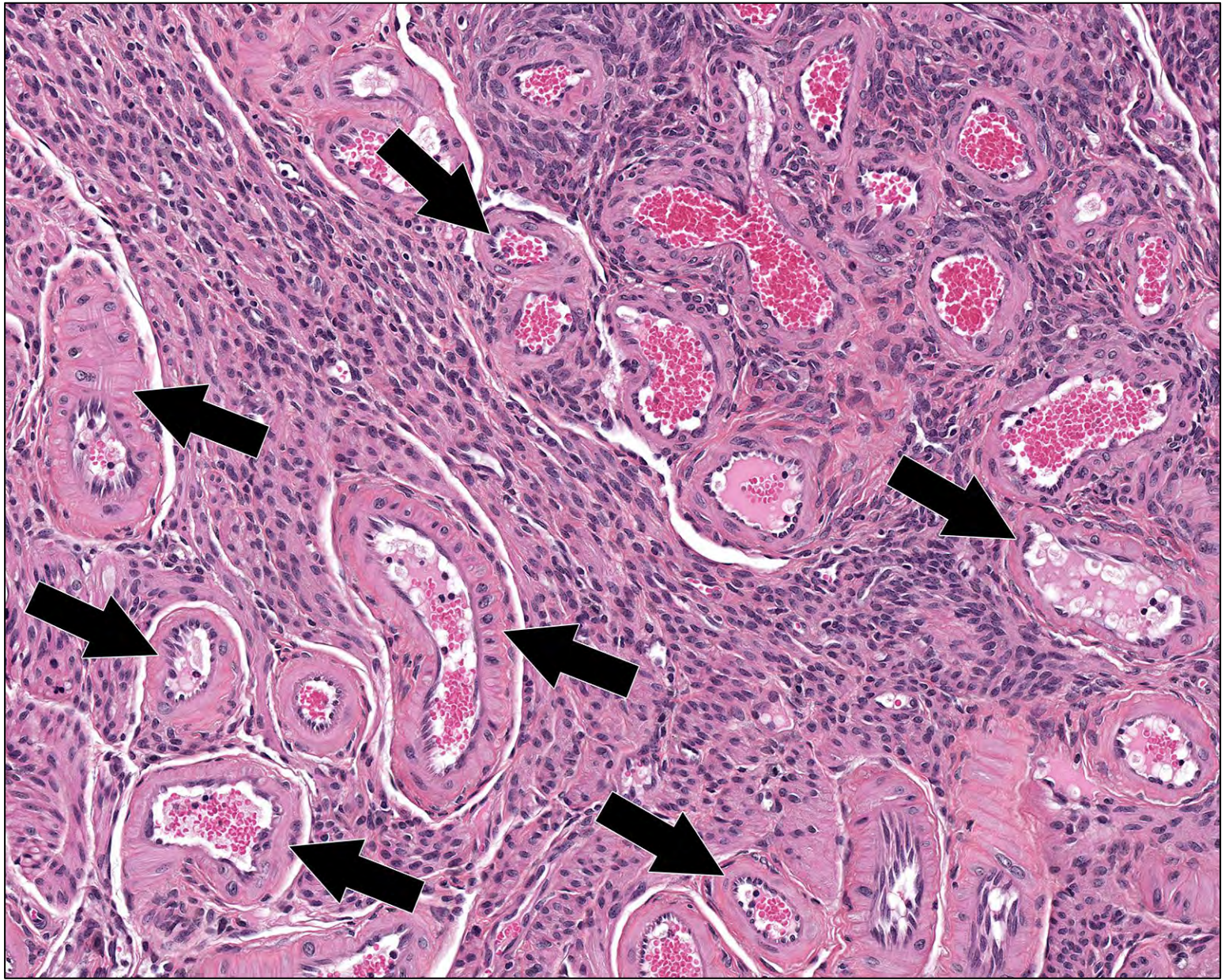


Next slide

Congenital Lesions

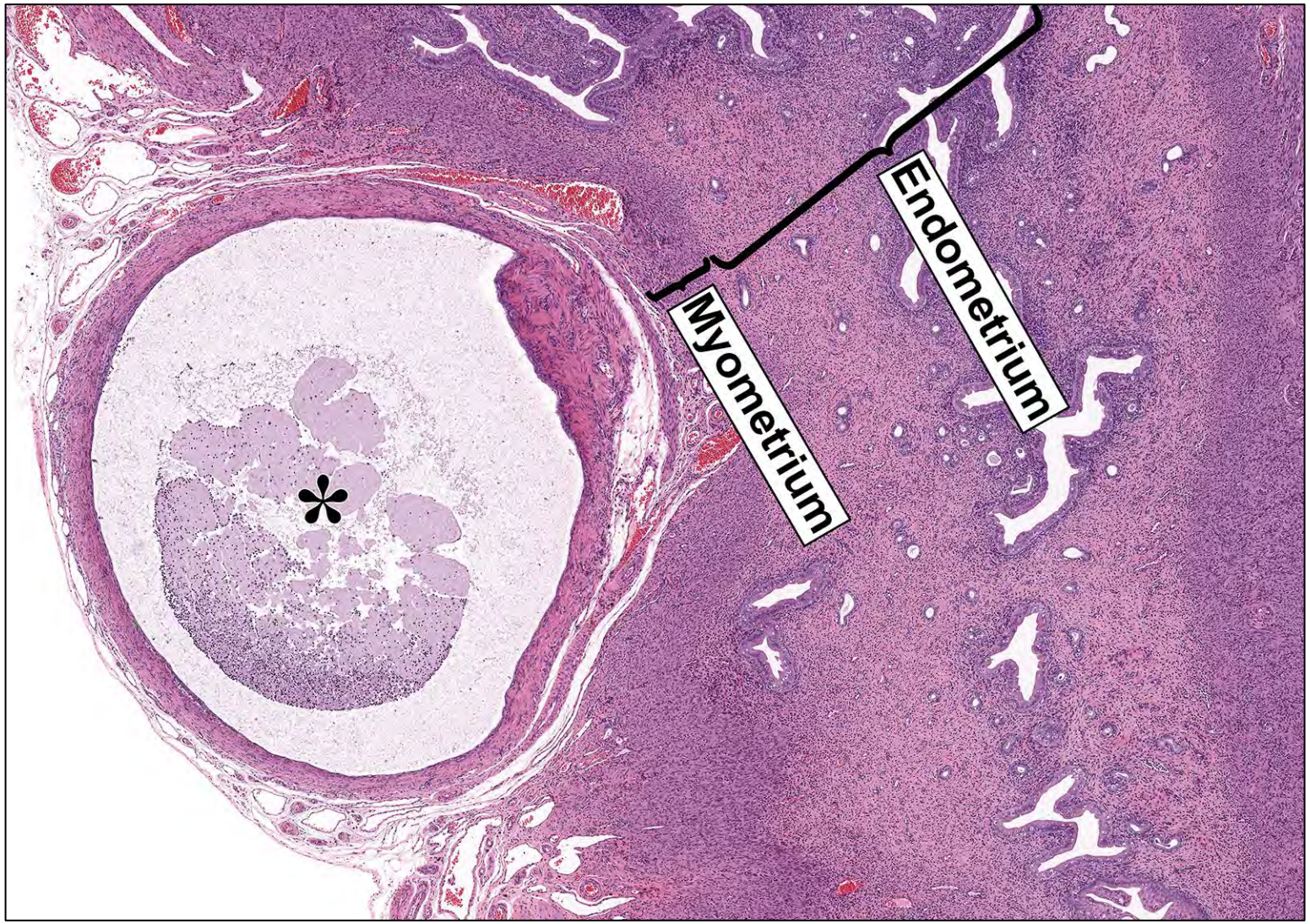
The vessels walls in this hamartoma are complete and contain a variably thick tunica media/smooth muscle layer (arrows).

In contrast, a hemangioma/ hemangiosarcoma would lack this smooth muscle layer and consist only of neoplastic endothelial-lined cavities.



Another Congenital Lesion: What's your diagnosis?

