



CONTINUING EDUCATION IN TOXICOLOGIC PATHOLOGY REPRODUCTIVE SYSTEM

Third Conference

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STAGING OF ESTRUS CYCLE IN RATS

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Introduction

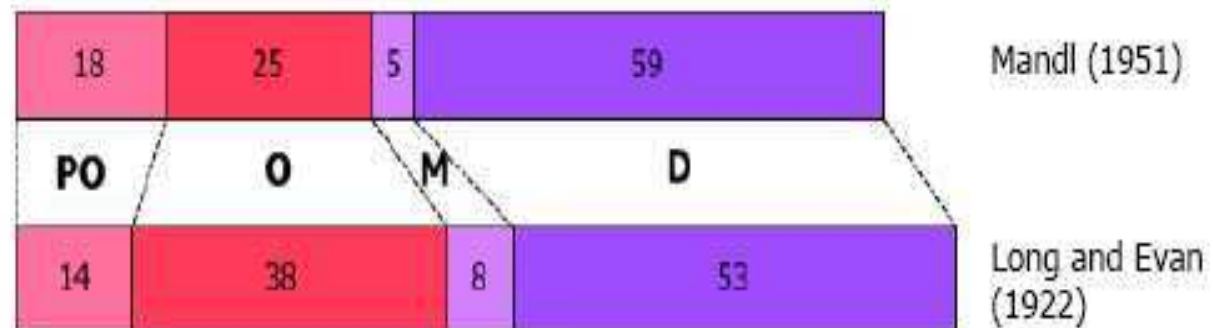
- The female reproductive tract is a **highly dynamic organ system**.
- Reproductive cyclicity characterized by the regular occurrence of an estrus cycle
- Numerous **well defined and sequential alterations** in reproductive tract histology, physiology and cytology
- **Knowledge of the normal histological appearance** of the reproductive tract at each stage of the oestrous cycle is essential

Estrus cycle

- PROESTRUS
- ESTRUS
- METESTRUS
- DIESTRUS

Mean oestrous cycle length

4.4 days (107 hours)



Mandl (1951)

Long and Evan (1922)

PO prooestrus O oestrus M metoestrus D dioestrus

- Many chemicals are found to interfere with the reproductive function of the female rat
- Significant **decrease in body weight and food intake** may also interfere with normal estrus cyclicity
- Interference is expressed as a change in normal morphology of the reproductive tract or a disturbance in the duration of particular phase
- These alterations can be recognized only if the pathologist has knowledge of the continuously changing histological appearance of the various components of the reproductive tract during the cycle

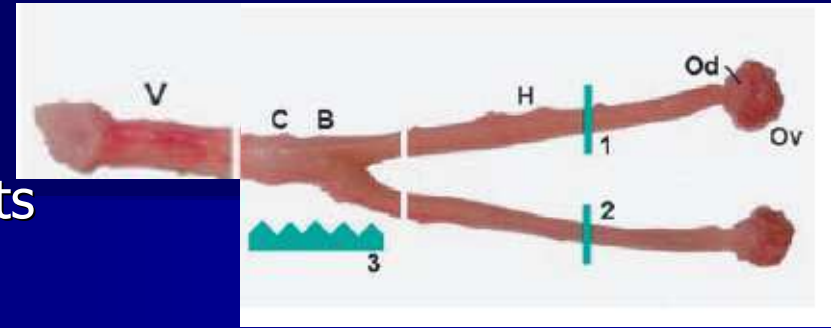
- Alterations in stages may manifest by

- More number of animals in a particular phase of the cycle
- Disturbance in the coordinated morphology of the components of the tract,
or
- Abnormal appearance of a particular region.

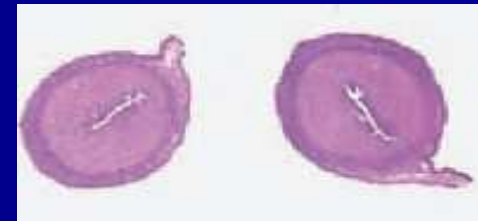
STAGING THE CYCLE

- Requires examination of individual reproductive organs, followed by an integrated assessment of the female reproductive tract.
- **Vagina undergoes the most characteristic morphological alterations** during the oestrous cycle, staging is initially based on the appearance of this organ.
- The uterus and ovary are then examined for compatible, synchronous histological changes
- In essence, all parts of the reproductive tract should “tell the same story”

- The histological changes occurring in vagina **do not occur uniformly** along its length.



- Hence, a **transverse section of the mid vagina** should be examined in order to ensure consistency in staging.



- Sampling from the caudal (posterior) one-third of the vagina should be avoided

- Transverse sections of the mid portion of both uterine horns, and medial sections of both ovaries are the minimal requirement for an adequate evaluation



Ovary and staging

- The stage of the reproductive cycle cannot easily be determined from the appearance of the ovarian follicles in a species like the rat, which has a short estrus cycle.
- Follicles in virtually all stages of development are generally present within the ovaries at all phases of the cycle.
- **Two or three generations of corpora lutea** may be present in an individual ovary from the preceding ovulatory cycles, as each corpora lutea persists morphologically for twelve to fourteen days
- **Thus, the use of corpora luteal morphology as an accurate tool for estrus staging can be confusing.**

STAGING



Key histological features of

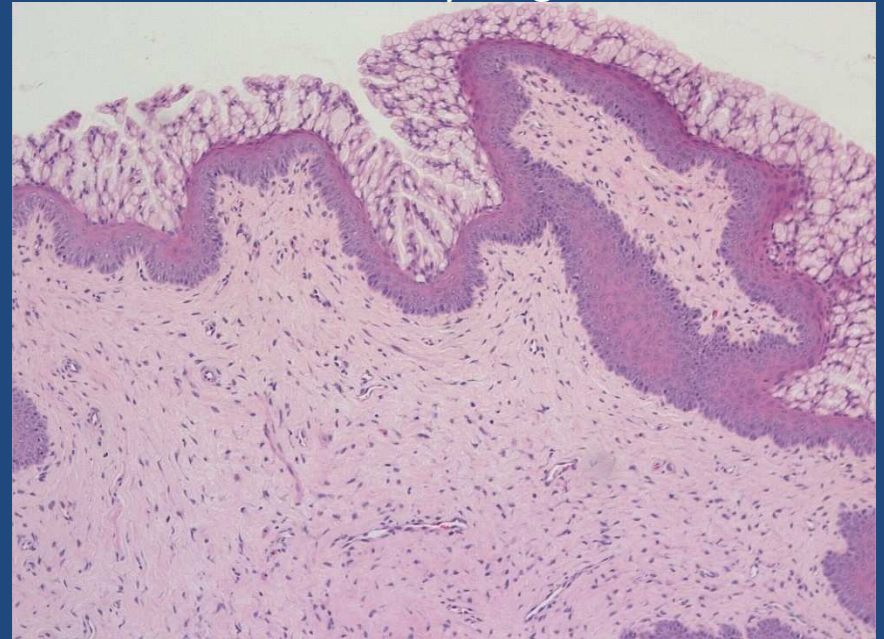
- PROESTRUS
- ESTRUS
- METESTRUS
- DIESTRUS
- PERSISTENT DIESTRUS
- PERSISTENT ANESTRUS

