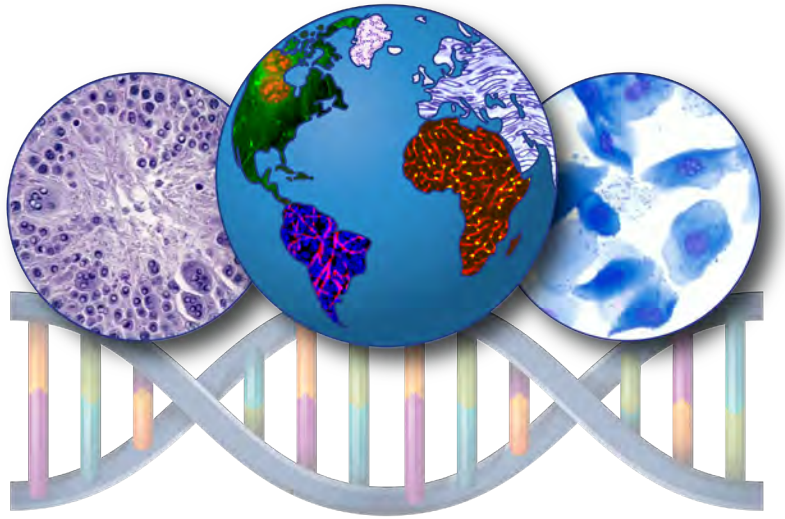
National Institute of
Environmental Health Sciences
Division of Translational Toxicology

Nonproliferative Lesions in the Rodent Uterus



Division of Translational Toxicology Global Toxicologic Pathology Training Program

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

Nonproliferative Lesions of the Uterus

- Adenomyosis
- Angiectasis
- Atrophy
- Cyst
- Decidual Reaction
- Dilatation, Lumen
- Fibrosis
- Infiltrate, Inflammatory Cell
- Inflammation
- Metaplasia, Squamous Cell
- Pigment

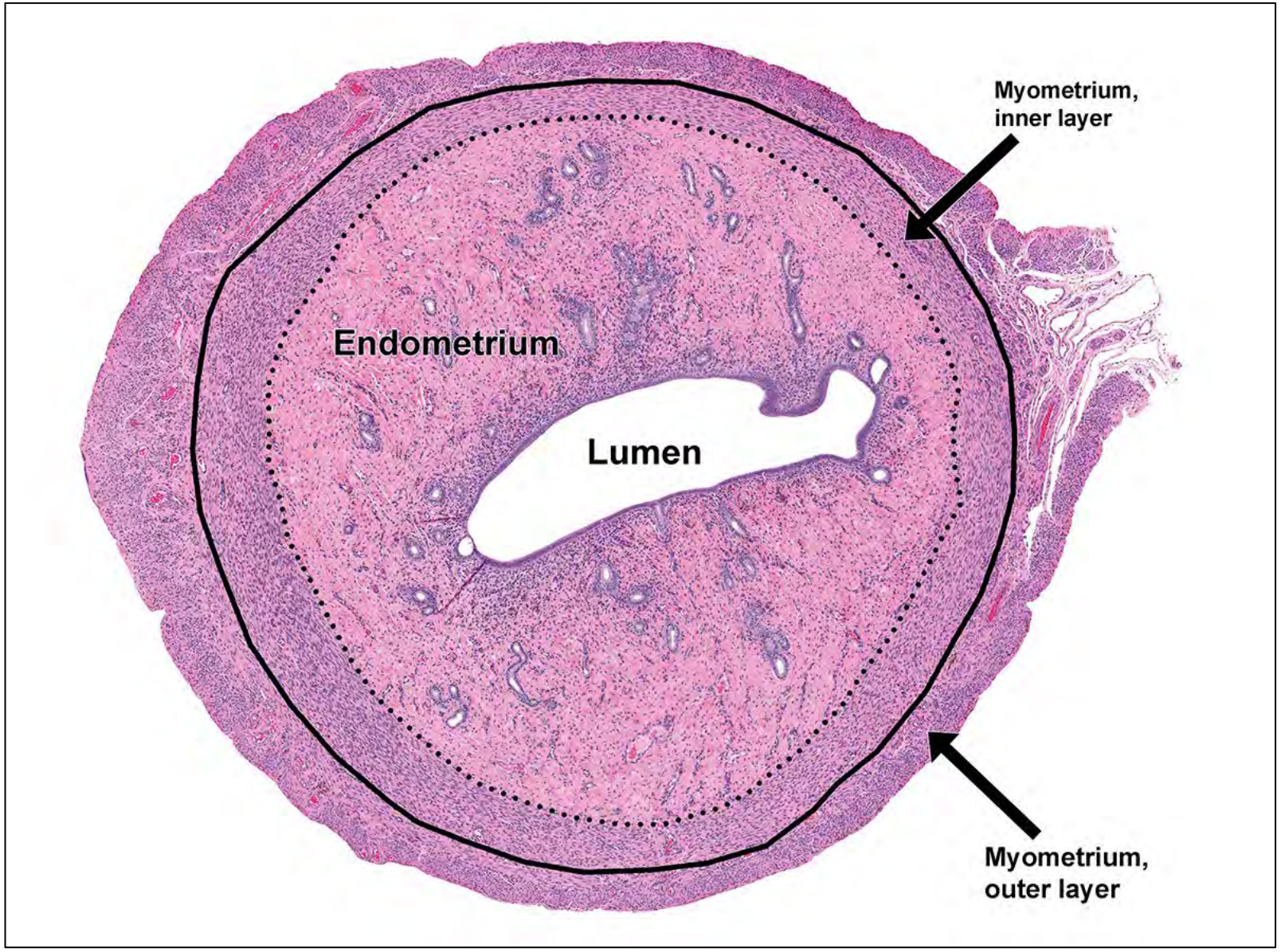
Normal Uterus Histology: Transverse Section

Endometrium

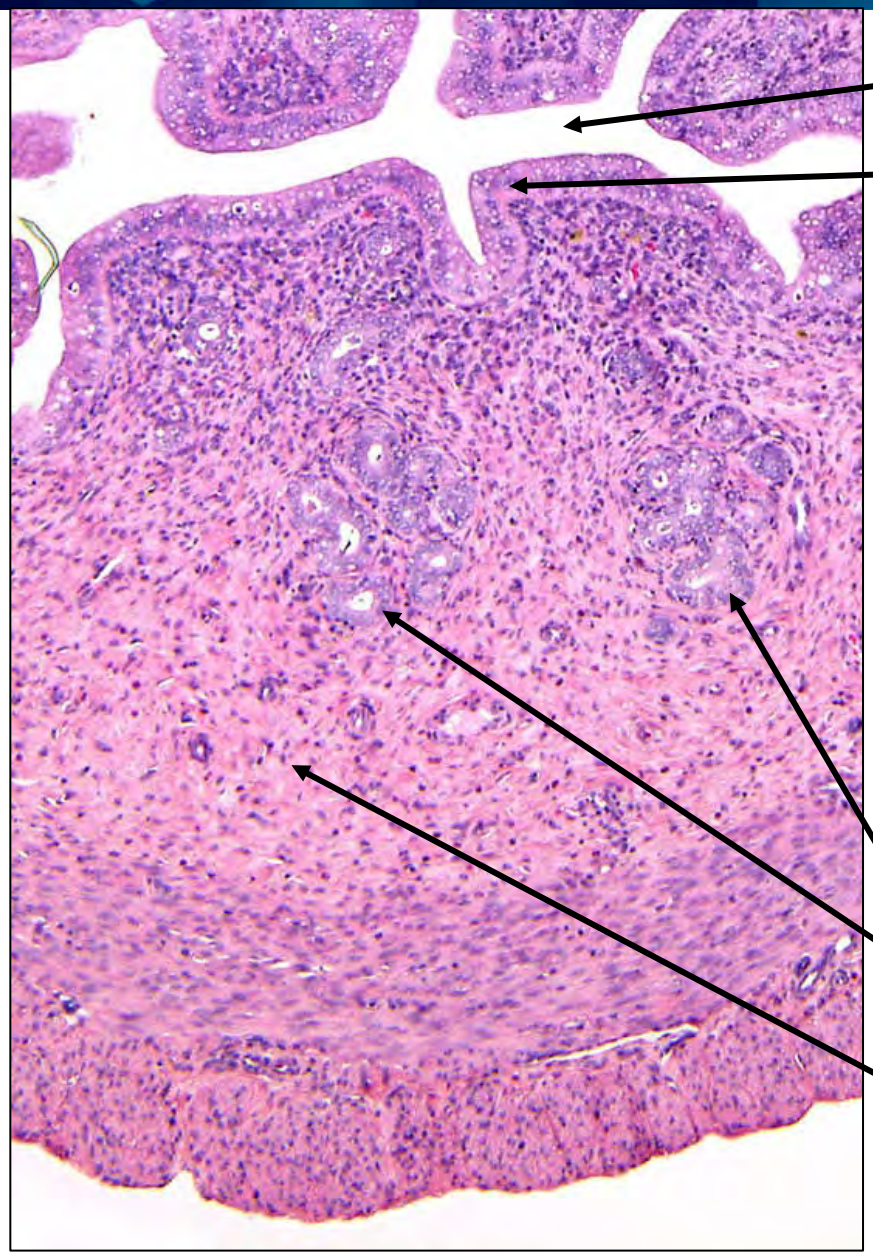
- The glandular, central portion of the uterus.
- A dynamic structure composed of tubular glands embedded in a specialized and highly-cellular stroma.
- The lumen of the uterus is visible as an elongated and central clear space.

Myometrium

- The smooth muscle layer surrounding the endometrium.
- Composes of an inner and outer layer of smooth muscle fibers.
  - Inner layer: Transversely oriented (“circular”)
  - Outer layer: Longitudinally oriented
- The DTT recommends longitudinal sectioning for uterine review. See the Normal Rodent Uterus module for further information.

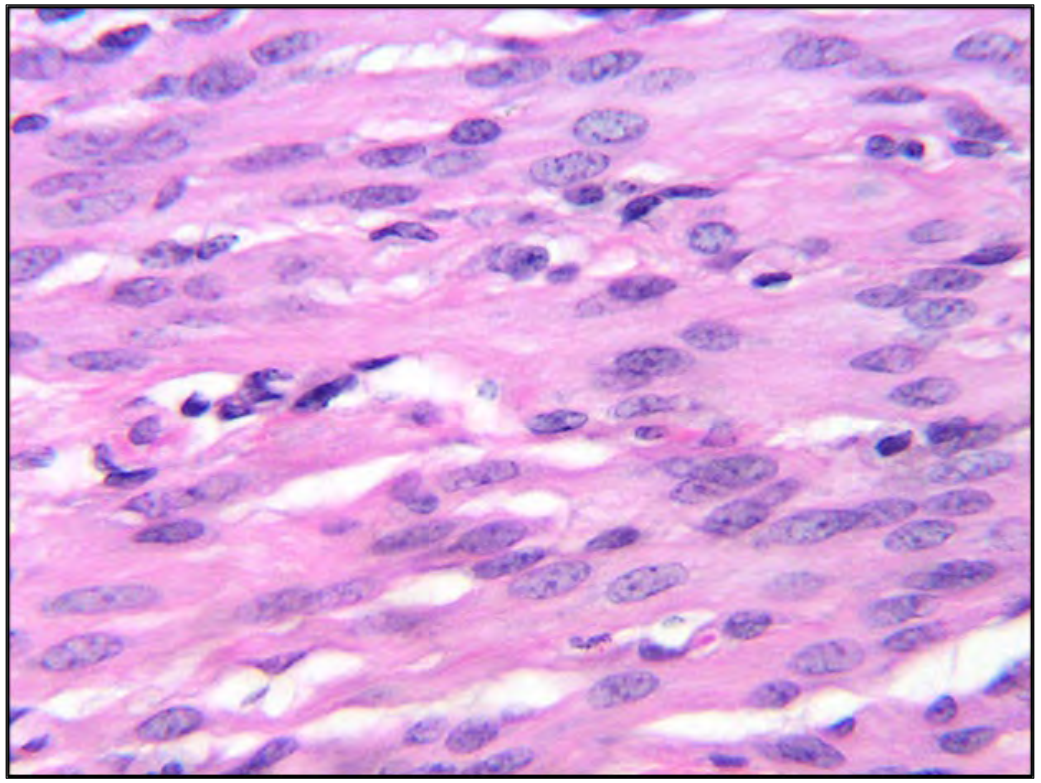


Normal Uterus: Endometrium



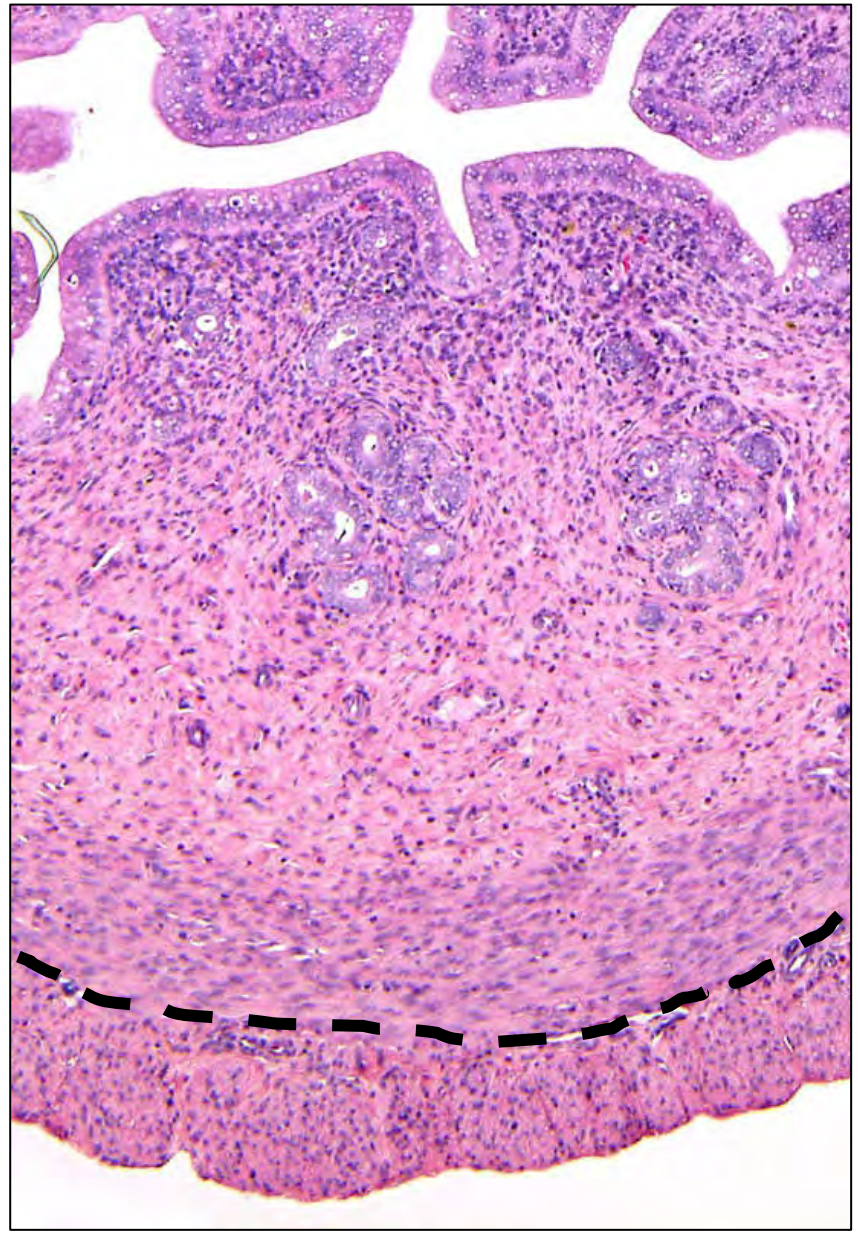
- The uterine lumen and endometrial glands are lined by epithelium, which like the stroma (and vaginal epithelium), undergoes hormone-induced (estrogen and progesterone) alterations throughout the estrous cycle.
- The underlying stroma is highly cellular and composed of spindle-shaped mesenchymal cells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