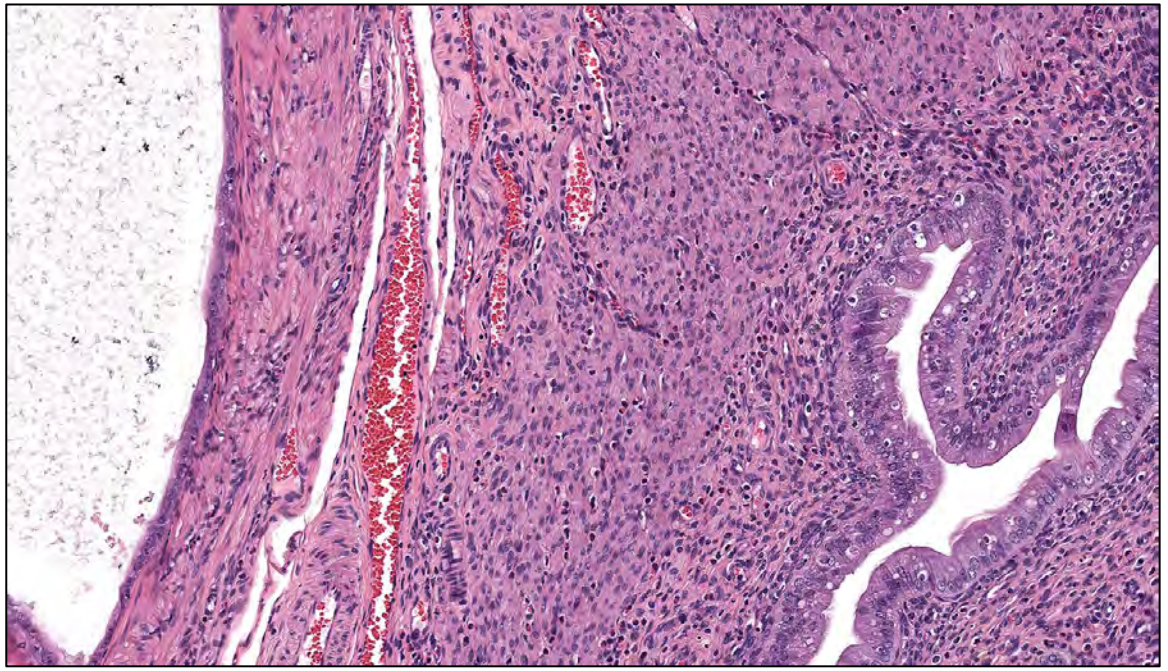


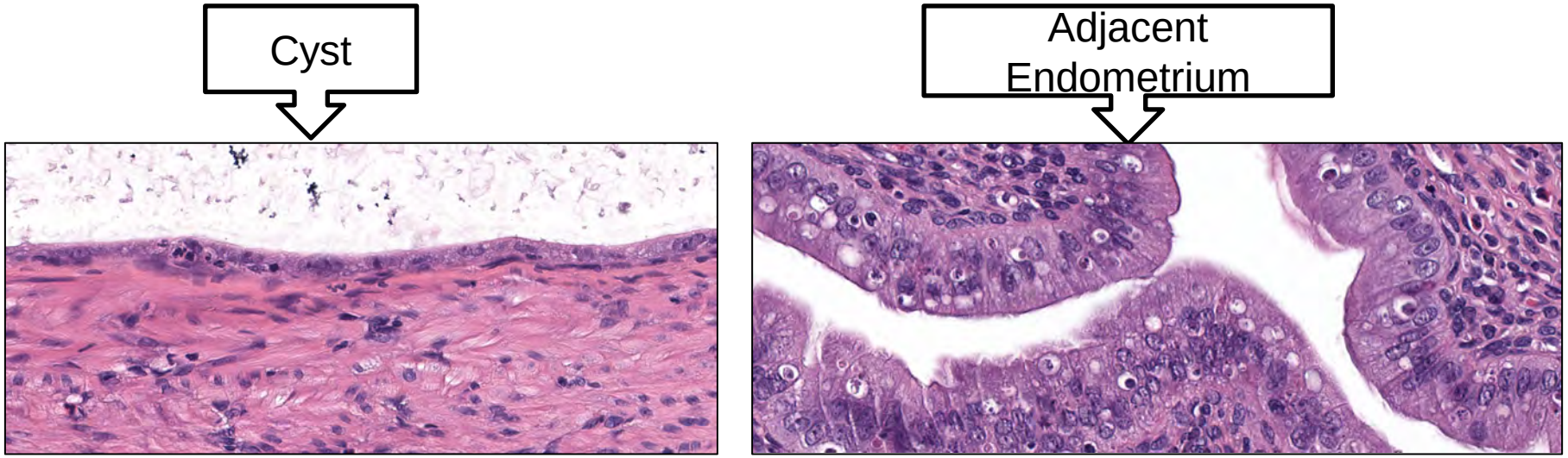


This cyst (*) is arising from the lateral surface of the uterus. What structure may it be derived from? **Advance to examine the cyst lining.**

What's your diagnosis?