PROESTRUS

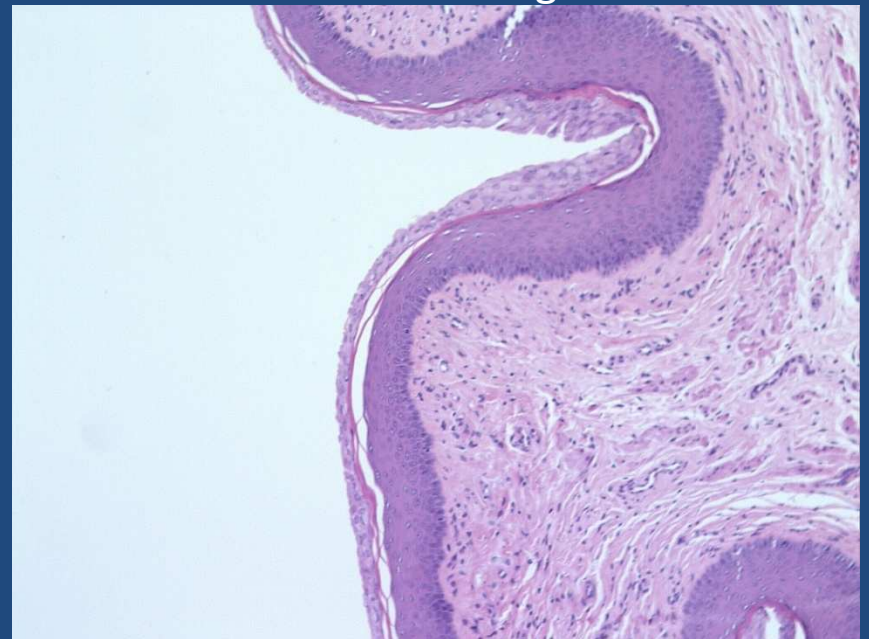
Vagina

- Mitotic figures present
- Little if any degeneration or desquamation
- Formation of stratum granulosum (defines start), superficial mucoid layer and stratum corneum progressively
- At end of stage, fully cornified and generally showing superficial mucoid layer with some desquamation of mucoid cells.

Early stage



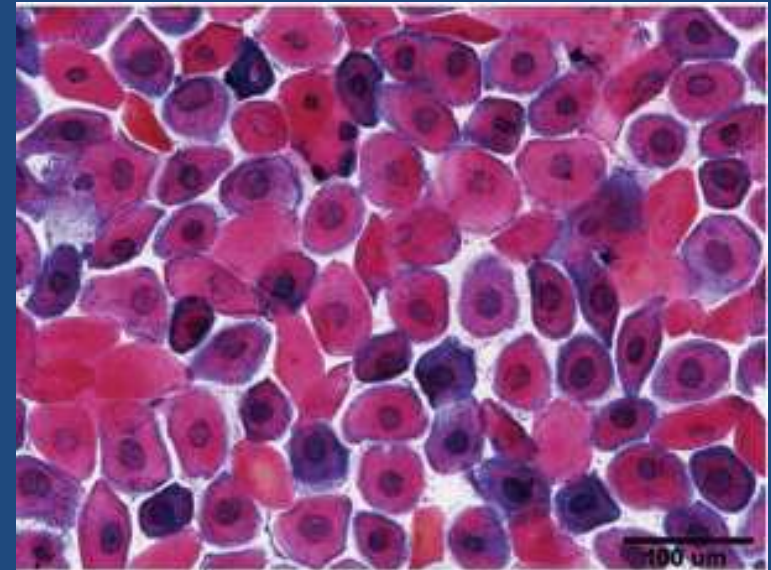
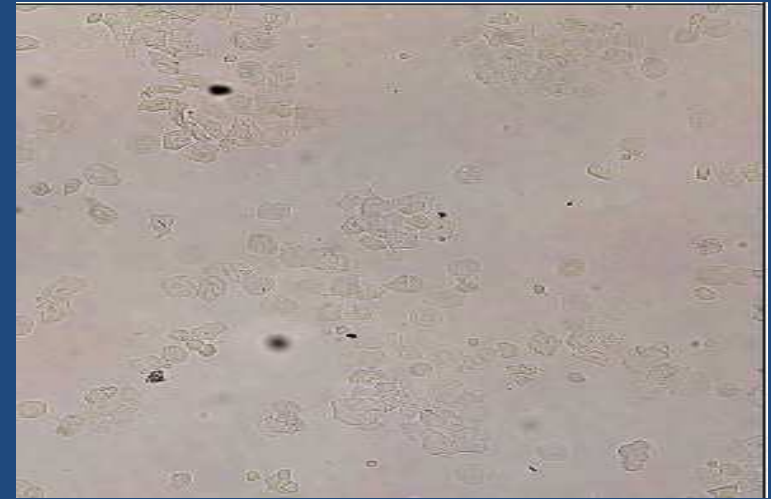
Late stage



Vaginal smear- Proestrus



- The vaginal smear is consistent with this histological appearance, showing a disappearance of leukocytes
- Presence of sheets of, or isolated, nucleated epithelial cells, which become progressively acidophilic with the appearance of cornified cells.



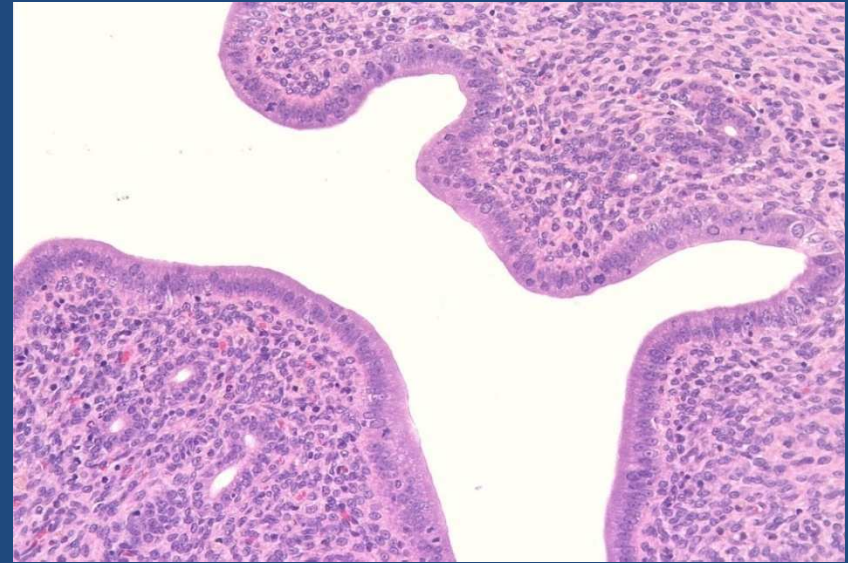
PAP Stained smears, Hubscher *et al*, 2004

PROESTRUS

Uterus

- Epithelium cuboidal to columnar. Mitoses present in epithelial cells with little or no degeneration
- Stromal edema
- Little inflammatory cell infiltration.
- Dilatation, particularly toward end of stage.