Normal Uterus: Myometrium



Myometrium

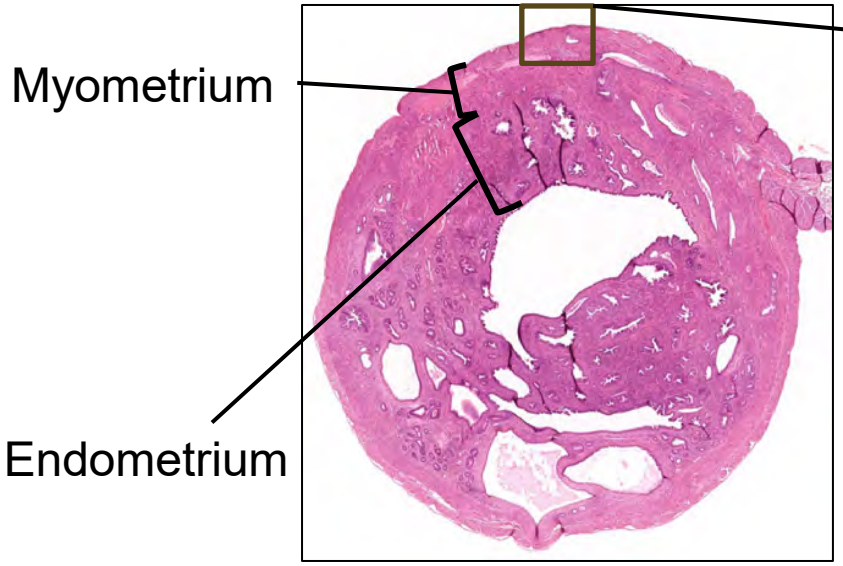
- Smooth muscle cells (myofibers) are elongate with tapered ends and oval (sometimes “cigar-shaped”) nuclei.
- Myofibers are bound together in branching bundles that form the contractile units.

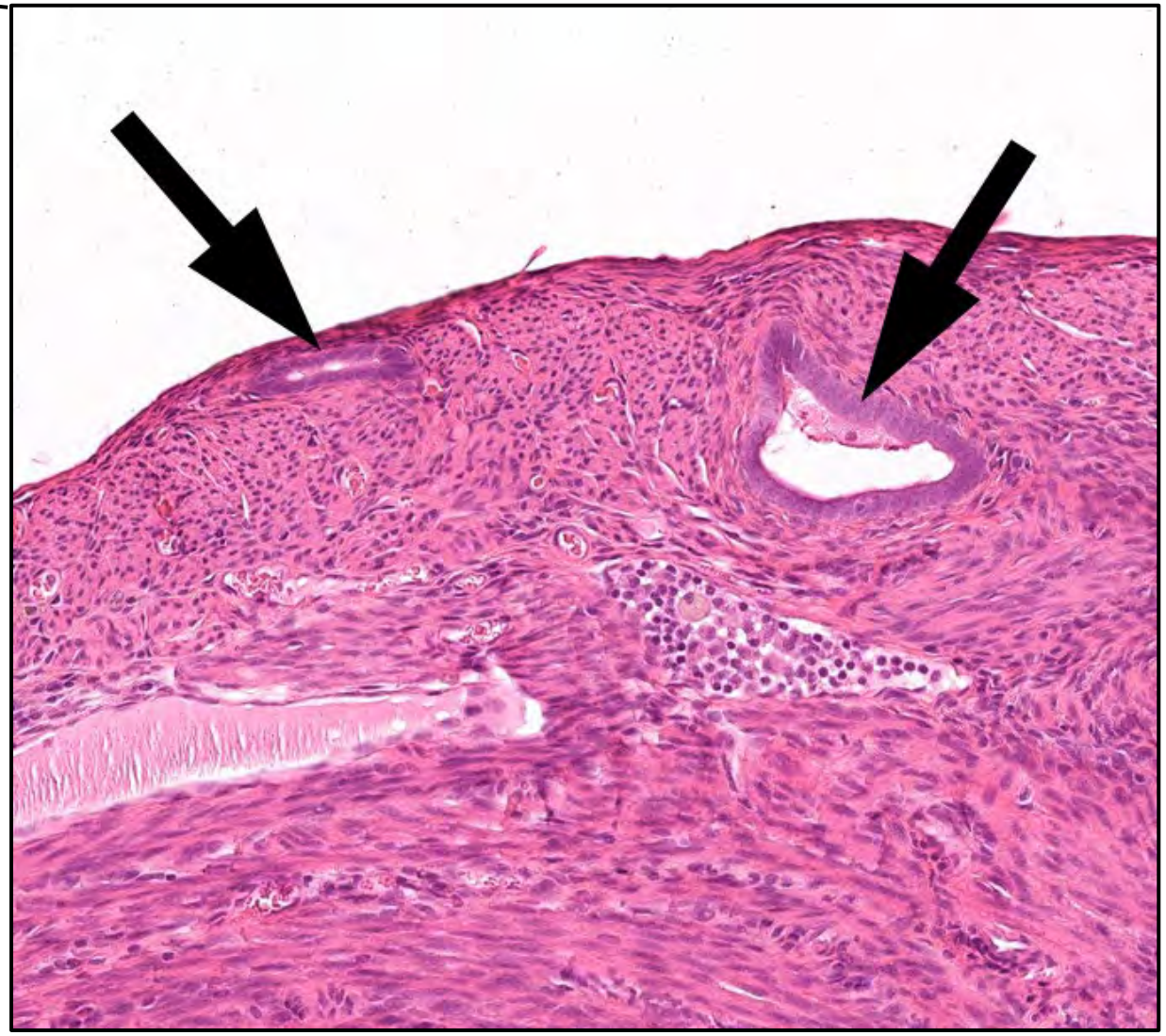


Uterus – Adenomyosis

- Commonly found in aging mice and less frequently in aging rats (Dixon et al., 2014).
- Thought to be related to hormonal imbalance.
- Consists of histologically normal (well-differentiated) endometrial glands and stroma confined to the myometrium.
- Does not spread into abdominal cavity (a characteristic of endometriosis in primates).
- No cellular atypia or crowding of endometrial epithelium lining the glands within the myometrium.
- Differentiate from adenocarcinoma by lack of cellular pleomorphism (size and shape variability), multilayering of cells, and vascular or serosal invasion – features of adenocarcinoma.

Uterus – Adenomyosis





Uterus – Adenomyosis, mouse.

- Normal appearing endometrial glands found within the myometrium, an abnormal location (arrows).
- Hyperplasia, glandular, cystic (covered in the Proliferative Lesions in the Rodent Uterus module) is also present.

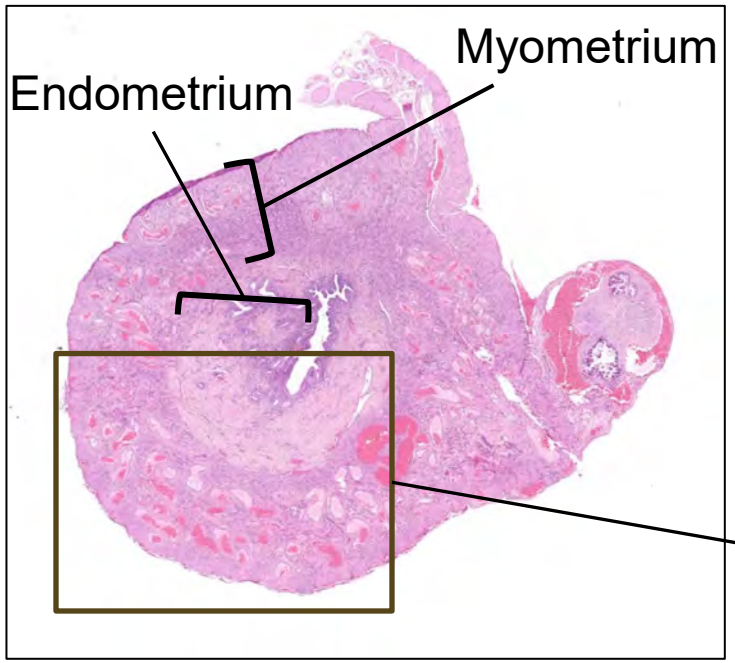
Comparative Pathology: Endometriosis

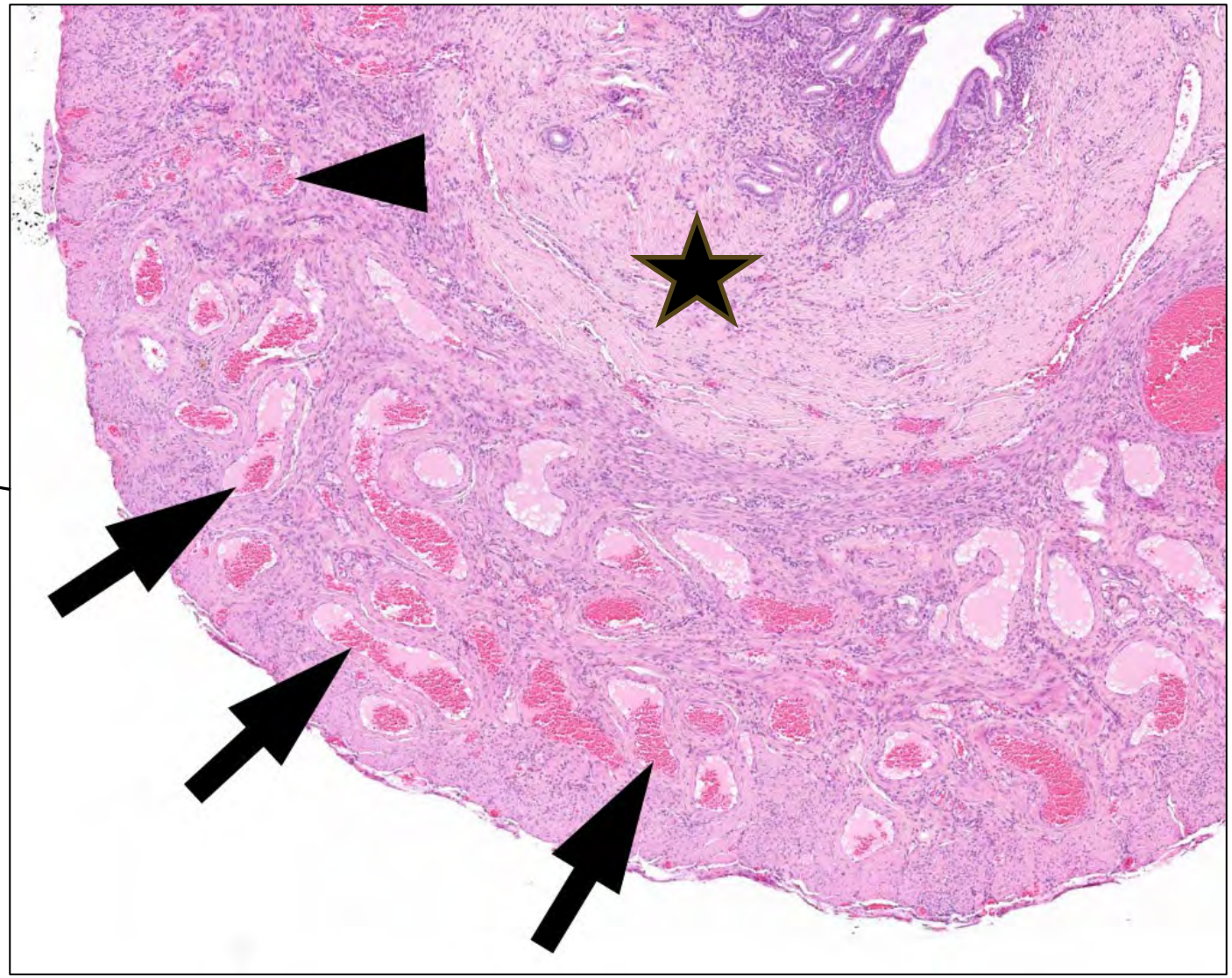
- In rats and mice, do not describe adenomyosis as endometriosis.
  - Adenomyosis, the presence of endometrial glands and stroma in the myometrium, is an uncommon finding in rodents, minipig, rabbit, dog, and nonhuman primates; termed adenosis in the minipig.
  - Endometriosis, the presence of ectopic endometrial tissue outside the uterus, is seen in species that menstruate (humans and Old-World nonhuman primates).
- Endometriosis is one of the most common gynecologic disorders in humans and nonhuman primates (Jagirdar et al., 2012).
  - Ectopic endometrial tissue may be observed on uterine, ovarian, and colonic serosal surfaces; however, it can also be observed at more distant sites like the abdominal wall or thoracic cavity (Atkins et al., 2017; Assaf & Miller, 2011).
  - Criteria necessary to make the diagnosis of endometriosis:
    - Presence of glandular epithelium resembling endometrial glands.
    - Some of the glands are surrounded by dense endometrial stroma.
    - Evidence of current or past hemorrhage (red blood cells outside of vessels and/or macrophages containing red cell breakdown products, namely hemosiderin).

Uterus – Angiectasis

- More common in mice than rats (Dixon et al., 2014).
- Presence of multiple dilated, blood vessels lined by normal endothelial cells, without an increase in the number of vessels.
  - Usually seen in the myometrium; may distort normal architecture but does not compress surrounding tissue.
- Often associated with Uterus – Hyperplasia, Glandular, Cystic (see Proliferative Lesions in the Rodent Uterus module).
  - May be associated with thrombosis (clot), hemorrhage or inflammation.
- Differentiate from hemangioma (a benign, expansile proliferation of capillaries), hemorrhage (blood outside of vessels), congestion (slight distention of vessels by blood), and lymphangiectasis (dilated lymphatic vessels).