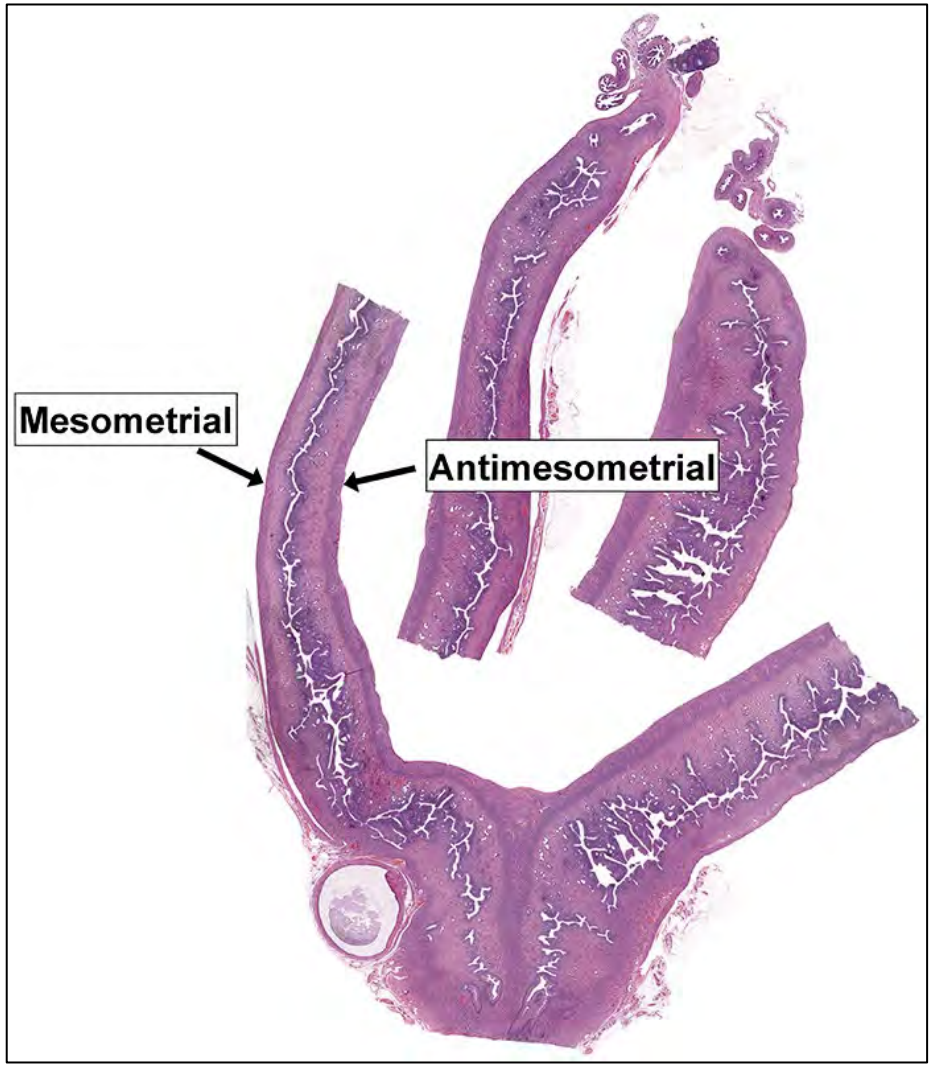
- The epithelial lining of the lumen of the endometrium is taller than that lining the cyst (columnar vs. cuboidal), but has similar basophilic cytoplasm
- What two structures could this cyst be arising from? ***Recall the embryologic development of the reproductive tract.***





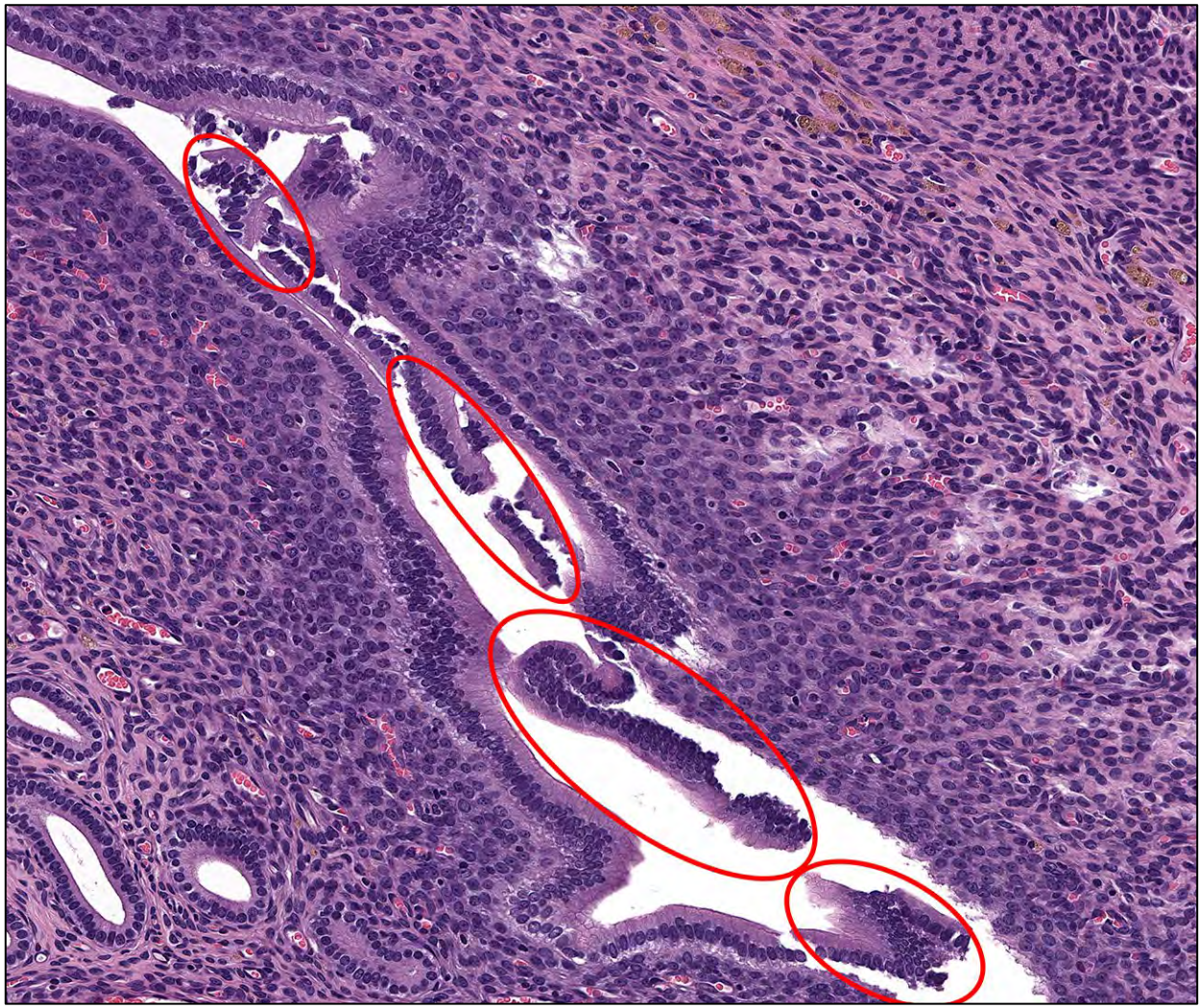
Mesonephric and Paramesonephric Duct Remnants