Early stage



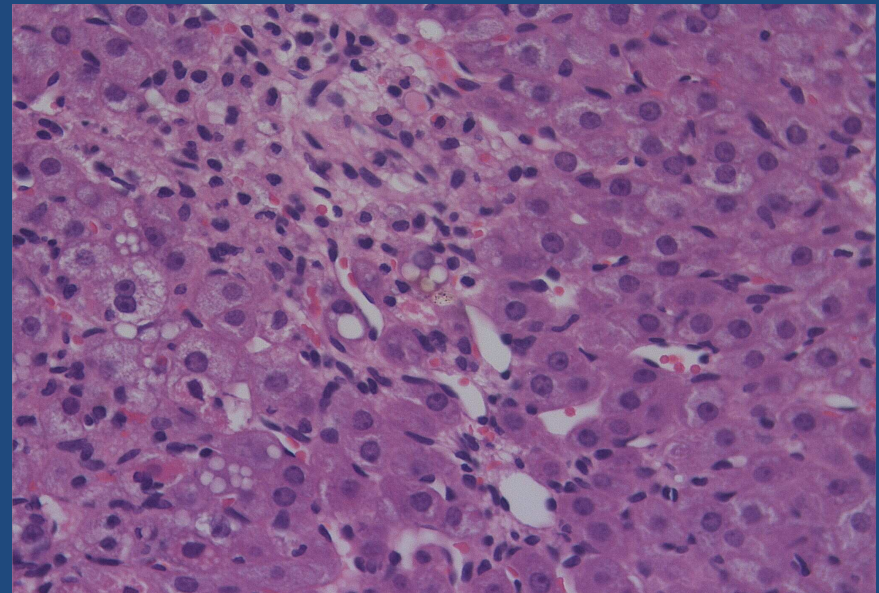
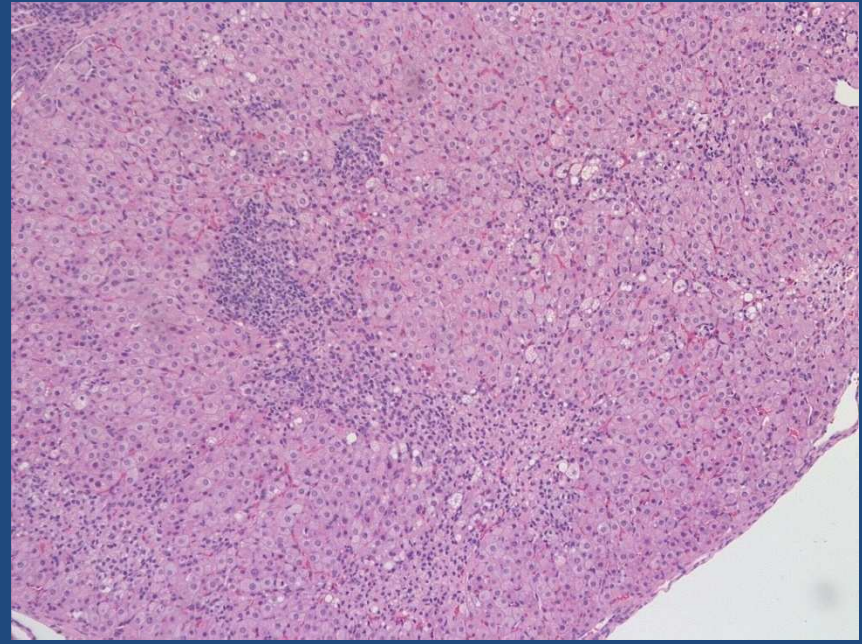
Late stage



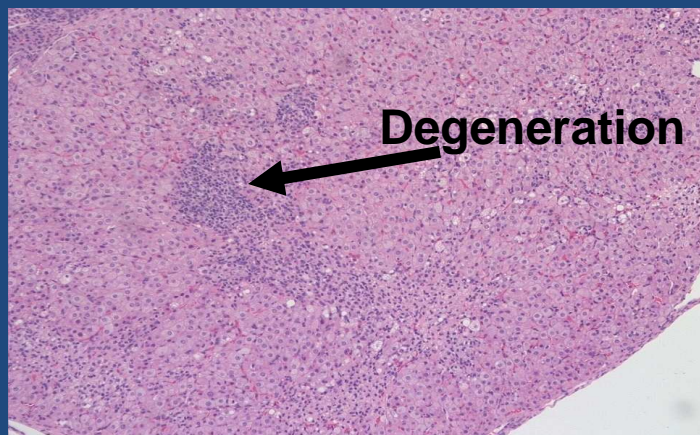
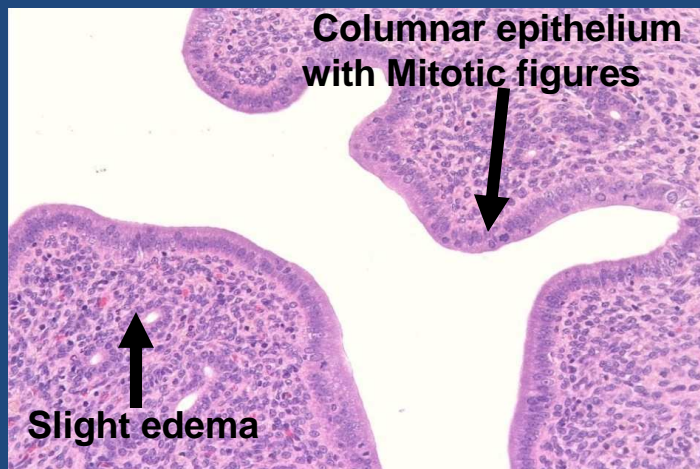
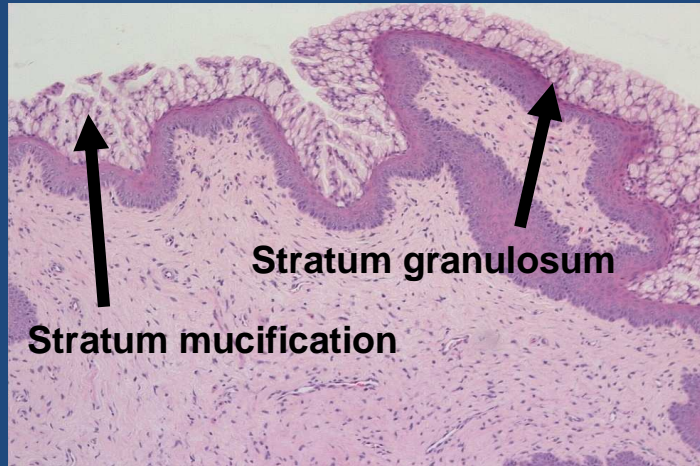
PROESTRUS