Uterus – Angiectasis



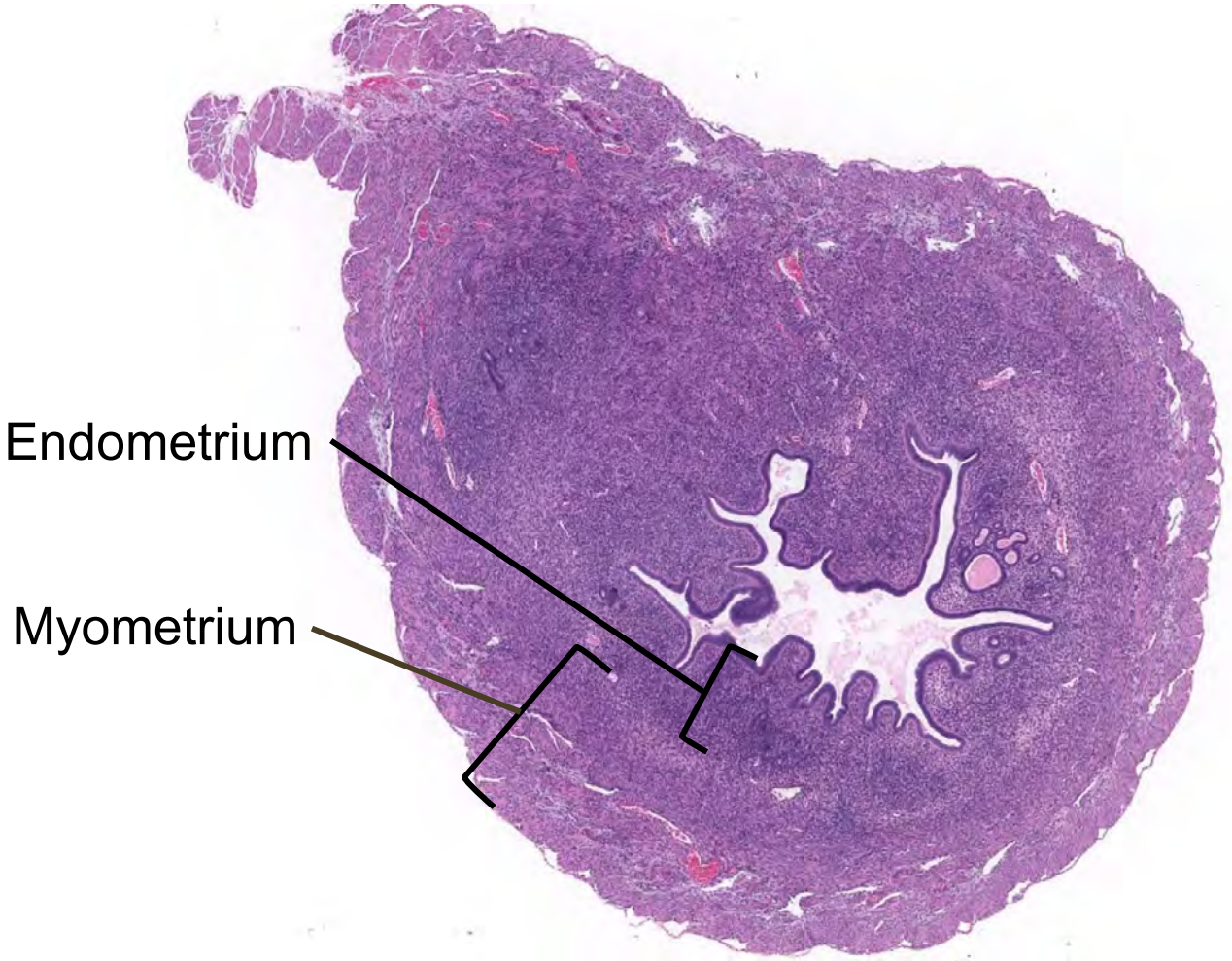


Uterus – Angiectasis, rat.
Many blood vessels are dilated (arrows) within the myometrium. Normal blood vessel (arrowhead). Endometrial fibrosis (star) also present; covered later in this module.

Uterus – Atrophy

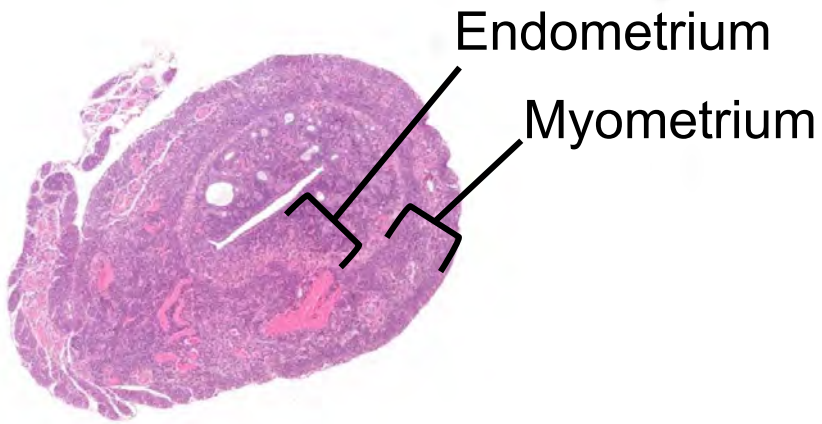
- Common in aged rodents in response to decreased estrogen and related ovarian atrophy (Dixon et al., 2014).
  - Also induced by changes (e.g., ovariectomy) or agents (e.g., cyclophosphamide) that cause ovarian damage or alter steroid production by the ovary.
- Associated with decreased organ weight, grossly decreased size, and thin uterine horns.
- Generally, atrophy affects all layers of the uterus.
  - Some agents may cause selective atrophy of only certain layers.
- Reduced height of luminal and glandular epithelium.
  - Decreased number and/or size of the endometrial glands, stroma may be collapsed or hyalinized (homogeneously pink-staining or eosinophilic, glassy appearance), myometrium thin.
  - Luminal or glandular epithelium lack signs of hormonal influence.
- Must be differentiated from hypoplasia (failure to develop) and immaturity of the uterus.
  - In aged animals, may need to differentiate from fibrosis (uterus typically normal in size with replacement by collagen and fibroblasts).

Uterus – Atrophy (Continued)



Uterus – Normal, mouse
2X magnification

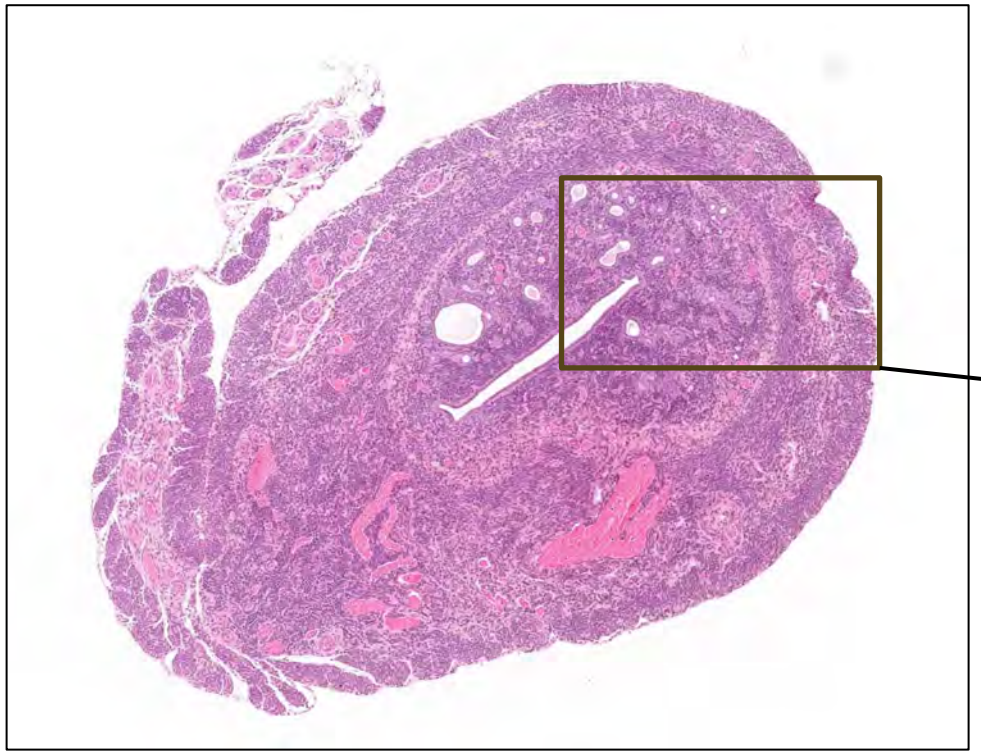
Note the thickness of both endometrium and myometrium is not decreased.



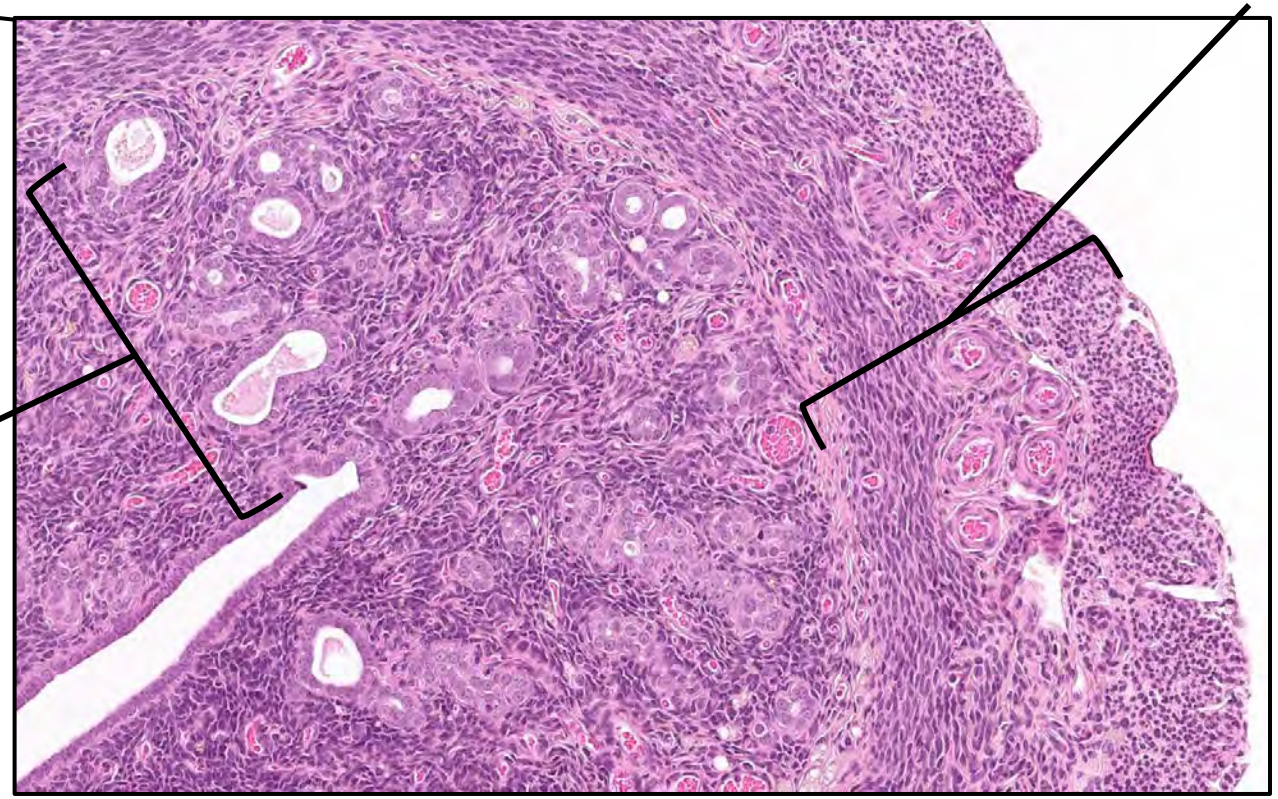
Uterus – Atrophy, mouse
2X magnification

Note the thickness of both endometrium and myometrium is markedly decreased.

Uterus – Atrophy (Continued)

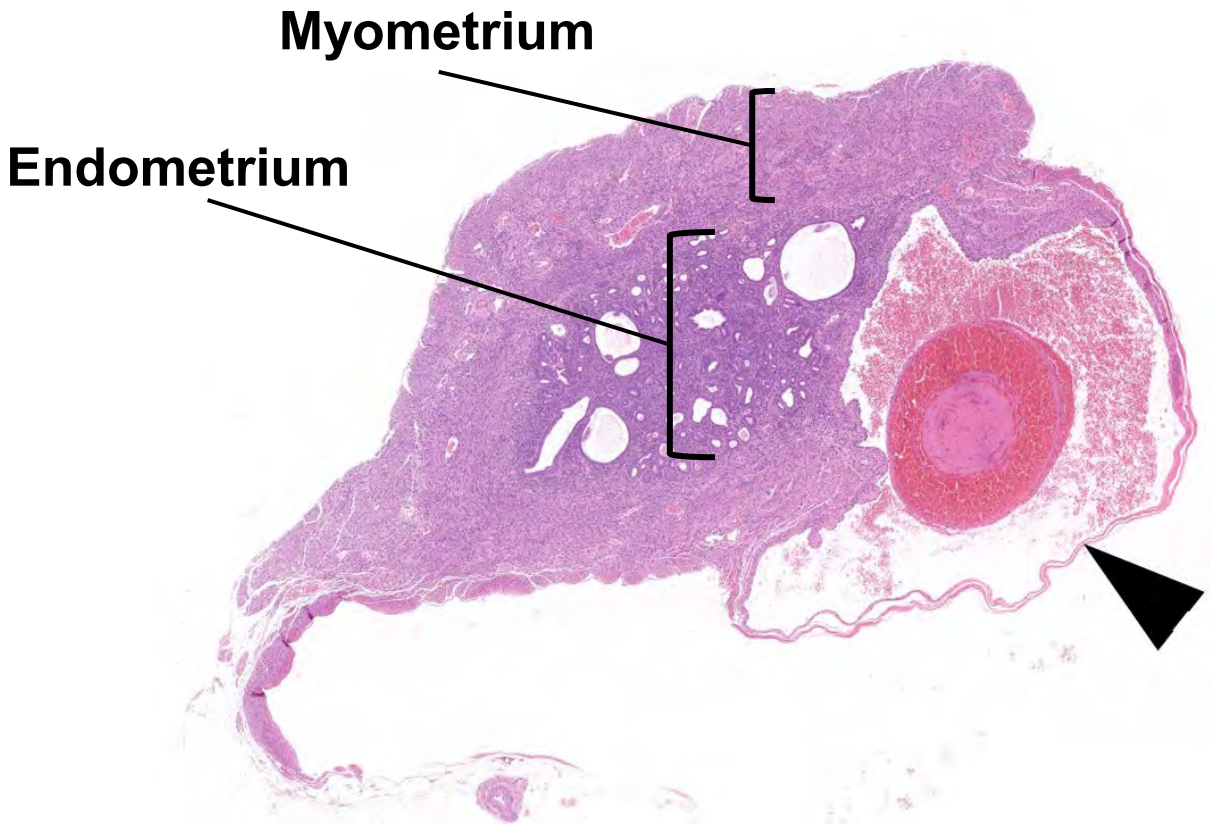


Uterus – Atrophy, mouse.
The uterine horn is small (same image as previous slide). The endometrial stroma is collapsed. The myometrium is thin. Cytoplasm of stromal cells and smooth muscle cells is decreased, resulting in high density of nuclei.