- Diagnosis: Mesonephric or paramesonephric duct remnants
  - Remnants of the male (mesonephric/Wolffian ducts) or female (paramesonephric/Müllerian ducts) embryonic structures can be found incidentally in either sex (Dixon et al., 2014)
- Microscopic or grossly evident cystic structure(s), generally along the mesometrial (lateral) aspect of the female reproductive tract
  - Mesometrial: side with broad ligament/mesometrium insertion
  - Antimesometrial: opposite side
- Immunohistochemistry (IHC) is necessary to distinguish between mesonephric and paramesonephric duct remnants
  - A study using IHC for Paired box 8 (PAX8) and GATA binding protein 3 (GATA3) for paramesonephric-derived and mesonephric-derived cysts, respectively, suggested most of these cysts in female rats arise from the paramesonephric duct (Jackson-Humbles et al., 2018)



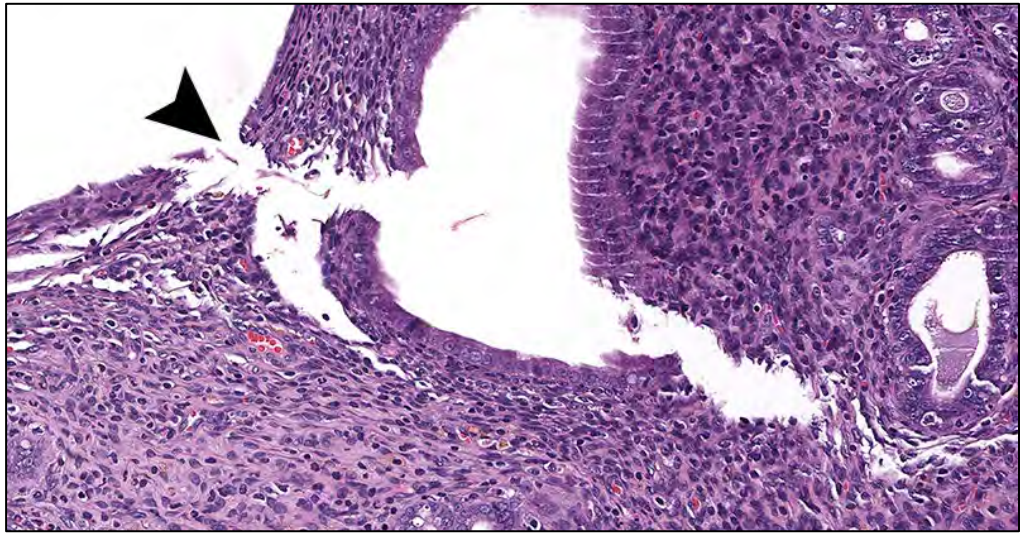
Uterine Histology: Artifacts

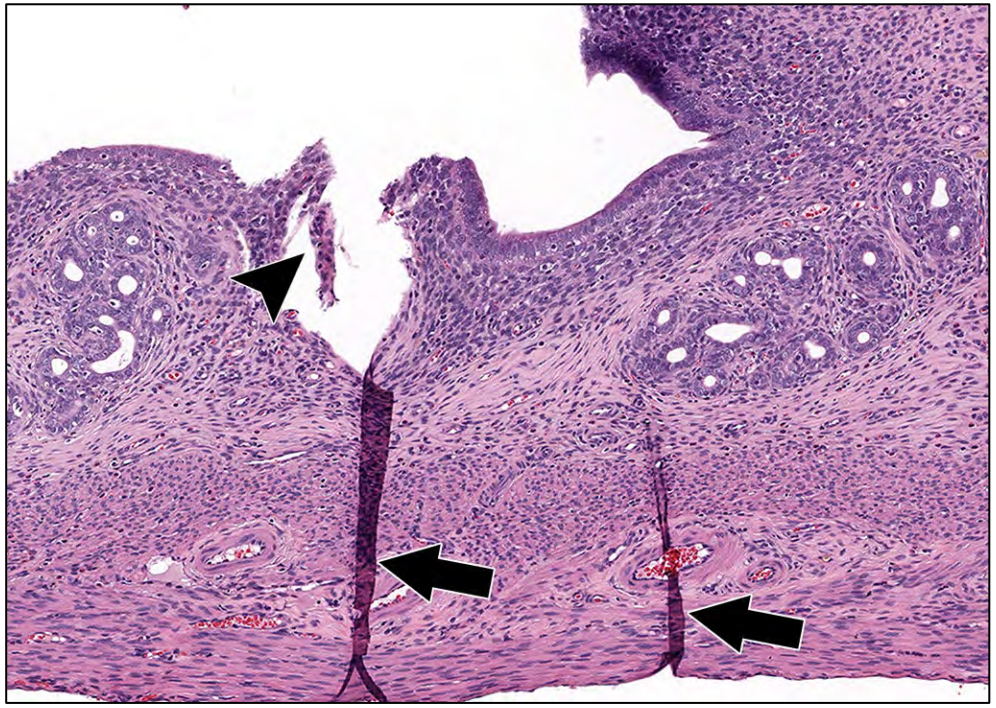