Ovaries

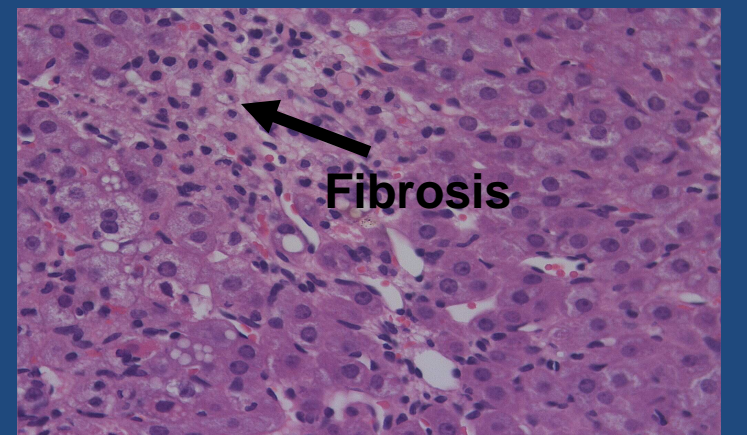
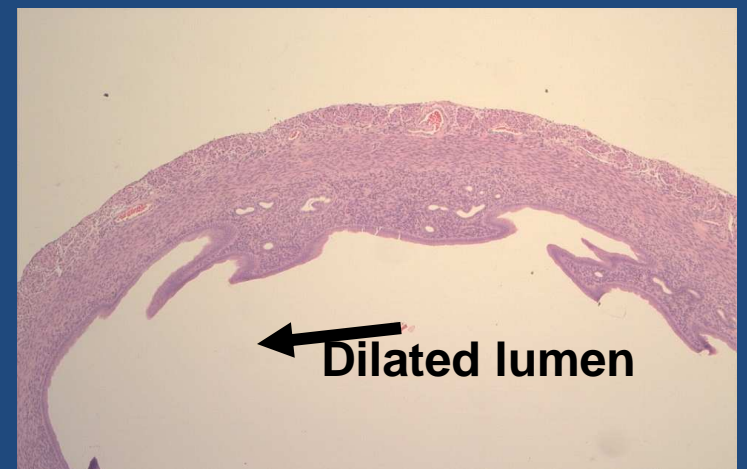
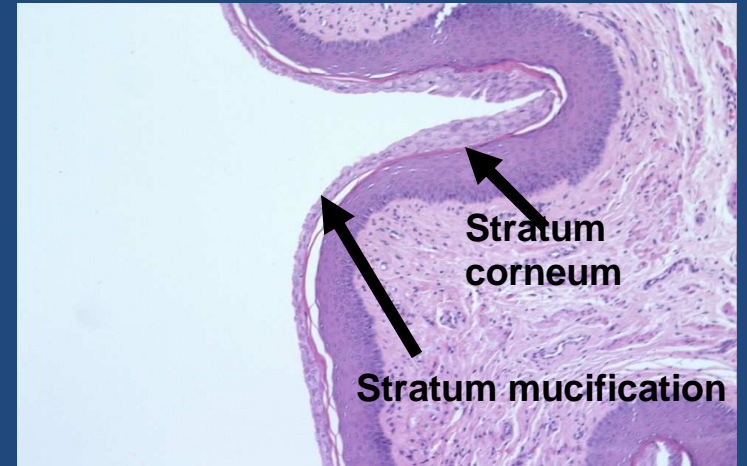
- Corpora lutea often degenerate
- Cytoplasmic vacuoles generally present
- Fibrous tissue proliferation in central cavity.



EARLY PROESTRUS



LATE PROESTRUS

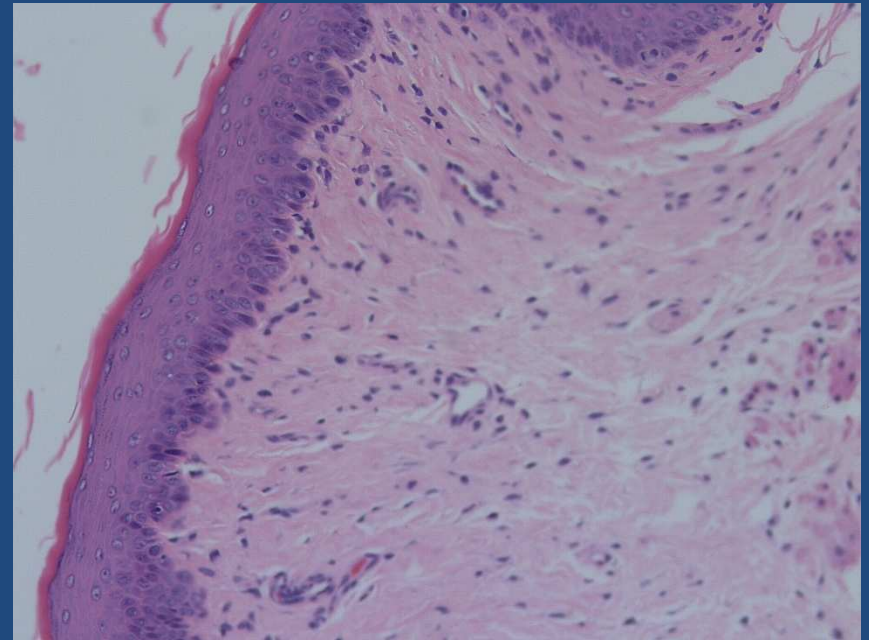


ESTRUS

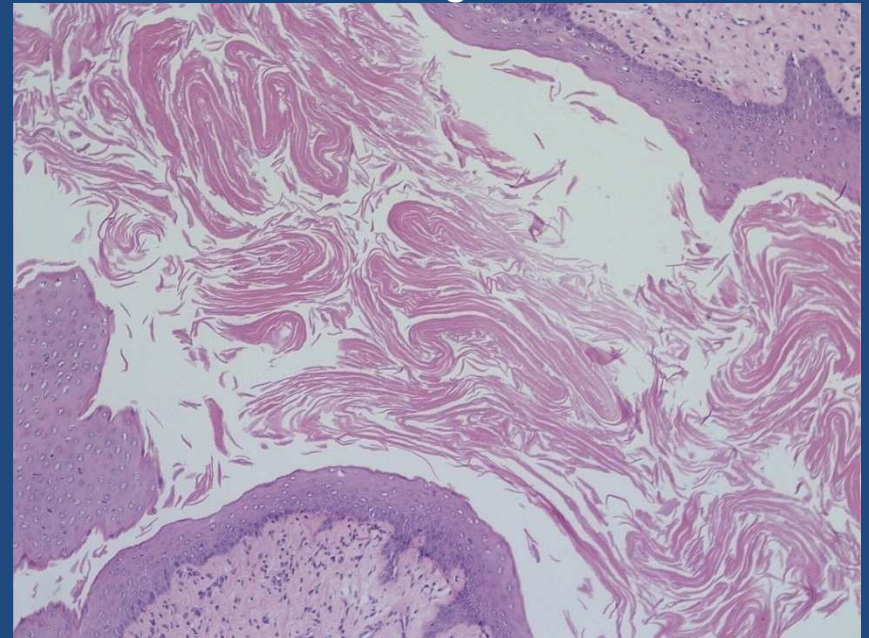
Vagina

- Progressive shedding of superficial mucoid and cornified layers
- Reduction in height of epithelium
- Cell debris present
- Loss of mitotic figures
- At the end of the stage- complete detachment of the cornified epithelium