Uterus – Cyst

- Dilated, fluid- (e.g. blood) or keratin-filled structure.
- Can occur in any level of the uterus (e.g., modifiers glandular, myometrial or serosal).
- Epithelial lining often obvious and may be atrophied.
- May have squamous cell metaplasia and/or keratinization of the cyst lining.
- If has a smooth muscle wall, considered a Mesonephric Duct Remnant (a congenital finding discussed in normal uterus module).
- If the origin cannot be identified, diagnose **Cyst, NOS (Not Otherwise Specified).**
- Should be differentiated from Hyperplasia, Glandular, Cystic lesion.



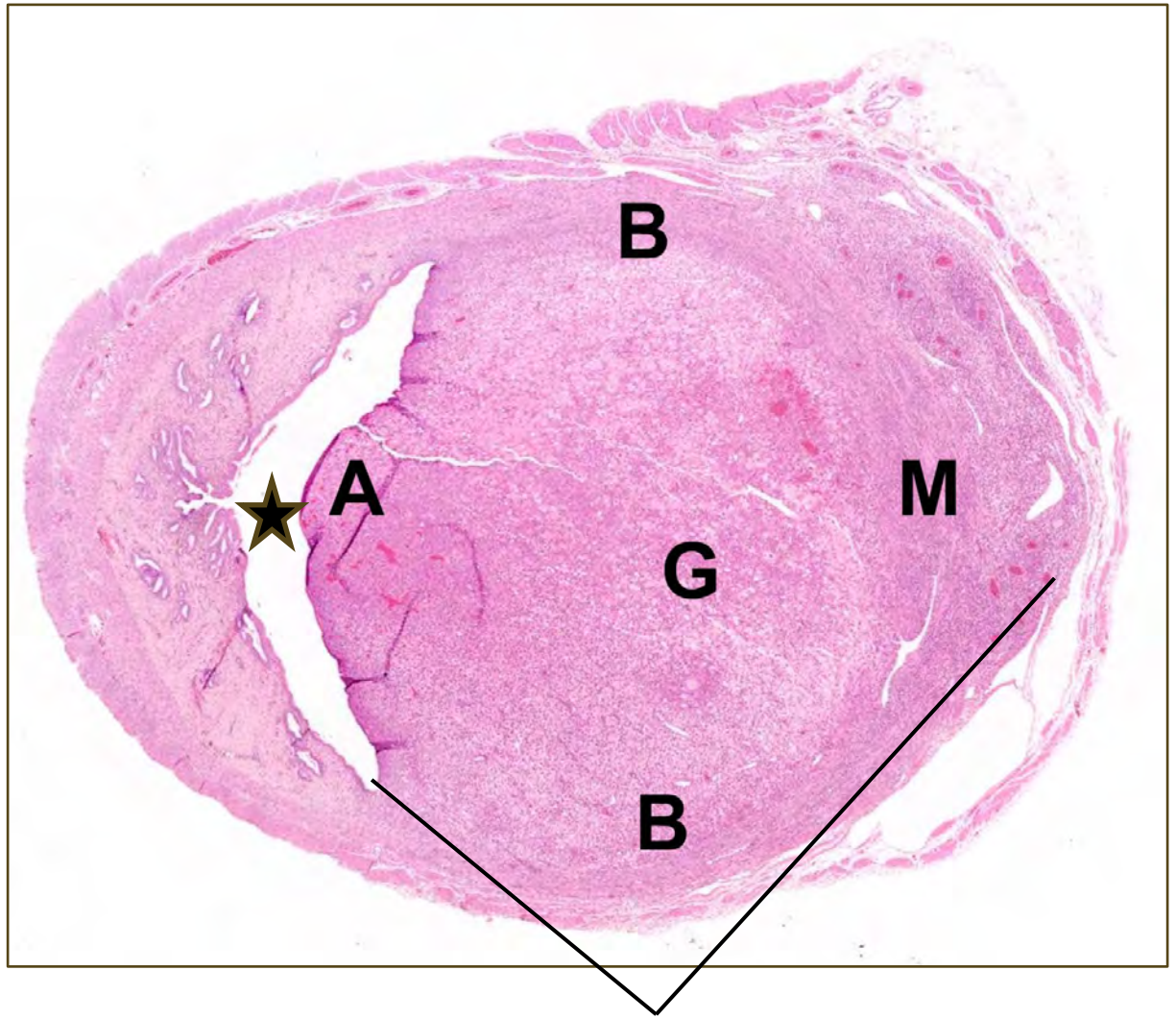
Uterus – Cyst, Myometrial, mouse.
A blood-filled cyst (arrowhead) is present in the myometrium.

Uterus – Decidual Reaction

- Cellular response in the endometrium that resembles normal decidual implantation sites.
- Not a neoplasm.
  - "Deciduoma" is not accepted terminology in toxicologic pathology.
- Rare, spontaneous condition in most strains of mice and rats.
- Reaction occurs with pseudopregnancy.
- Can also be induced by nonspecific stimuli.
  - Growth hormone.
  - Mechanical or electrical stimulation.
  - Endometrial trauma.
  - Intrauterine injection of salt solution, oil, or air.
- Has a defined limited course which ends in degeneration and regression in about 12 days in rodents (Dixon et al., 2017).
- Differentials include normal decidual reaction during pregnancy, focal decidualization, and stromal polyp.

Uterus – Decidual Reaction (Continued)

- Discrete round nodules in the endometrial stroma; can be single or multiple, unilateral or bilateral.
- Consists of several well-defined regions (not all may be seen microscopically):
  - A: Antimesometrial region
  - B: Basal zone
  - G: Glycogenic area
  - M: Mesometrial region and metrial gland
  - Star: Uterine lumen
- Antimesometrial is the side of uterus or lesion that is opposite to the mesometrial side (side containing the mesentery and broad ligament).

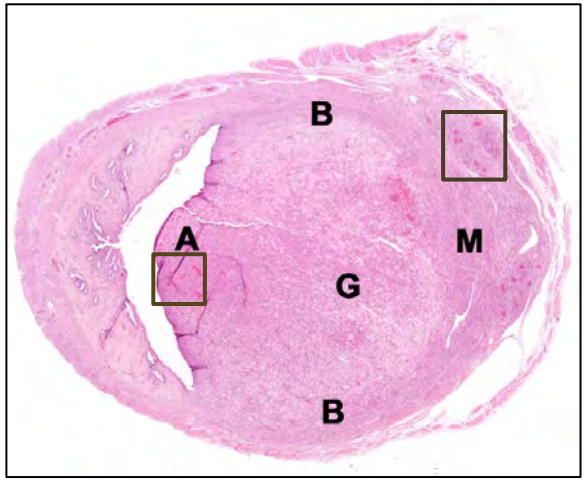


Uterus – Decidual Reaction, rat.

Uterus – Decidual Reaction (Continued)

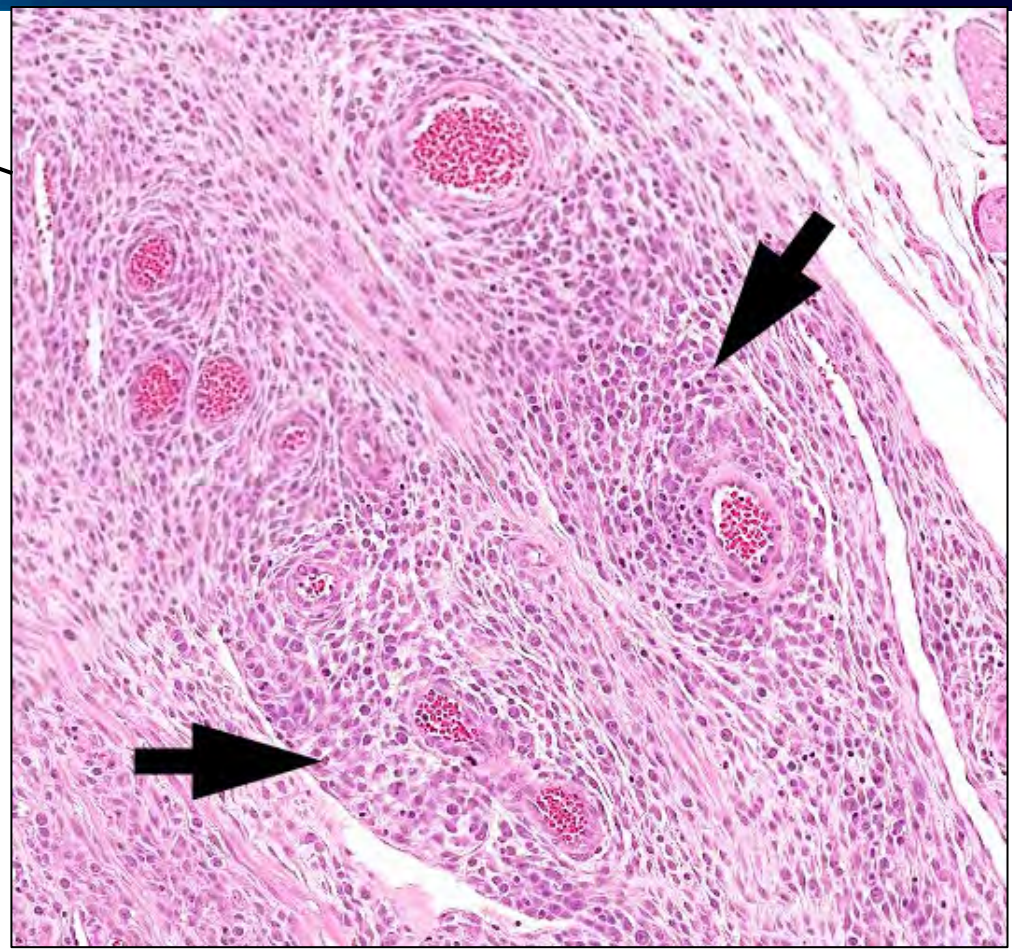
- **Metrial Gland and Mesometrial Region (M):**
  - Metrial gland is within the myometrium on the mesometrial side.
    - Large, round granulated cells, abundant cytoplasm that also whorl around vessels.
    - Fibroblastic stromal cells separate clusters of metrial gland cells.
  - Mesometrial region composed of cells like those in the metrial gland and spiny cells with long cytoplasmic processes and abundant glycogen.
- **Basal Zone (B):**
  - Separates the decidual reaction from the myometrium.
  - Consists of remnants of the endometrial stroma and glands.
- **Antimesometrial Region (A):**
  - Closely packed large cells.
  - Abundant eosinophilic cytoplasm with vesicular nuclei.
- **Glycogenic Area (G):**
  - Between the mesometrial and antimesometrial regions.
  - Loosely arranged cells that resemble the spiny mesometrial cells; abundant glycogen.

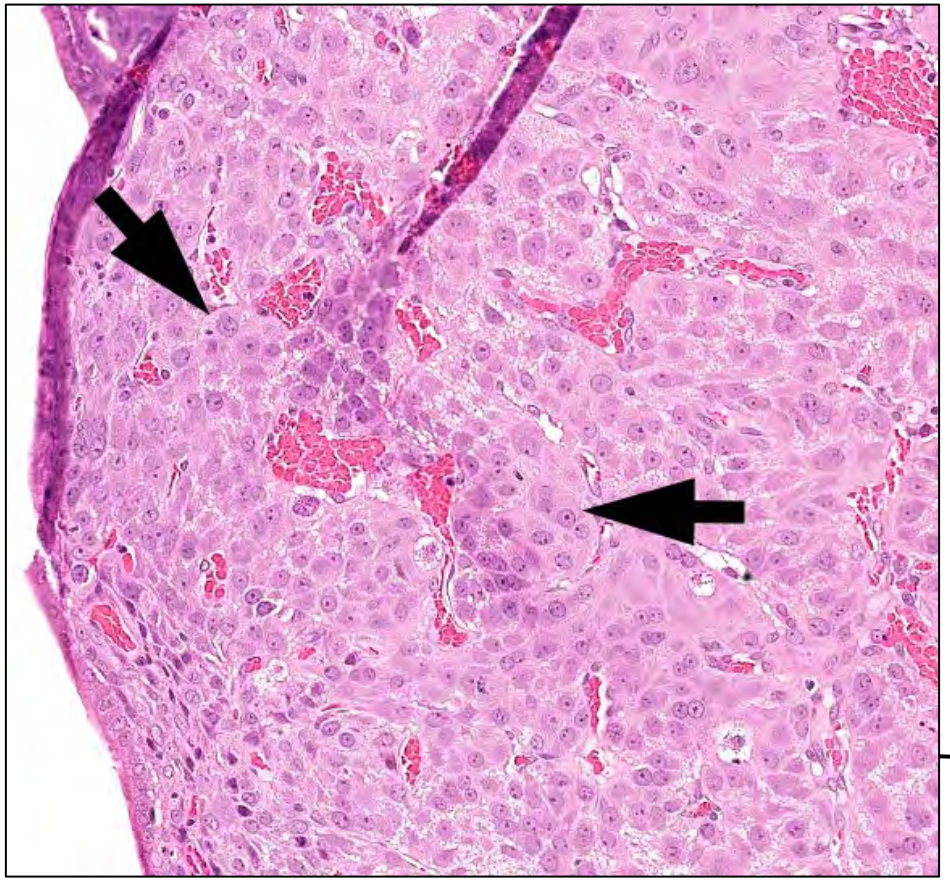
Uterus – Decidual Reaction (Continued)



Rat uterus

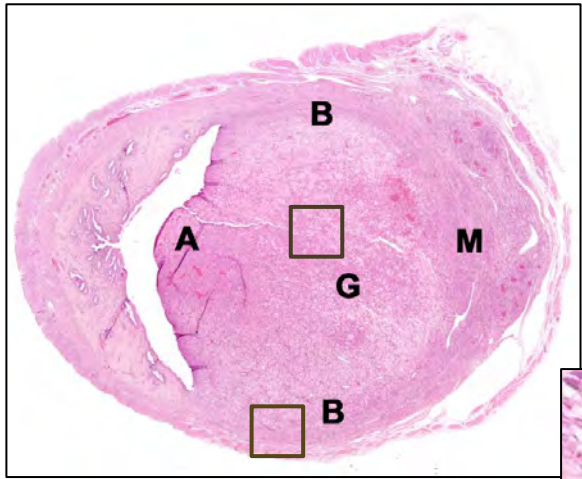
(Upper right inset) Metrial gland cells whorl around blood vessels (arrows).