- The uterus is a complex organ with variably-textured components, which makes the generation of sections without processing, staining, or trimming artifacts challenging
- Loss and/or sloughing of the epithelium (red circles) can occur as a postmortem (after death), autolytic change, or from a trimming artifact
  - Separation of the glandular epithelium from its basement membrane can occur as early as 30 minutes postmortem (Dixon et al., 2014)
  - Distinguishing this from antemortem (before death) epithelial loss, ulceration, can be difficult if the ulceration is peracute (occurring extremely suddenly). However, ulceration is generally associated with other changes (inflammation, hemorrhage, necrosis)

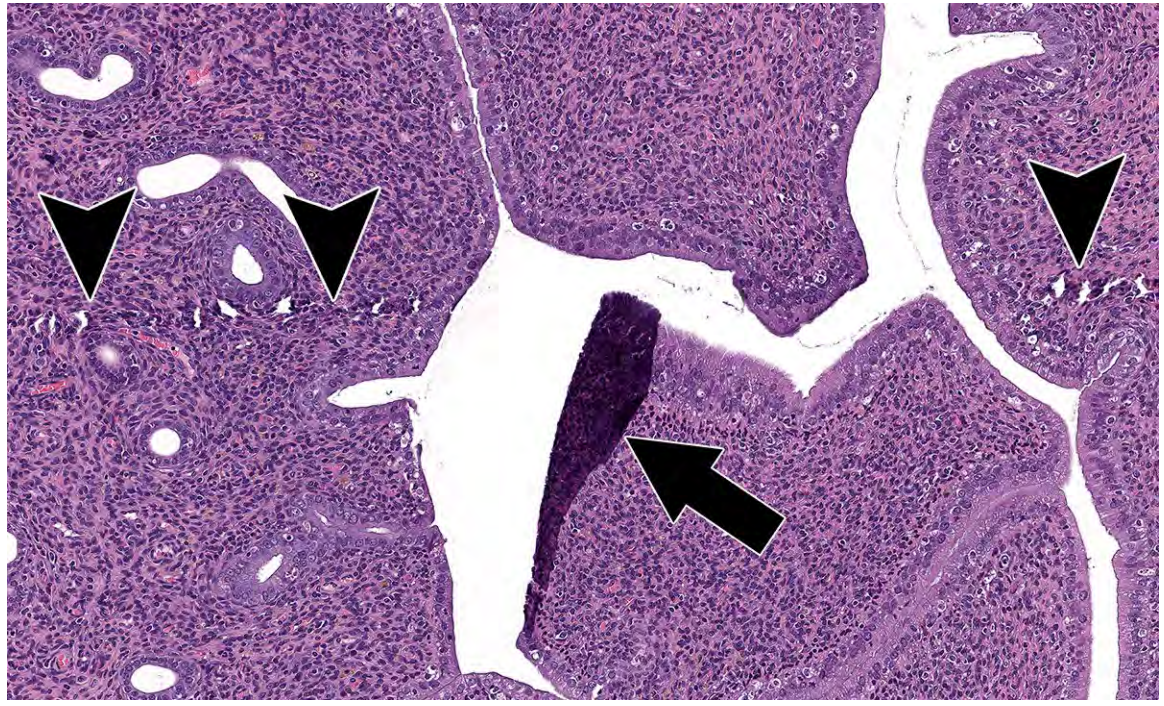


Uterine Histology: Trimming Artifacts

- Sectioning artifacts (arrowheads) may appear as large-fragmented tissue tears (right and lower left photos) or as repeated linear fractures (knife mark, lower right photo)
- Folds in the uterus (arrows) are also common and can mask important pathologic lesions