Early stage



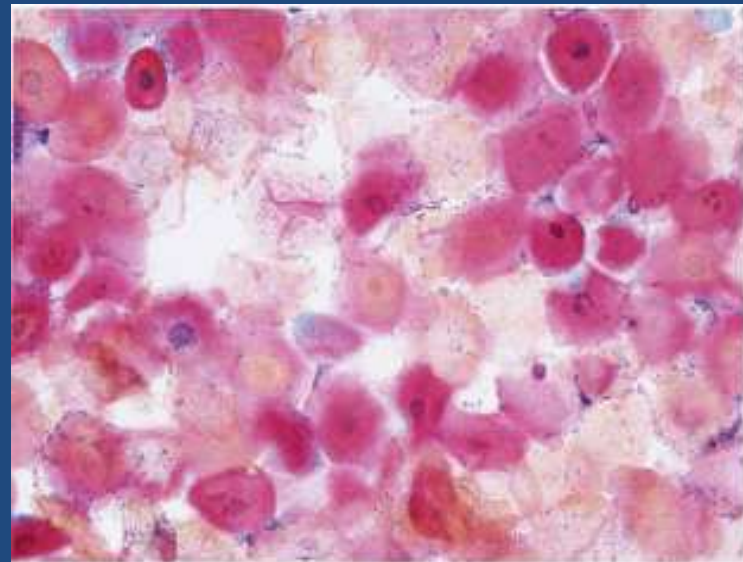
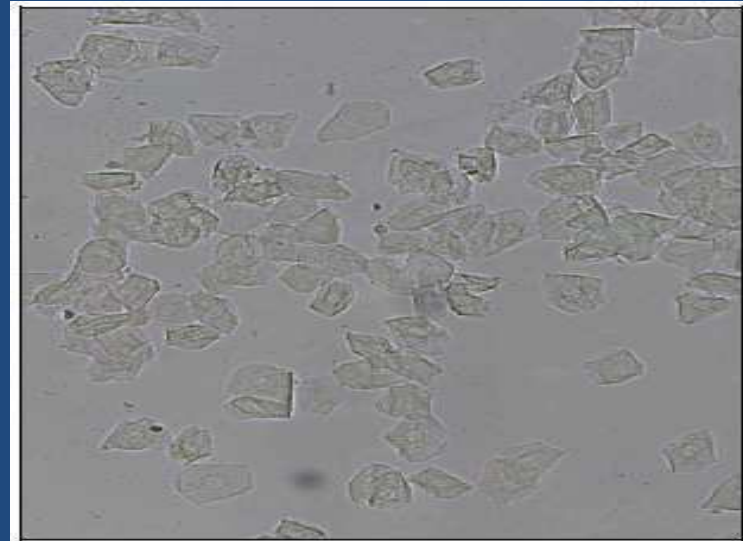
Late stage



Vaginal smear- Estrus



- The vaginal smear shows non-nucleated cornified cells,

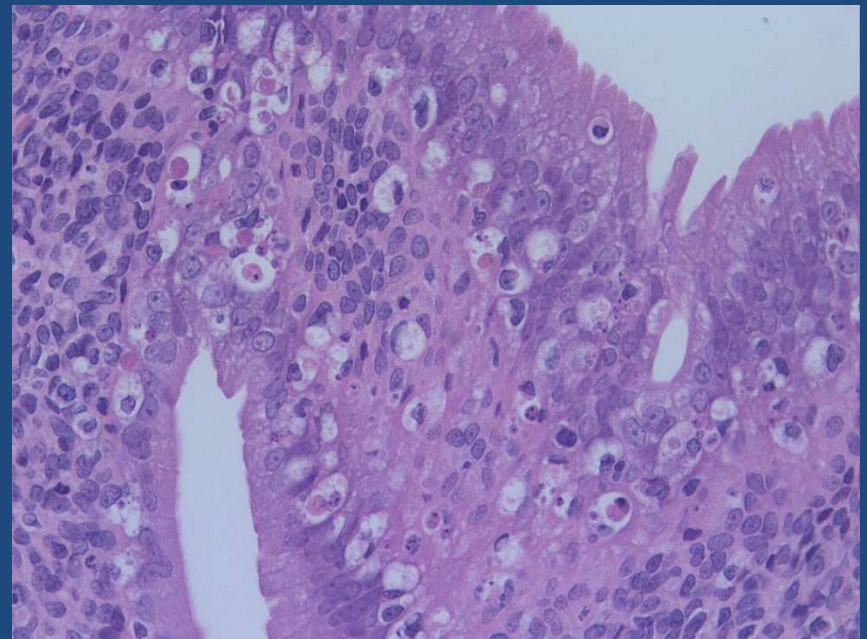
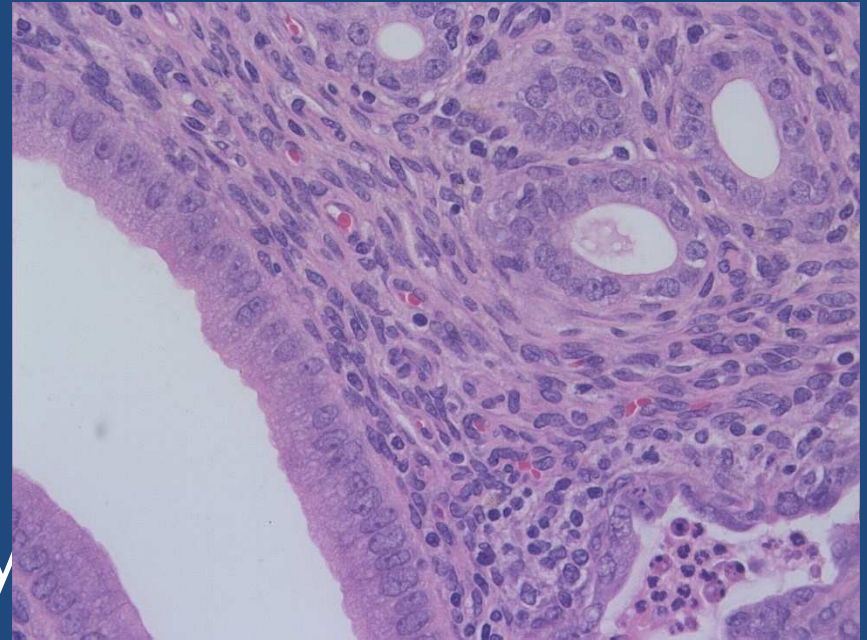