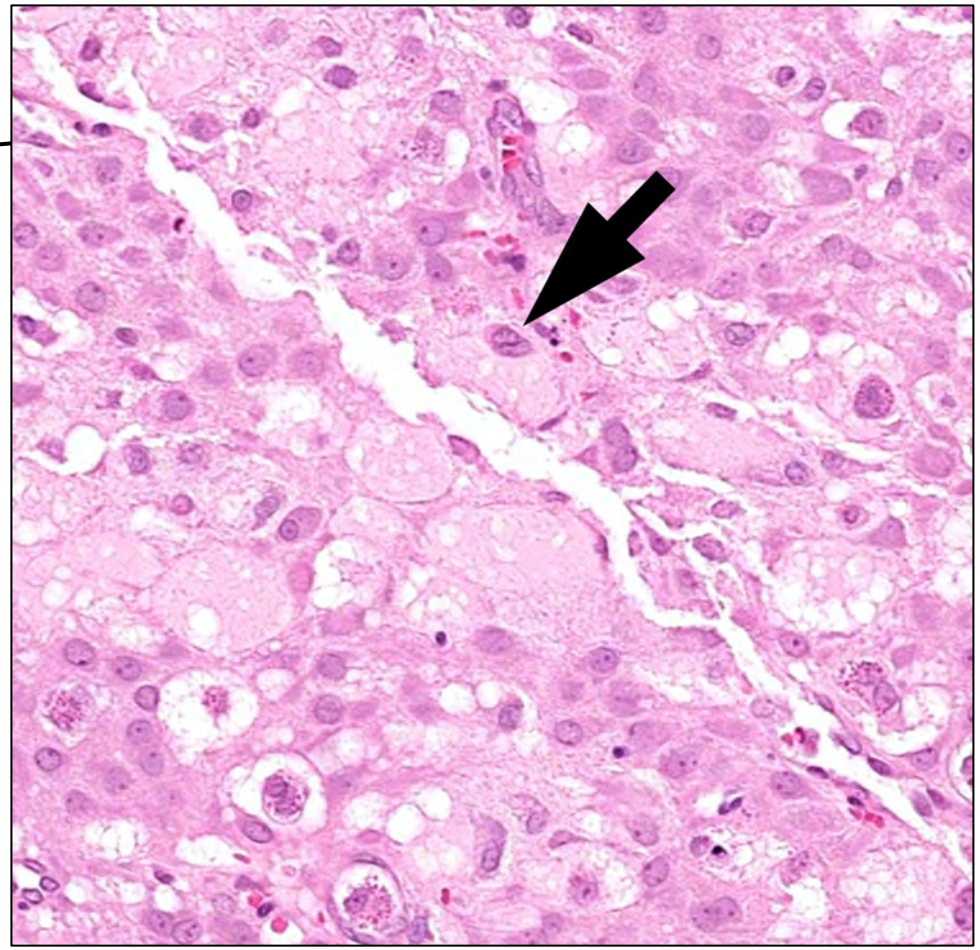
(Lower left inset) Antimesometrial cells with abundant eosinophilic cytoplasm (arrows).

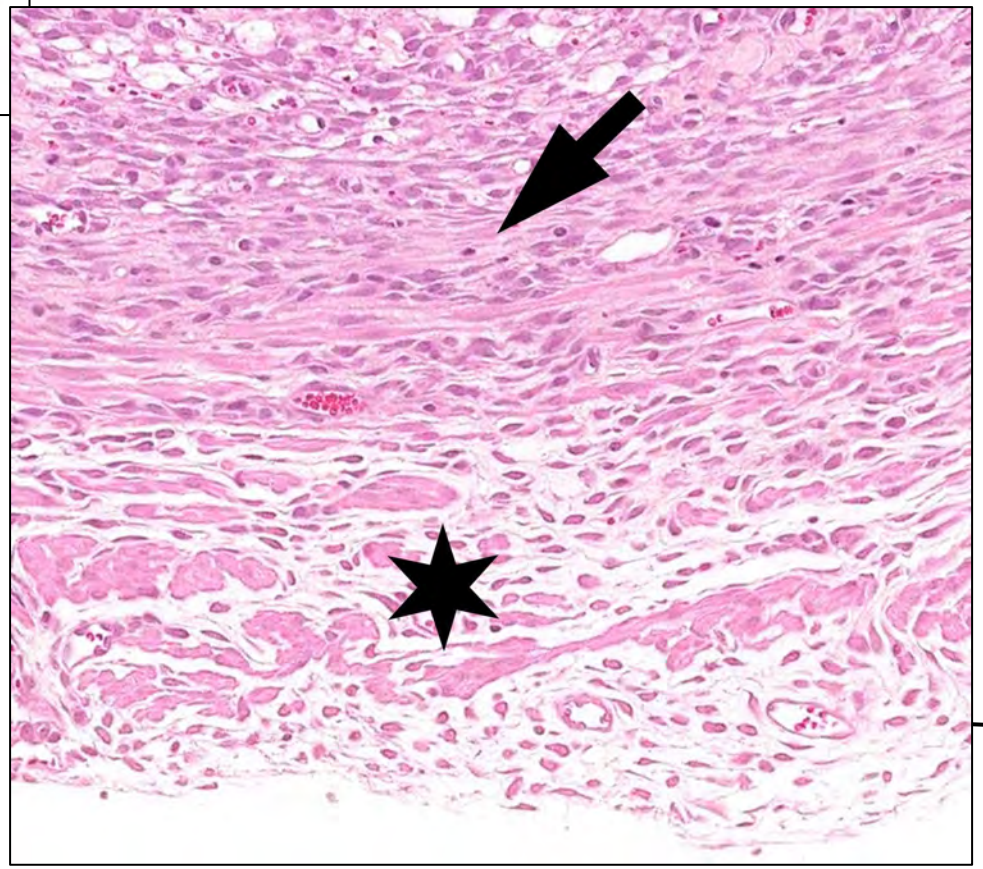
Uterus – Decidual Reaction (Continued)



Rat uterus

(Upper inset) Cells in the glycogen area are large with abundant cytoplasmic glycogen (arrow).



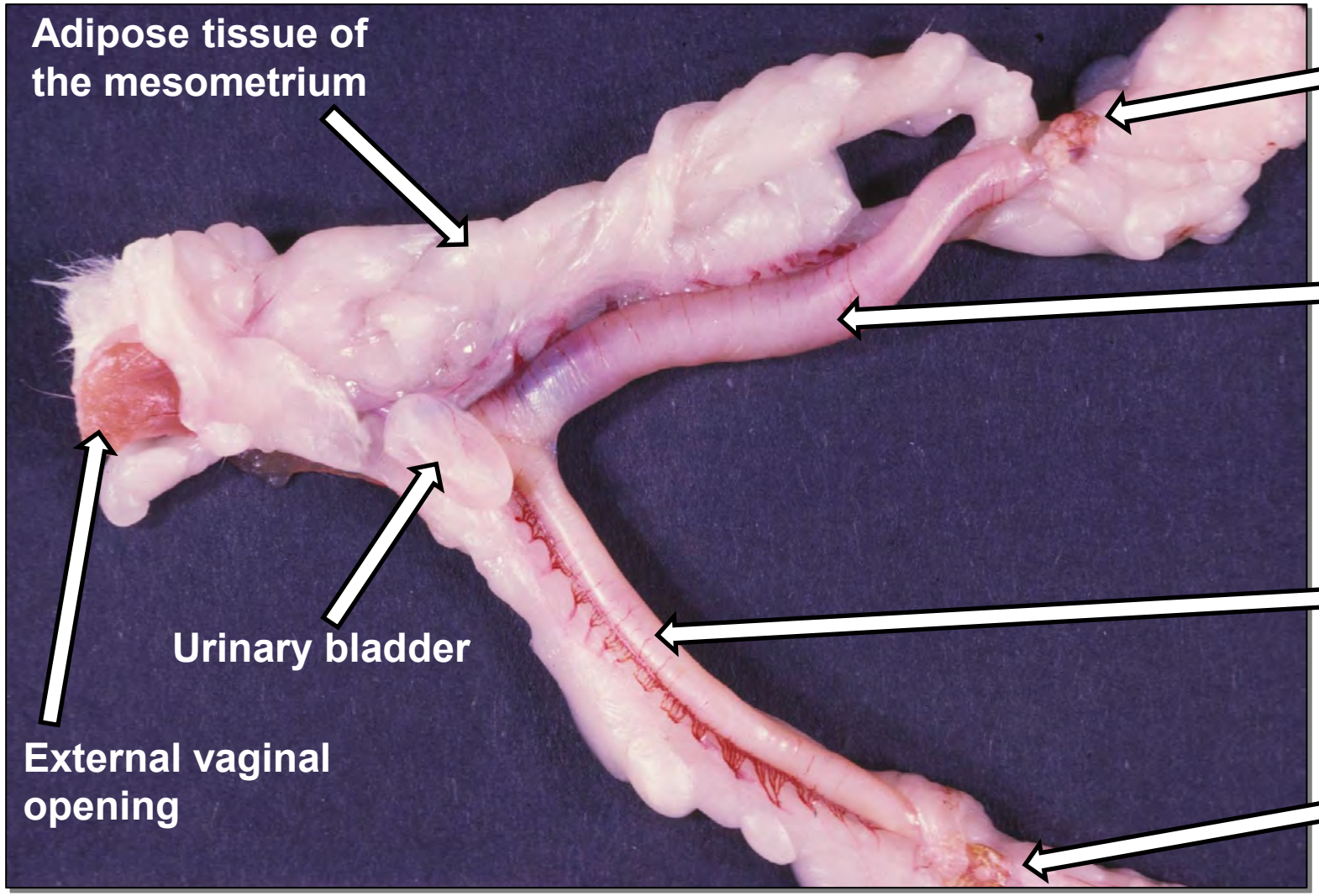


(Lower inset) Endometrial stroma in basal region (arrow) separates decidual cells from myometrium (asterisk).

Uterus – Dilatation, Lumen

- Division of Translational Toxicology (DTT) preferred terminology: **Dilation.**
- Commonly seen as a gross finding at necropsy.
- Dilatation of uterus due to the accumulation of serous (clear, watery) proteinaceous fluid.
  - May also be due to accumulation of mucus or suppurative exudate.
- Usually bilateral (both horns) but can be unilateral (single horn).
- Uterine wall thin and epithelium attenuated (flattened) with severe and prolonged distention.
- Distention may be a normal physiologic response to estrogen during proestrus or early estrus stages of the estrous cycle (*do not diagnose dilatation in these situations*).
- Increased incidence of uterine dilatation in toxicity studies can be a response to hormonal perturbations that increase estrogen dominance.

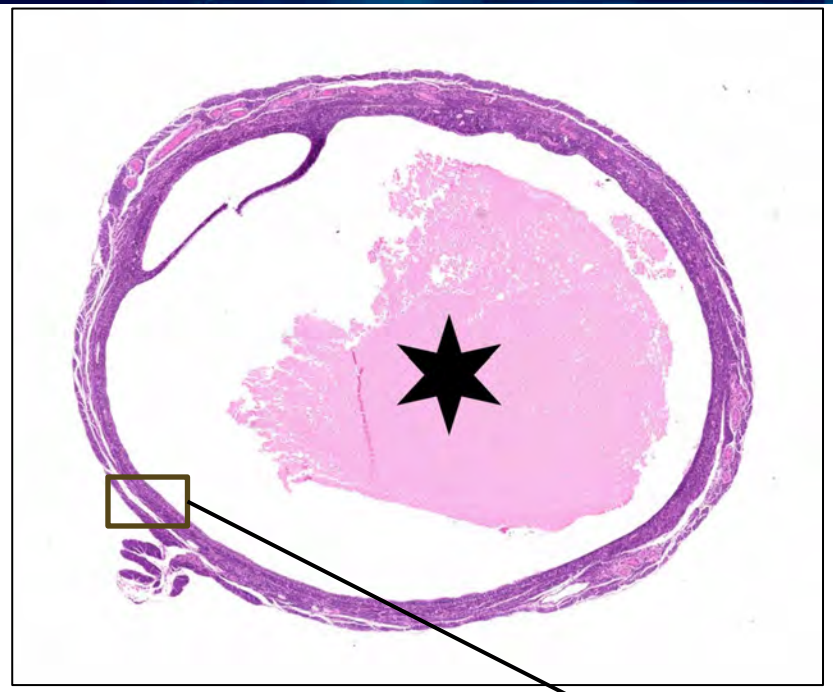
Uterus – Dilatation, Lumen (Continued)



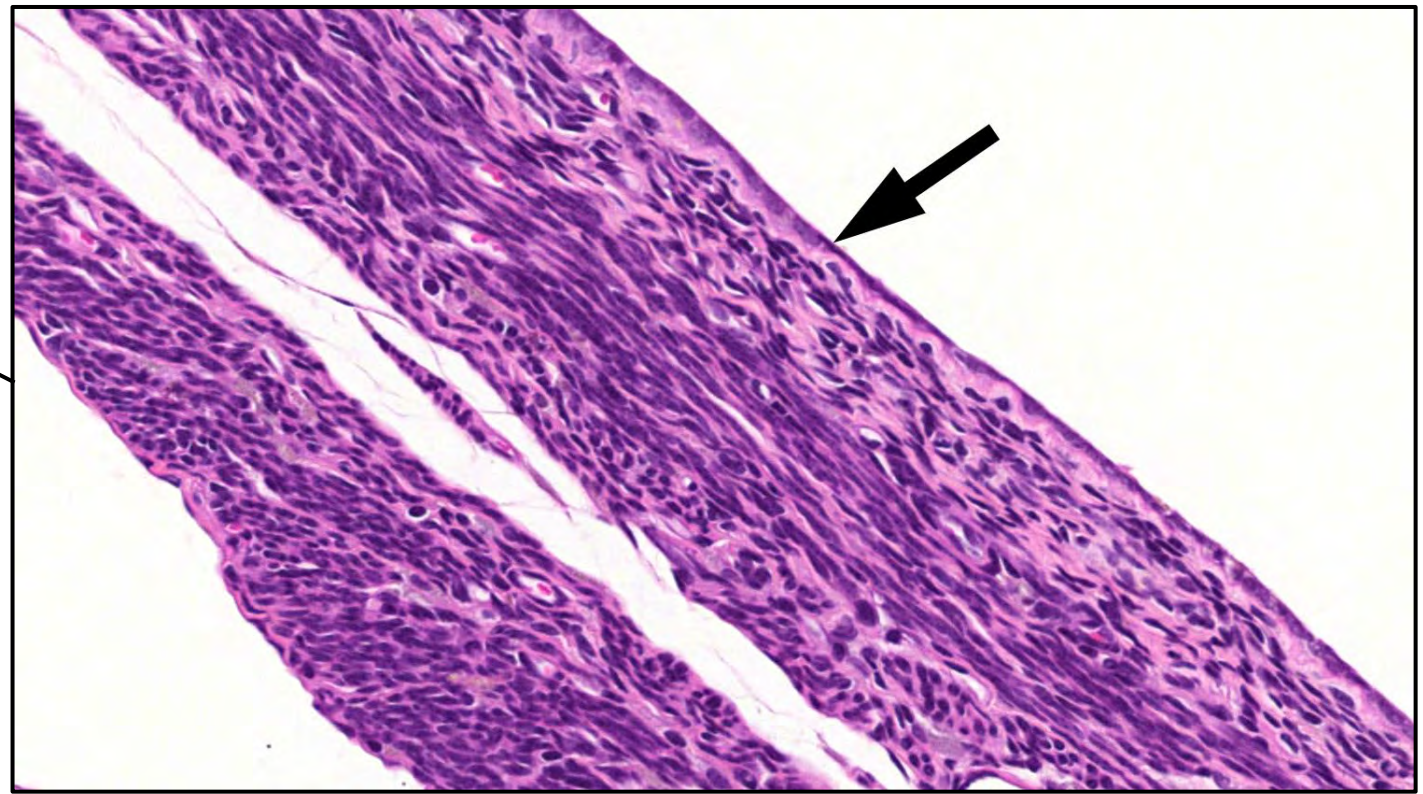
Rat uterus – dilatation

Image courtesy of Dr. Ron Herbert

Uterus – Dilatation, Lumen (Continued)