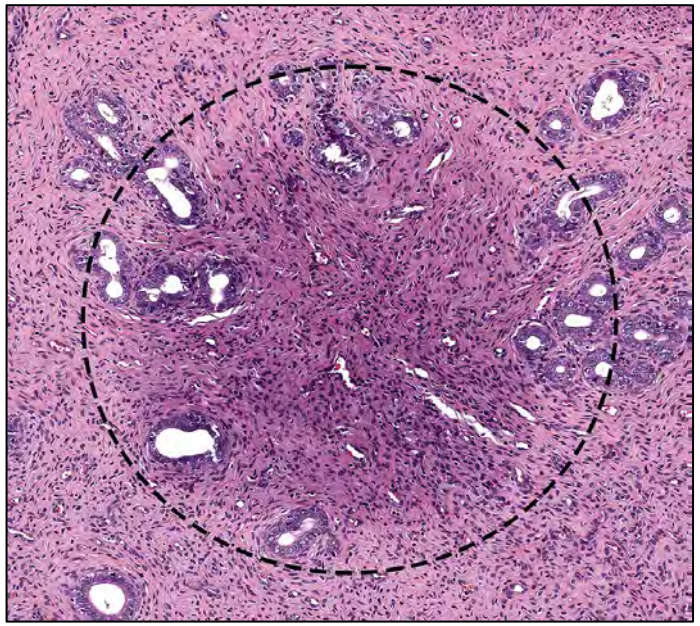


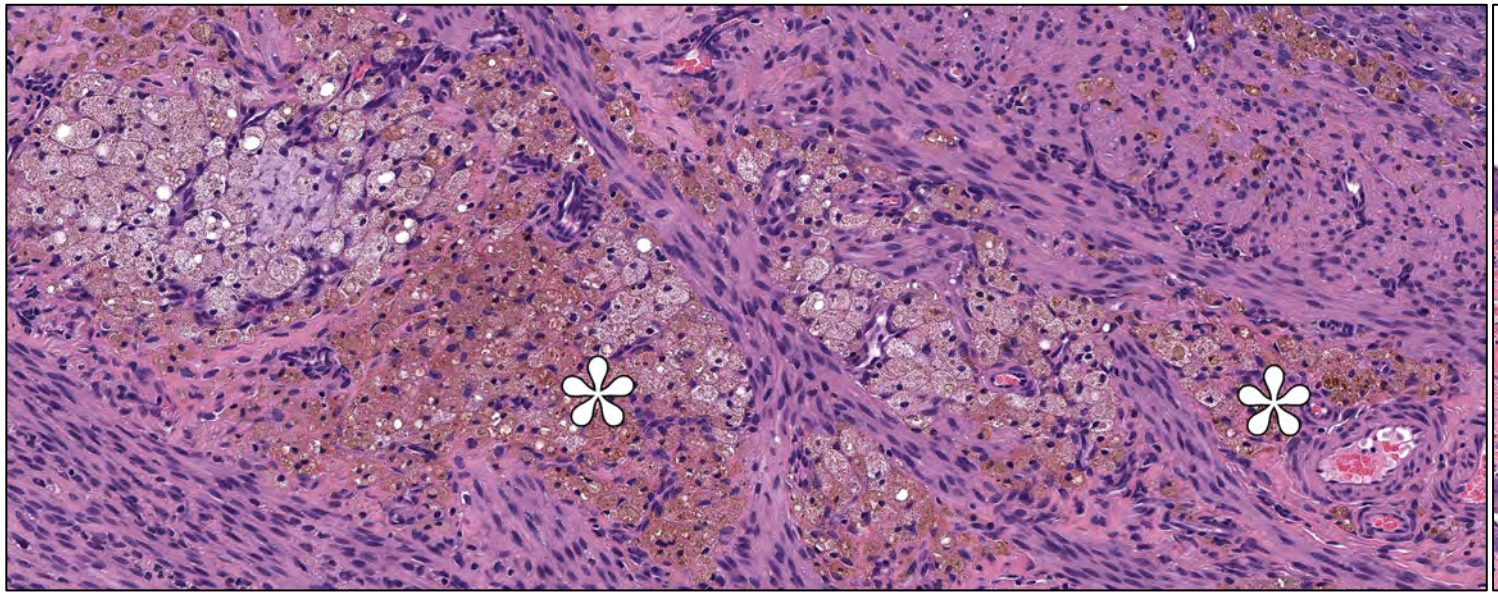


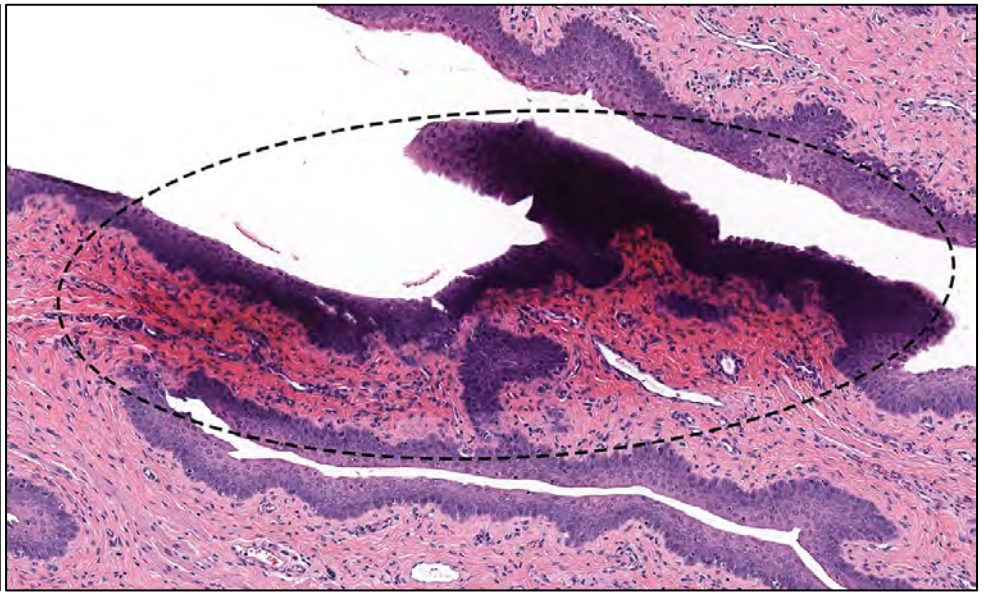


Uterine Histology: Staining Artifacts

- Staining artifacts, like the darkened pink areas in the images on the right and bottom right (black circles), can appear in any tissue