PAP Stained smears, Hubscher *et al*, 2004

ESTRUS

Uterus

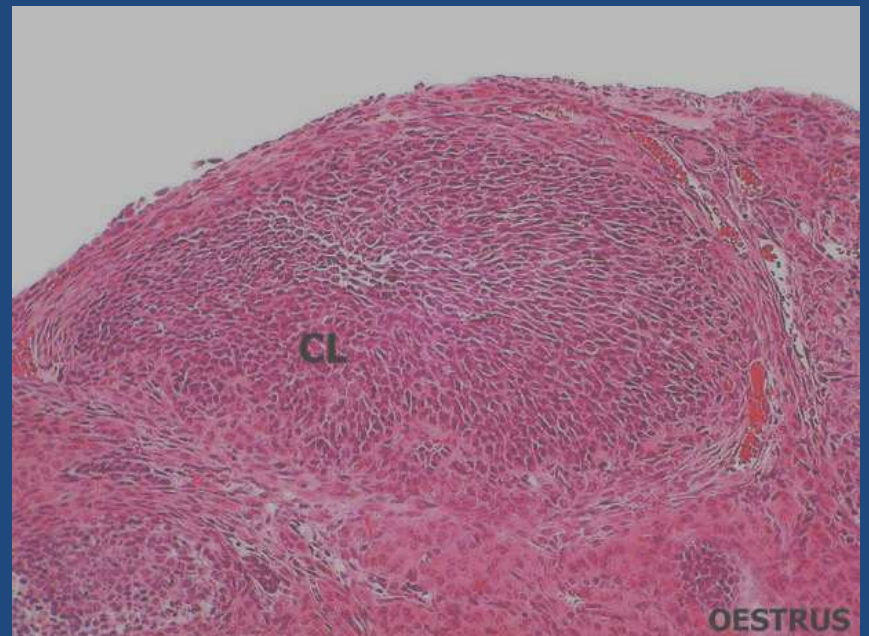
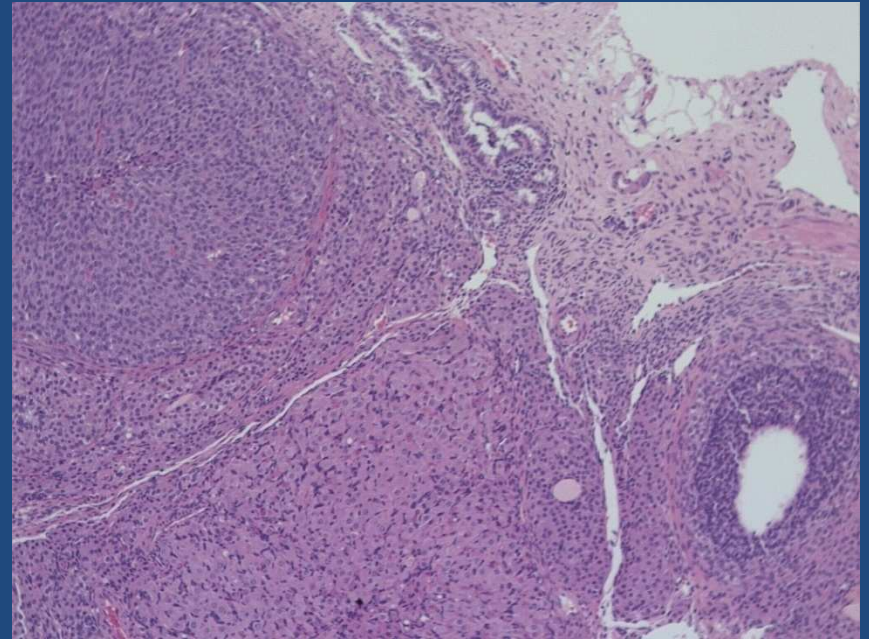
- Start of estrus defined by appearance of notable degeneration/necrosis of epithelial cells, glands generally first
- **Loss of mitotic activity**
- Leukocyte infiltration
- Dilatation may persist to late estrus.



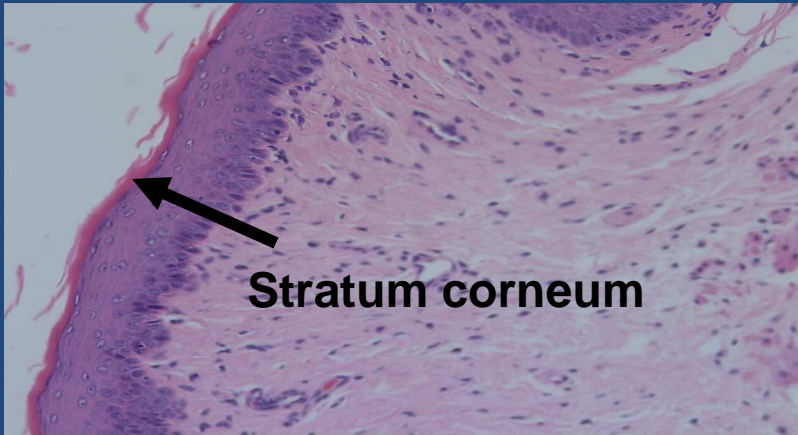
ESTRUS

Ovary

- Degenerate corpora lutea often present
- Some small corpora lutea with basophilic cell cytoplasm, central fluid-filled cavity, and no fibrous tissue.