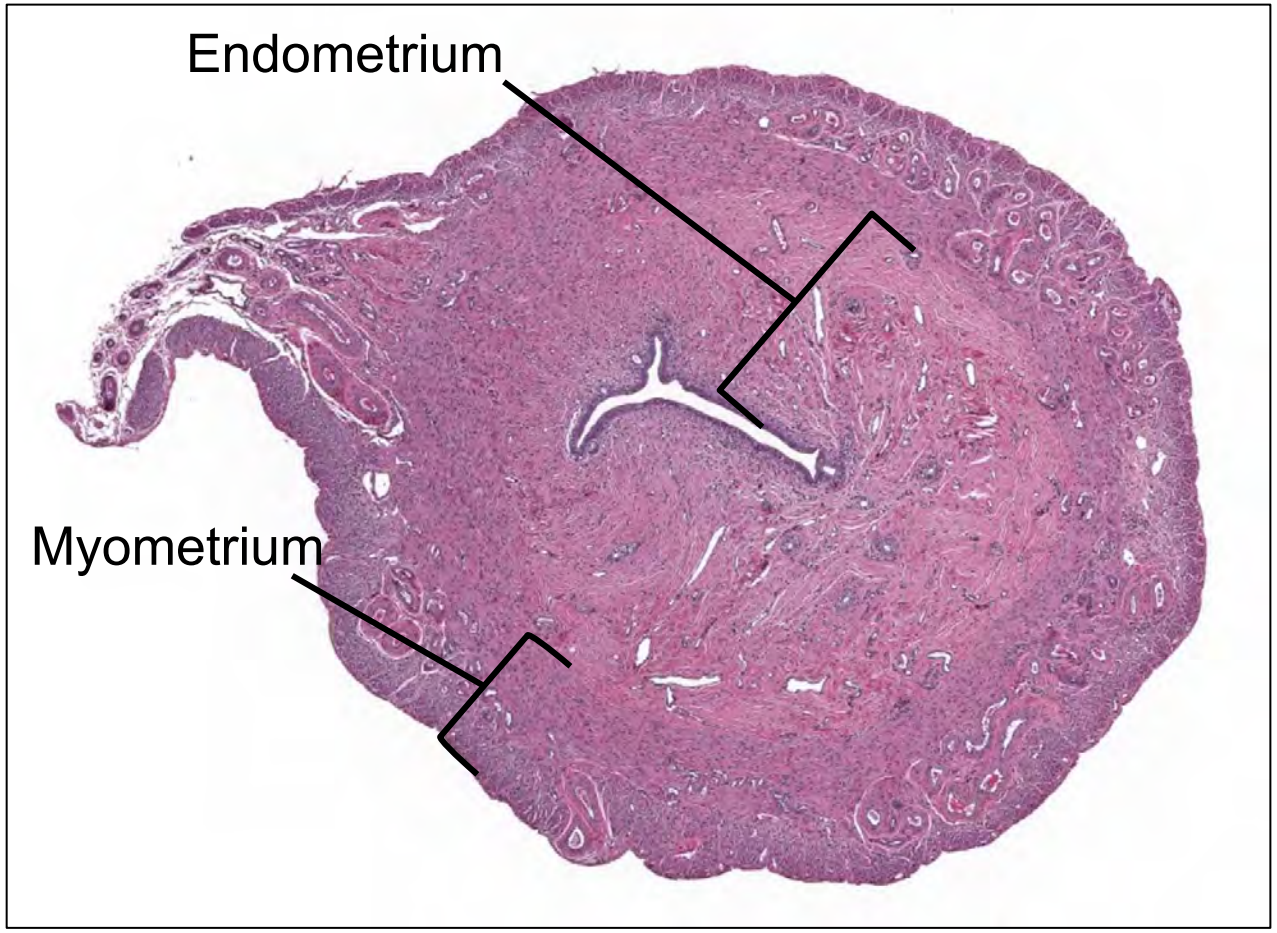
Uterus – Dilatation, Lumen, mouse.
Uterine wall is thin, and endometrial epithelium is attenuated (arrow). Fluid is present within the distended uterine lumen (asterisk). Attenuated epithelium supports this lesion is not associated with normal proestrus/estrus.



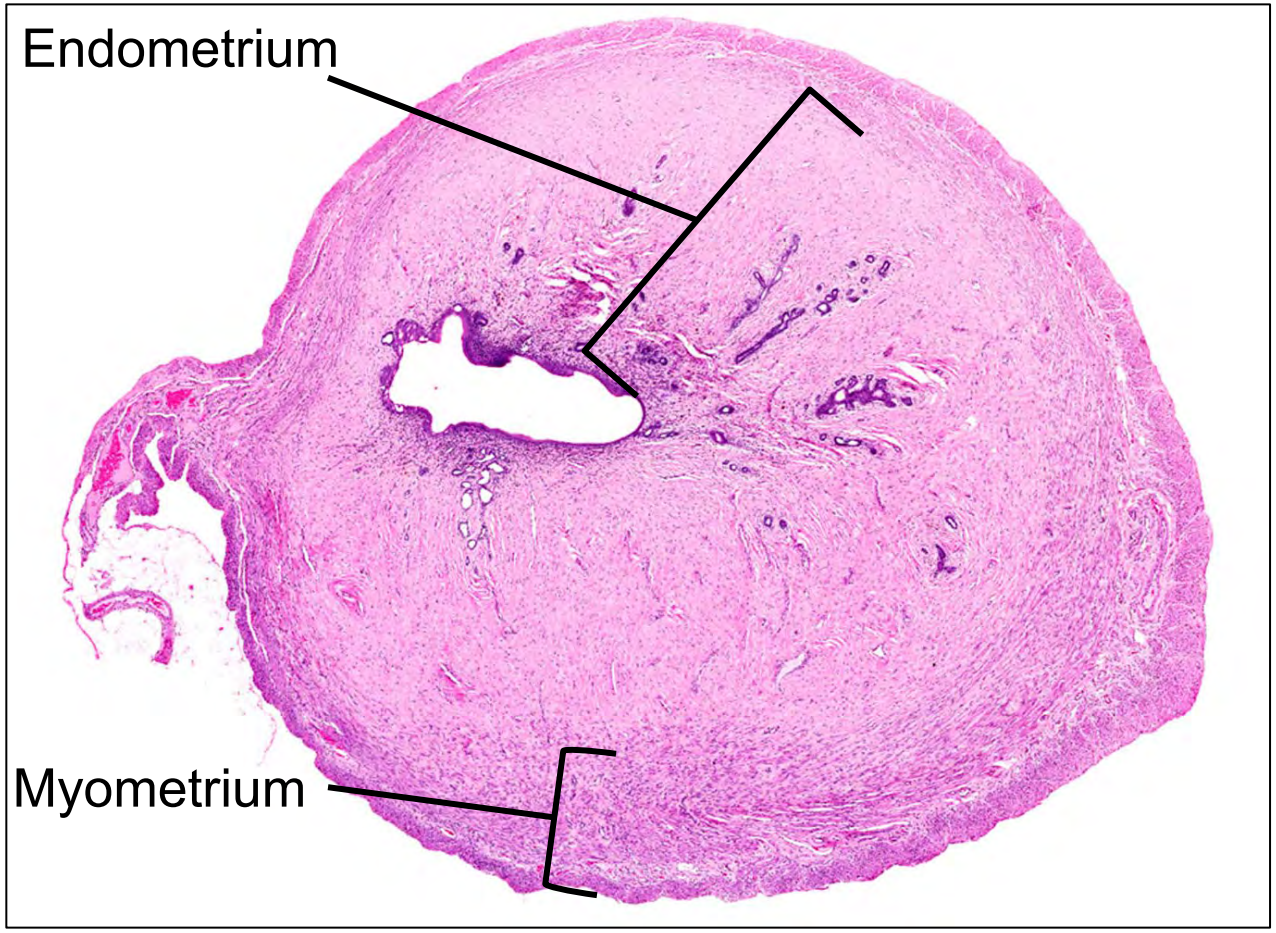
Uterus – Fibrosis

- Replacement of endometrial stroma or myometrium by fibroblasts, with or without an increase in collagen (fibrillar eosinophilic material).
- Presence of abundant mature collagen with few fibrocytes interspersed that is predominantly acellular is often referred to as “hyalinization.”
  - Differentiate from hyalinization of uterine atrophy based on size of uterus.
- May be a component of another lesion (e.g., inflammation, neoplasia).
- Has been associated with exposure of the estrogenic mycotoxin (fungal toxin) zearalenone in mice (Dixon et al., 2014).
- Modifiers: **stromal** or **myometrial.**
  - Stromal fibrosis often accompanied by progressive atrophy of endometrial glands as an age-related change in rats.
  - Myometrial fibrosis has been seen as a spontaneous lesion in older mice.

Uterus – Fibrosis (Continued)

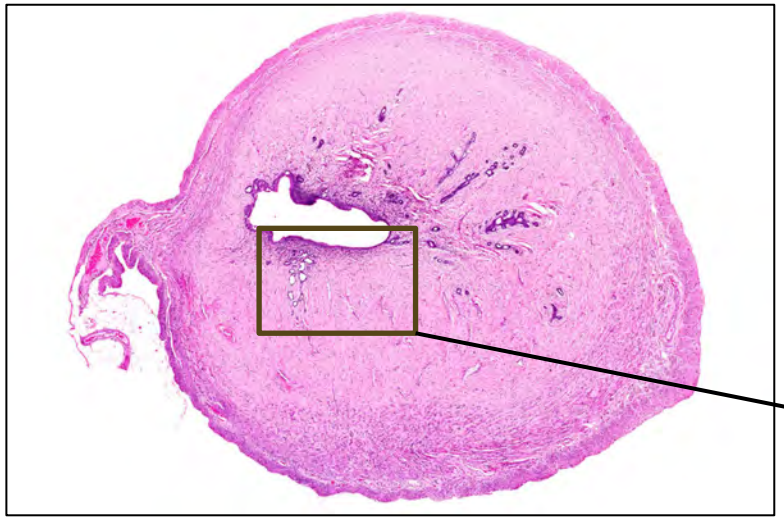


Uterus – Normal, rat.

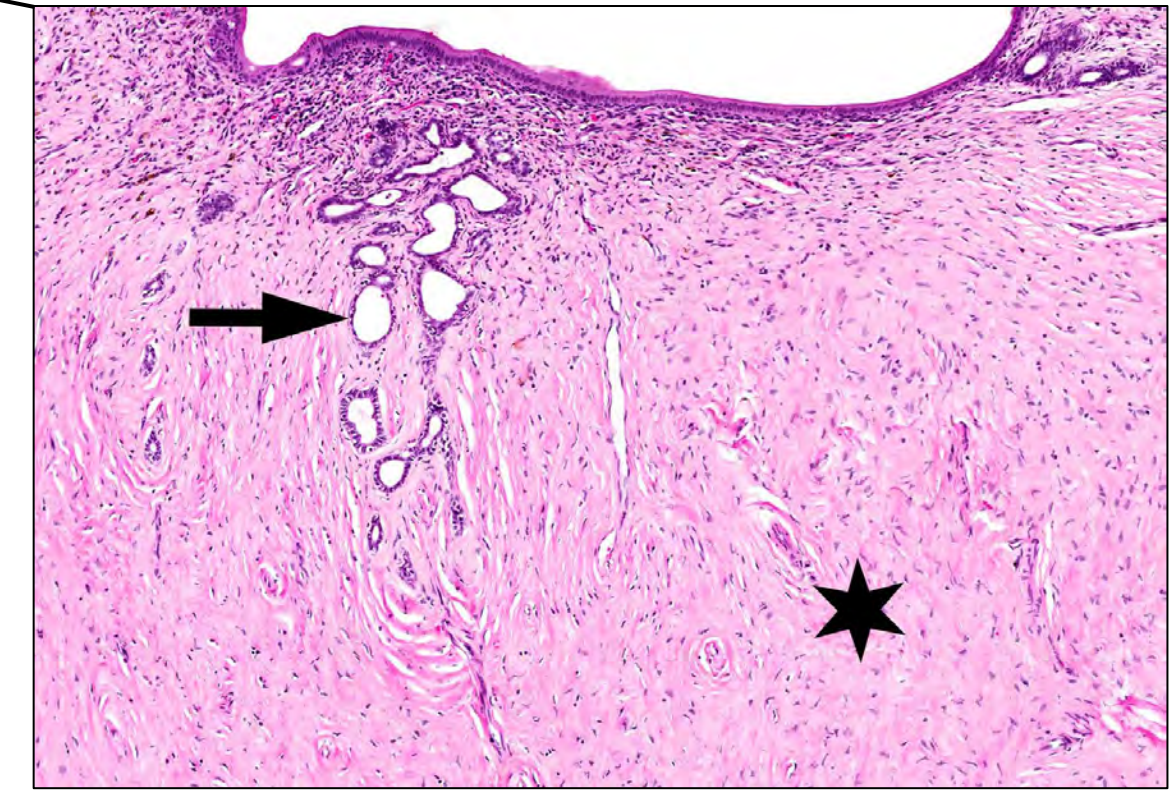


Uterus – Fibrosis, Stromal, rat.
Note thickened endometrium with replacement by hyalinized (pink) collagen.