- These must be distinguished from pathologic changes in staining, such as the pale areas observed in necrosis, or the accumulation of pigment (hemosiderin in the image below, white asterisks) which can occur with age or parity in the mouse and rat uterus







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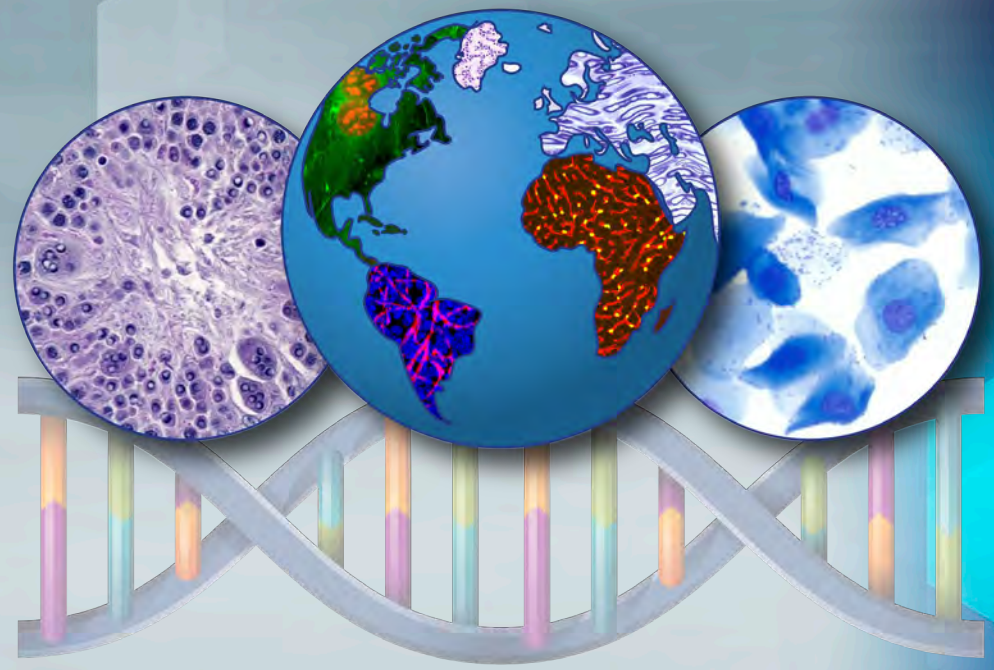
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