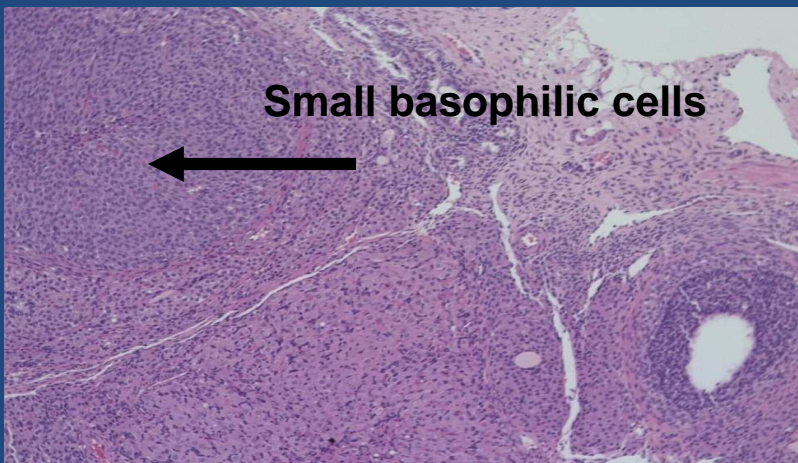
ESTRUS EARLY



Stratum corneum

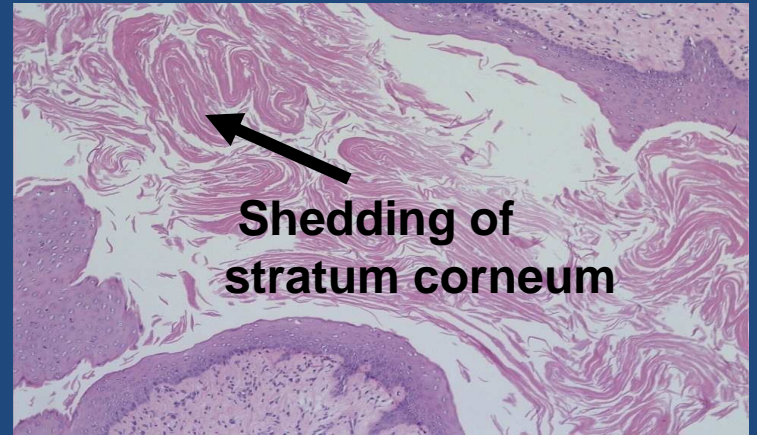


**Tall columnar epithelium
With no degeneration**

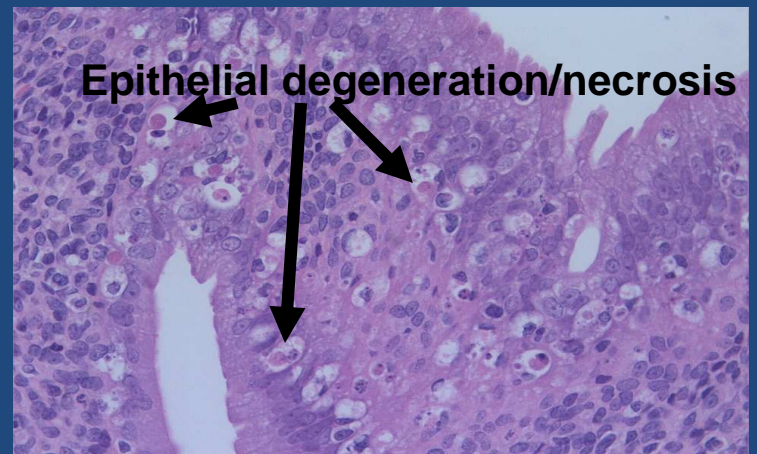


Small basophilic cells

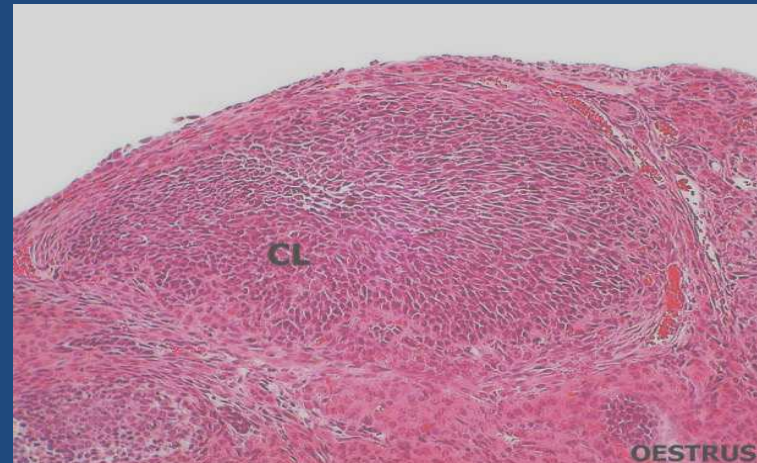
ESTRUS LATE



**Shedding of
stratum corneum**



Epithelial degeneration/necrosis

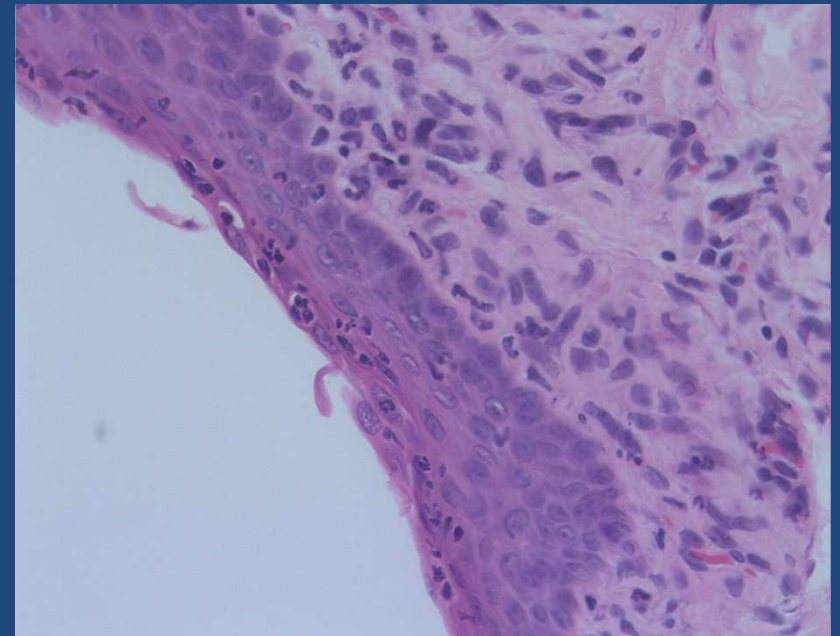
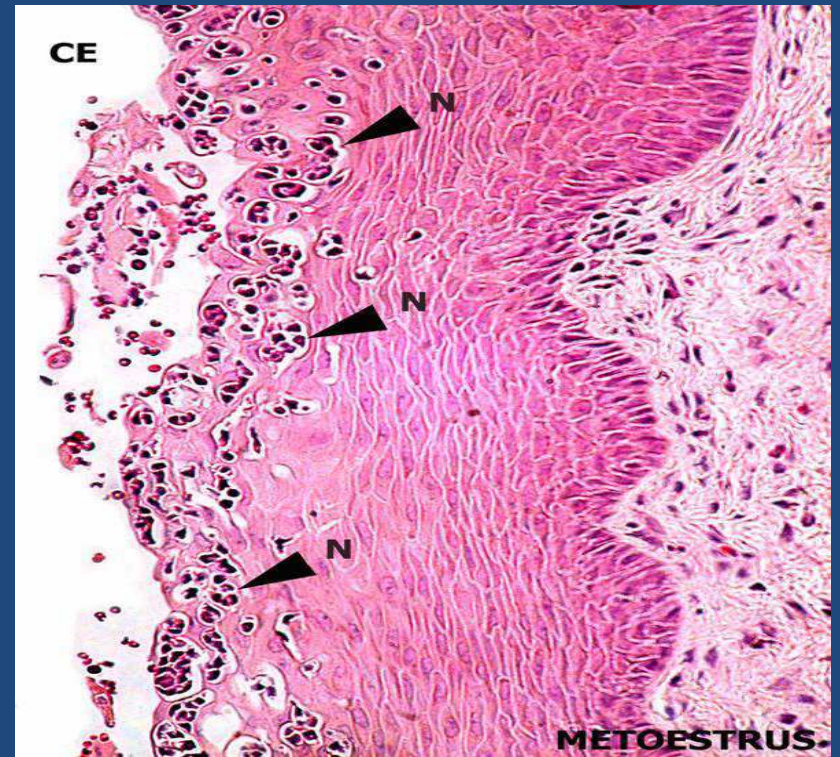