Uterus – Fibrosis (Continued)



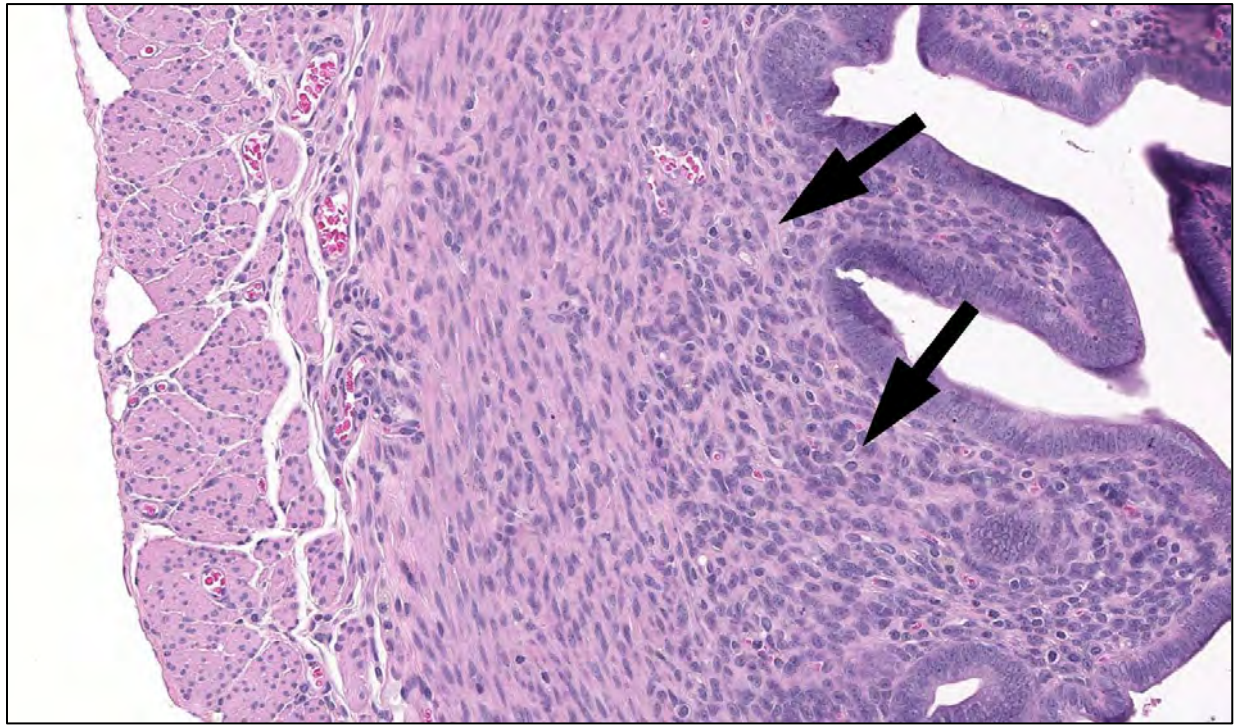
Uterus – Fibrosis, Stromal, Rat.
The thickened endometrial stroma is replaced by hyalinized collagen fibers (asterisk) with interspersed fibrocytes. Some of the entrapped endometrial glands are dilated (arrow).



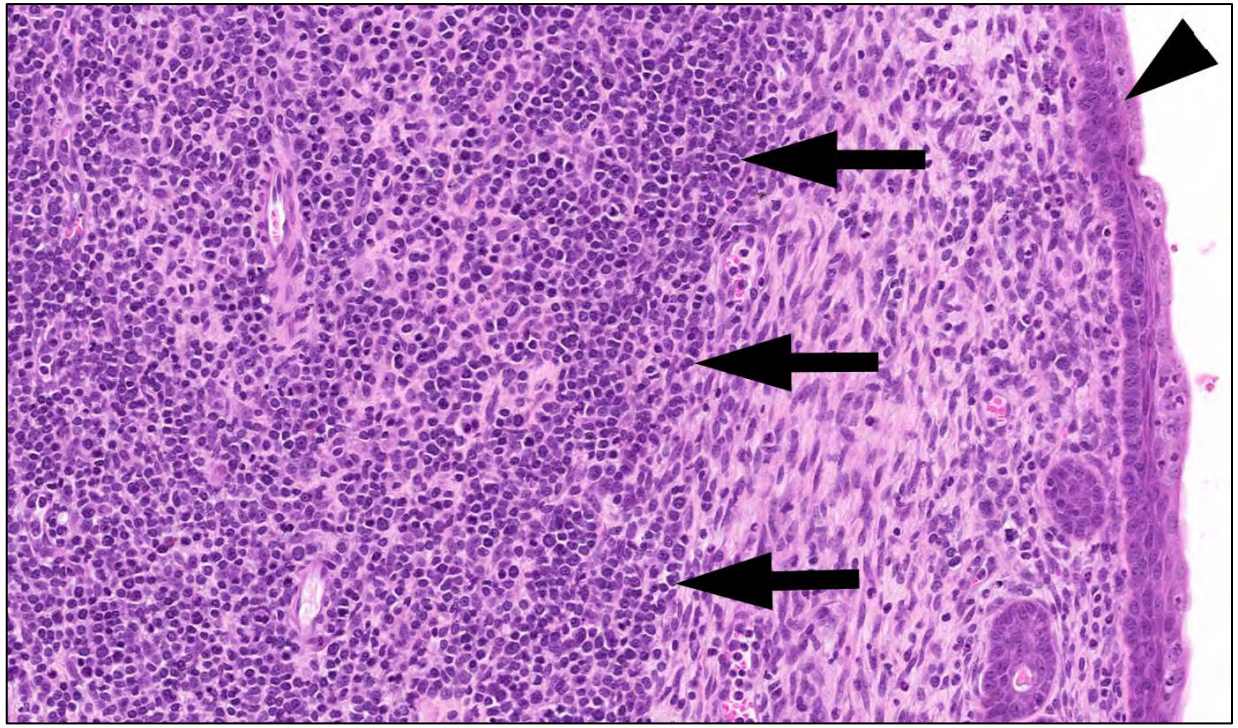
Uterus – Infiltrate, Inflammatory Cell

- **Infiltrate, Inflammatory Cell, [Modifier], [Location]**
  - Modifier: cell type (e.g., neutrophilic, lymphocytic, eosinophilic, mixed, etc.)
  - Location: e.g., endometrium
- The presence of inflammatory cells, with little or no evidence of other tissue damage or injury (e.g., edema, necrosis, congestion, fibrosis).
- Increased numbers of inflammatory cells compared to those that are normally present at certain estrous cycle stages.
  - Neutrophils are normally present in endometrium during metestrus and in endometrial epithelium during diestrus.

Uterus – Infiltrate, Inflammatory Cell (Cont.)



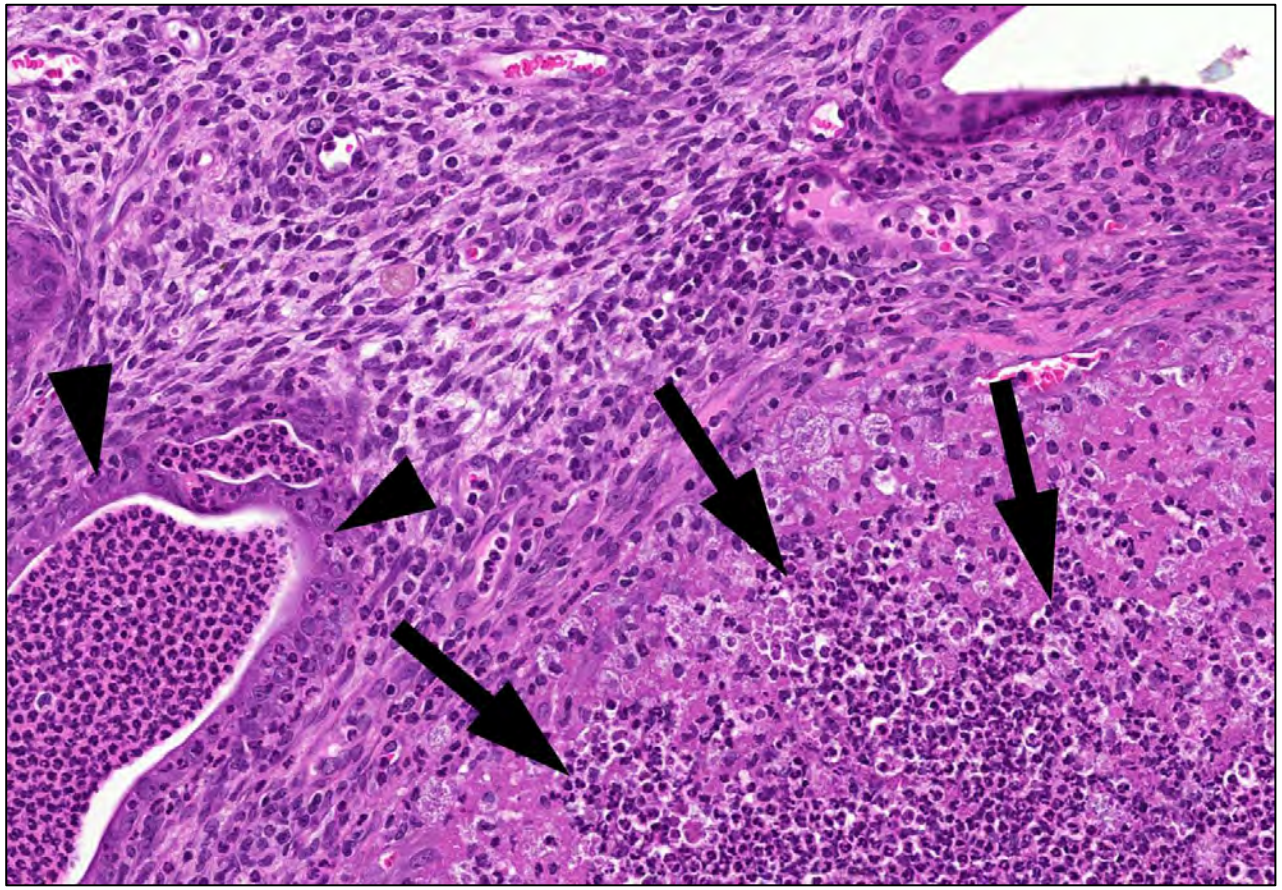
Uterus – Normal, mouse.
Note normal endometrial stroma (arrows).



Uterus – Infiltrate, Inflammatory Cell, Lymphocytic, Endometrium, mouse.
Note lymphocytes (to the left of arrows) in the endometrium. Squamous cell metaplasia of the endometrial epithelium (arrowhead) and glands is also present.

Uterus – Inflammation

- **Inflammation, [Modifier], [Locator]**
  - Modifier: cell type (e.g., neutrophilic, lymphocytic, mononuclear, mixed; also, suppurative and granulomatous)
  - Location: e.g., endometrium
- Evidence of inflammatory changes (e.g., edema, necrosis, congestion, fibrosis).
- May be limited to endometrium with little or no exudate or myometrial involvement.
- Endometrial lining may be eroded, ulcerated or metaplastic (e.g., switch to squamous cell).
- With prolonged inflammation, endometrial glands may be cystic (dilated) or atrophic (decreased size) due to periglandular fibrosis.



Uterus – Inflammation, Suppurative, Endometrium (arrows), **mouse**.
Endometrial gland with suppurative inflammation (arrowheads).

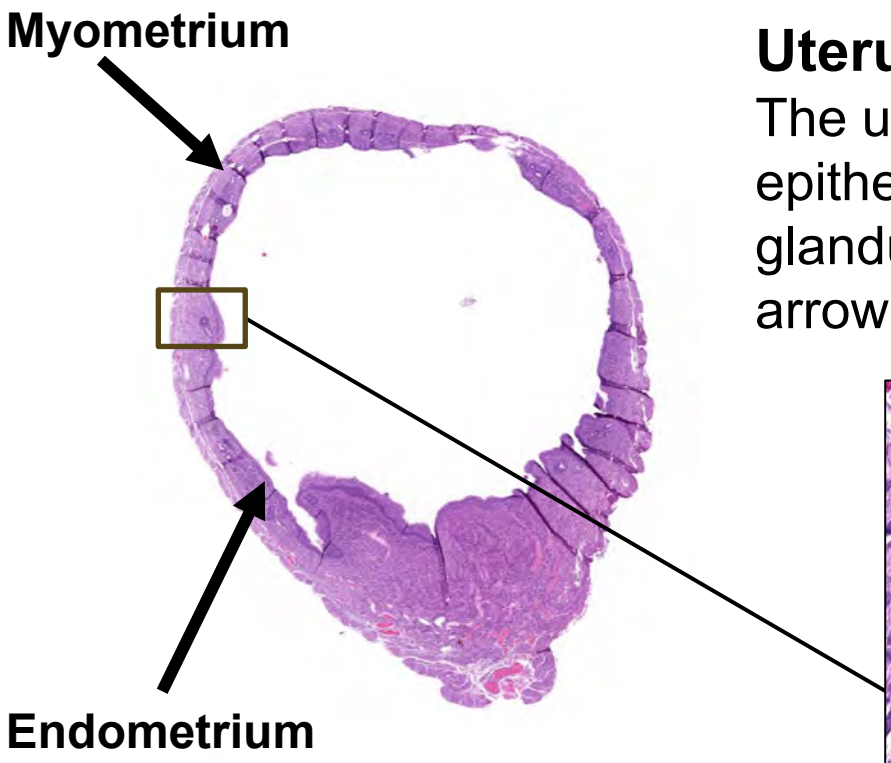
Uterus – Inflammation (Continued)

- **Inflammation** must be differentiated from **Infiltrate, Inflammatory Cell.**
  - **Inflammation** should be reserved for lesions with significant epithelial and stromal inflammatory changes.
- **Inflammation, Endometrium** frequently is a component of endometrial hyperplasia (proliferation of endometrial glands).
- In toxicologic pathology, suppurative exudation into the uterine lumen with or without involvement of the myometrium should be diagnosed as **Inflammation, Suppurative**; not as clinical terms "endometritis" or "pyometra."
  - Common sequela to infection of genital tract by *Mycoplasma pulmonis* and *Klebsiella oxytoca* in B6C3F1 mice (Dixon et al., 2014).
  - Estrogen treatment increases susceptibility in some rat strains (e.g., Wistar and Brown Norway rats) (Dixon et al., 2014).
- Endometrium or myometrium modifiers should not be used if inflammation affects whole uterus (transmural [affecting all layers]).

Uterus – Metaplasia, Squamous Cell

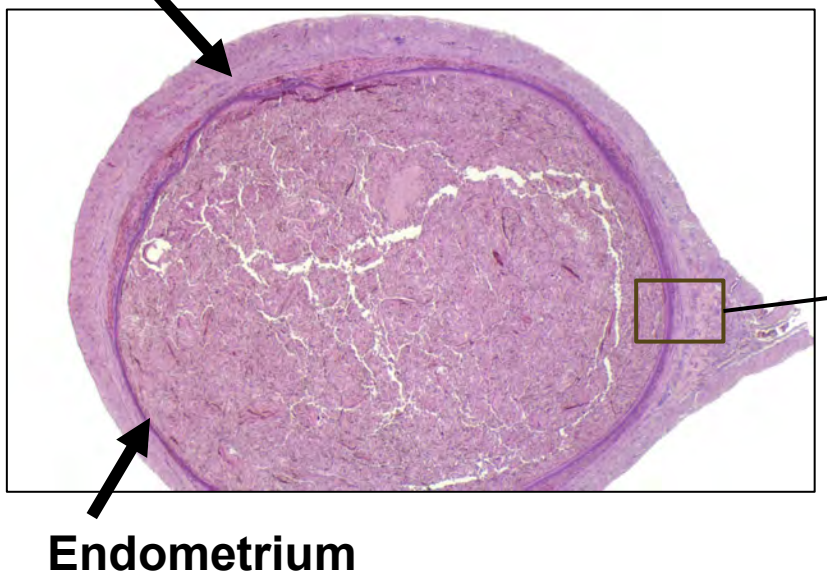
- Foci or areas of replacement of normal columnar epithelium of the endometrium by squamous epithelium.
  - Modifiers: non-keratinizing or keratinizing
- Keratinization is not usually prominent, but if present keratin can fill the uterine lumen.
- Spontaneously occurring change observed in the endometrium of aged rats and mice (Dixon et al., 2014).
- Associated with cystic endometrial hyperplasia (see Uterus - Hyperplasia, Glandular, Cystic in *Uterus Proliferative Lesions* module) or chronic endometrial inflammation.
- Also associated with continuous administration of synthetic estrogens, chlorinated hydrocarbons.
- Vitamin A deficiency may also induce this lesion.

Uterus – Metaplasia, Squamous Cell (Continued)

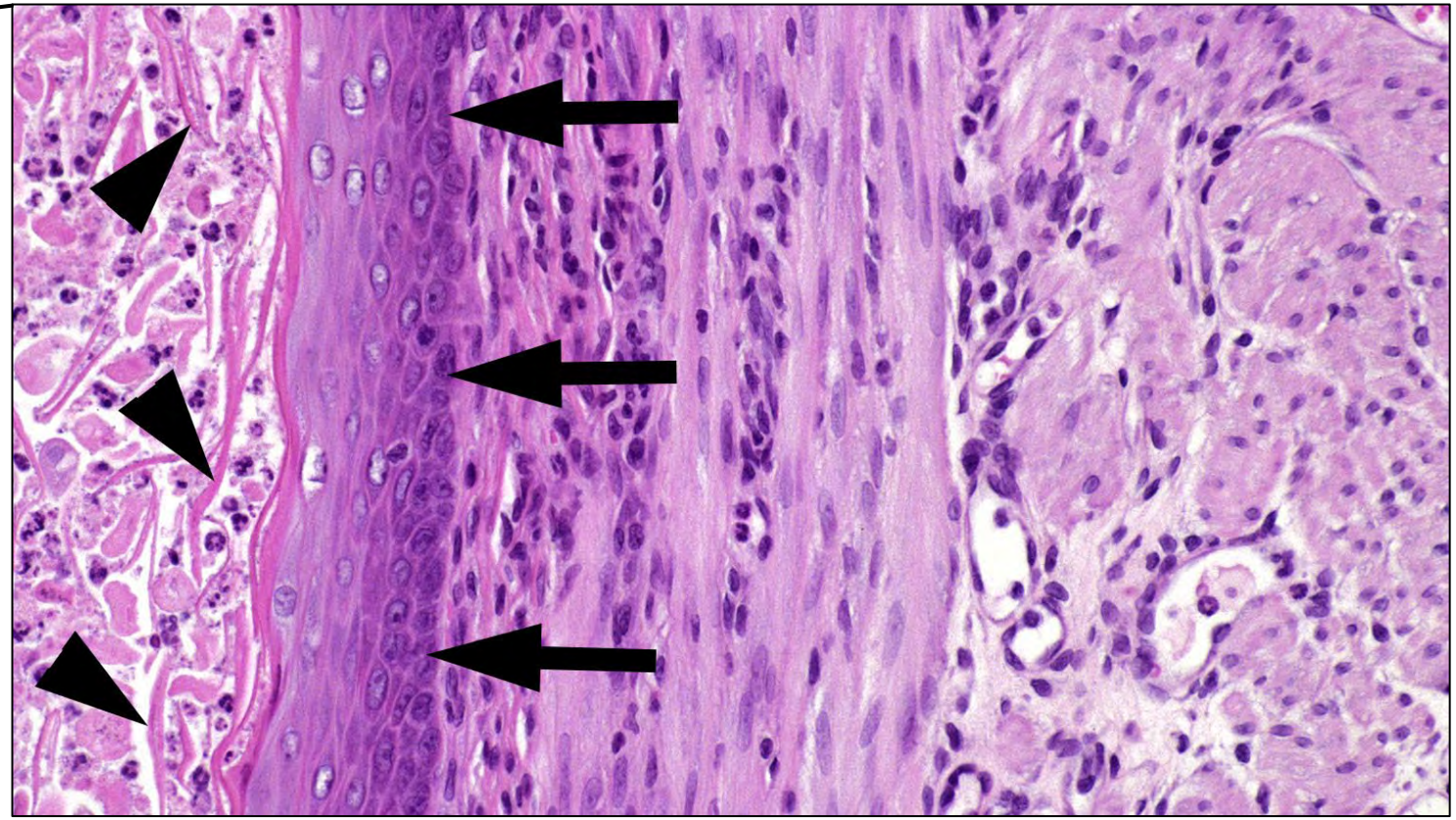


Uterus – Metaplasia, Squamous Cell, Non-keratinizing, rat.
The uterus is lined by stratified (multi-layered) non-keratinized squamous epithelium (inset, arrows) instead of normal columnar epithelium. The glandular epithelium is also replaced by squamous epithelium (inset, arrowheads). The uterine lumen is also dilated in the image on the left.

Uterus – Metaplasia, Squamous Cell (Continued)



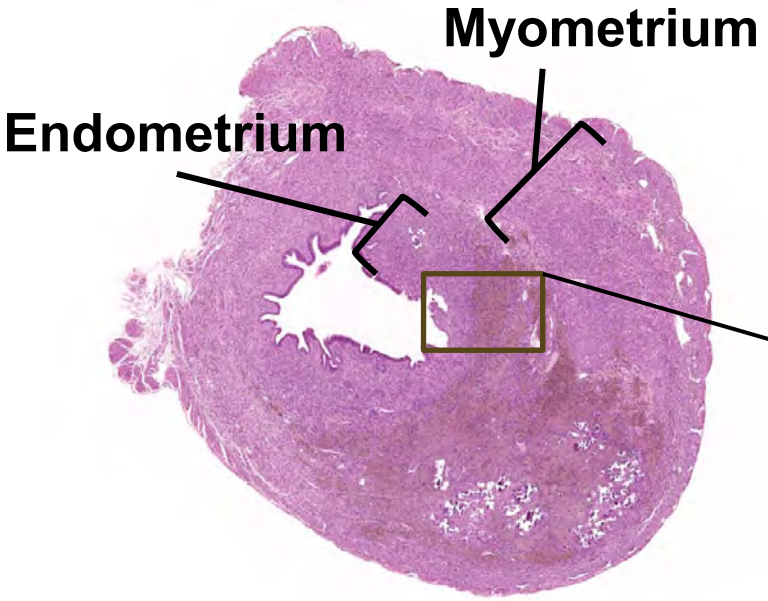
Uterus – Metaplasia, Squamous Cell, Keratinizing, rat.
The uterus is lined by stratified keratinized squamous epithelium instead of normal columnar epithelium (inset, arrows). Keratin (inset, arrowheads) and cellular debris fill the uterine lumen. The uterine lumen is also dilated in the image on the left.



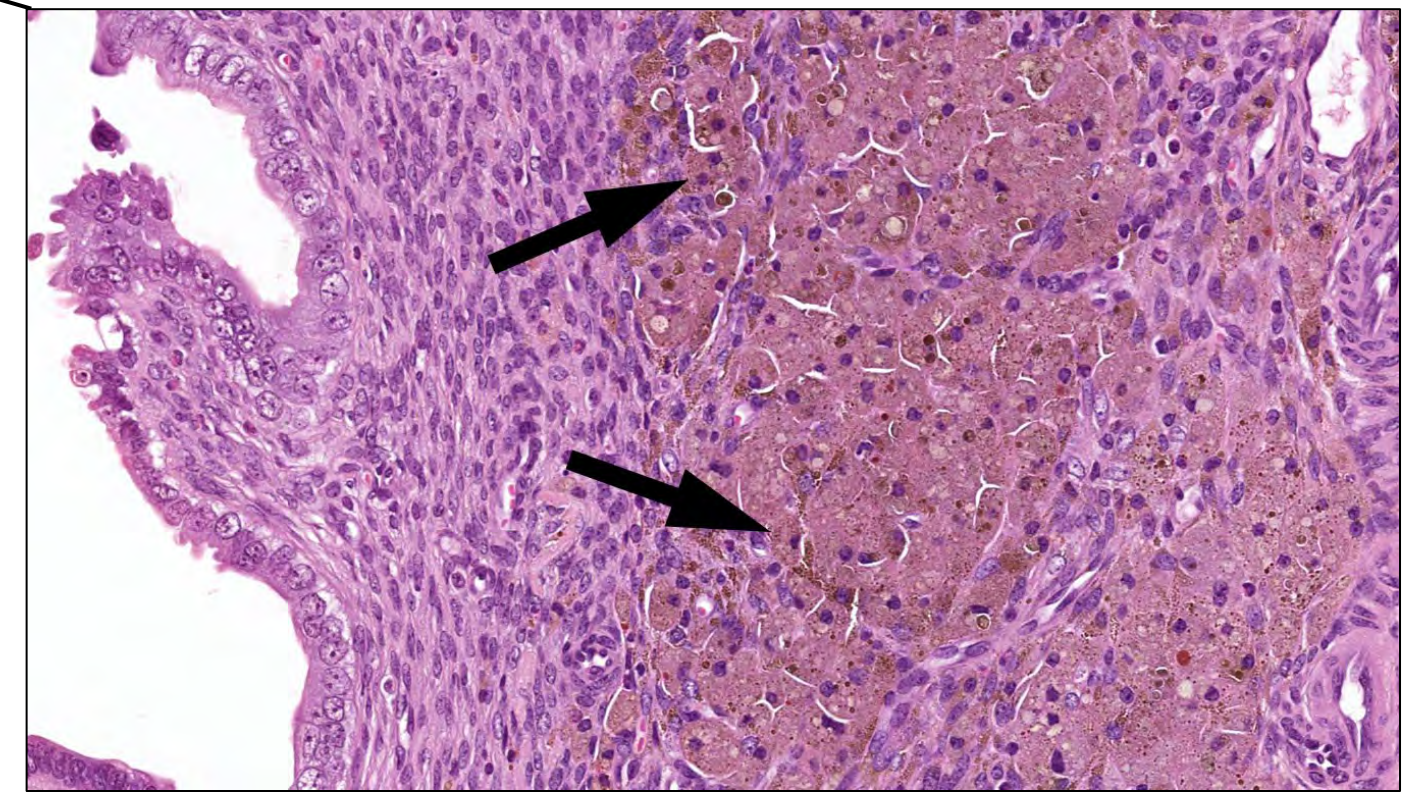
Uterus – Pigment

- Golden-brown to dark brown granular material.
- Pigment types (special stains required to differentiate):
  - Hemosiderin (iron-containing pigment derived from breakdown of erythrocytes)
  - Lipofuscin/ceroid (breakdown products of cell membranes)
  - Acid hematin (formalin pigment; artifact – do not diagnose)
- Do not diagnose if pigment is secondary to another lesion (e.g., hemorrhage).
- Hemosiderin-laden macrophages often seen in endometrium of aging rats (10-12 months of age) (Dixon et al., 2014).
- Hemosiderin also seen in multiparous rodent females at placentation sites secondary to hemorrhage (do not diagnose in this situation).

Uterus – Pigment (Continued)



Uterus – Pigment, mouse.
Pigment, most likely hemosiderin, is within the cytoplasm of macrophages (arrows) in the endometrium (inset) and myometrium.



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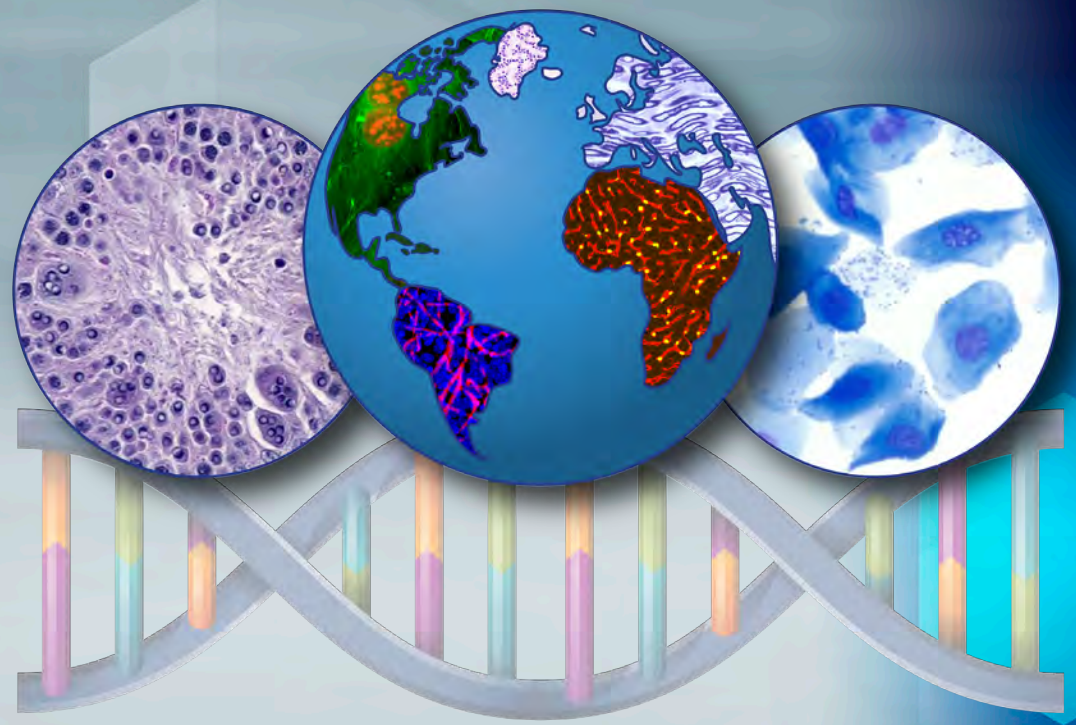

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