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METESTRUS

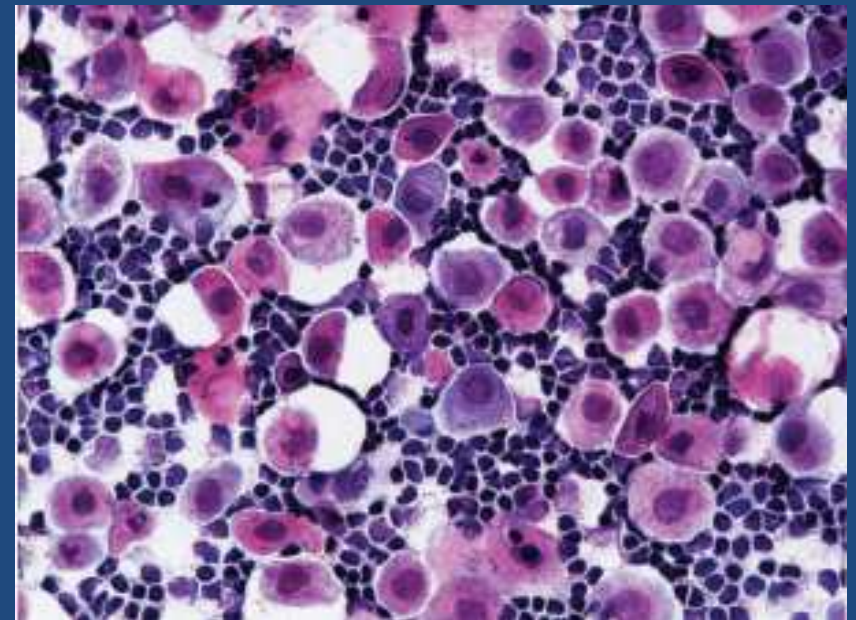
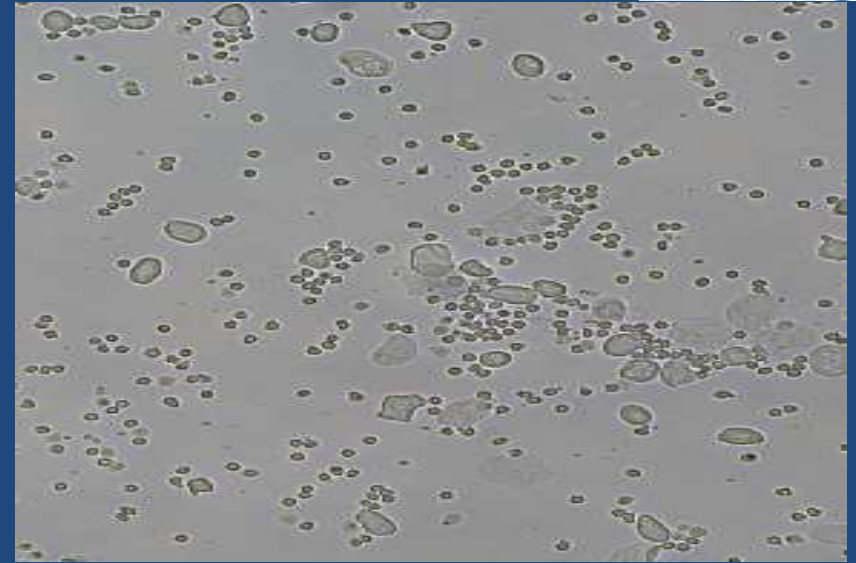
Vagina

- Start defined by virtually complete detachment of cornified layer (generally residual squames in lumen)
- Continued desquamation with loss of stratum granulosum and upper germinativum.
- Leukocyte infiltration.



Vaginal smear- metestrus

- The smear during this stage shows
 - Leukocytes,
 - Few cornified cells,
and
 - Basophilic cells.

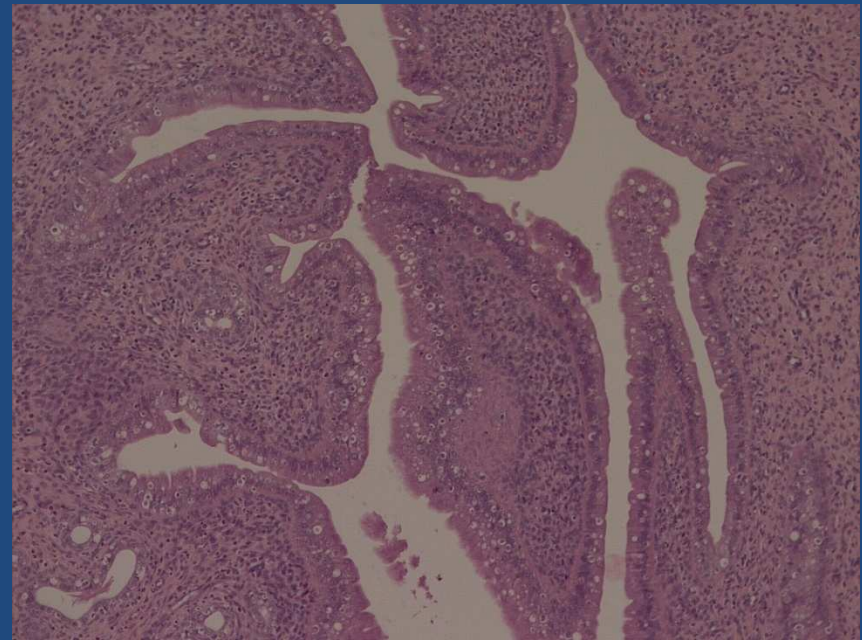


PAP Stained smears, Hubscher *et al*, 2004

METESTRUS

Uterus

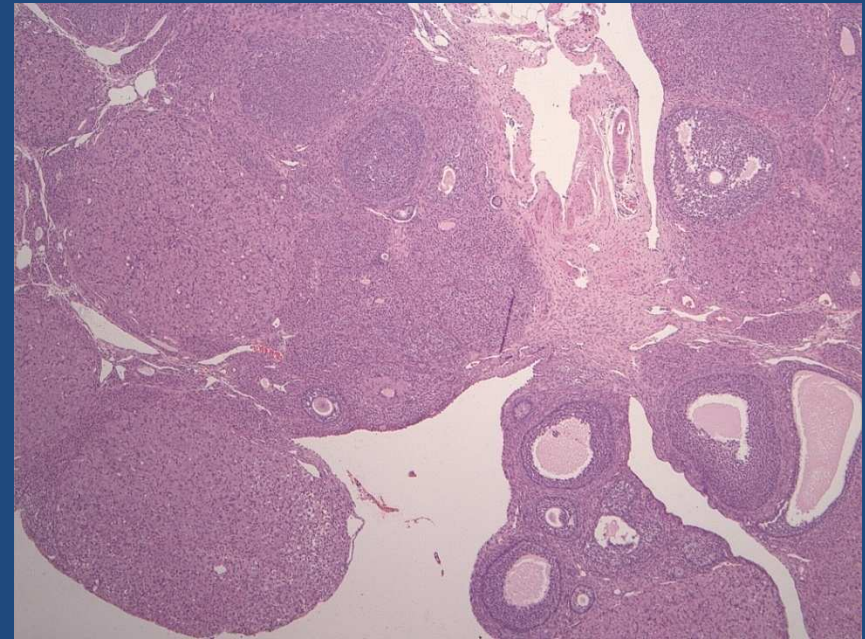
- Continued degeneration of endometrial epithelial cells
- Return of mitotic activity
- Both mitotic activity and degeneration seen together

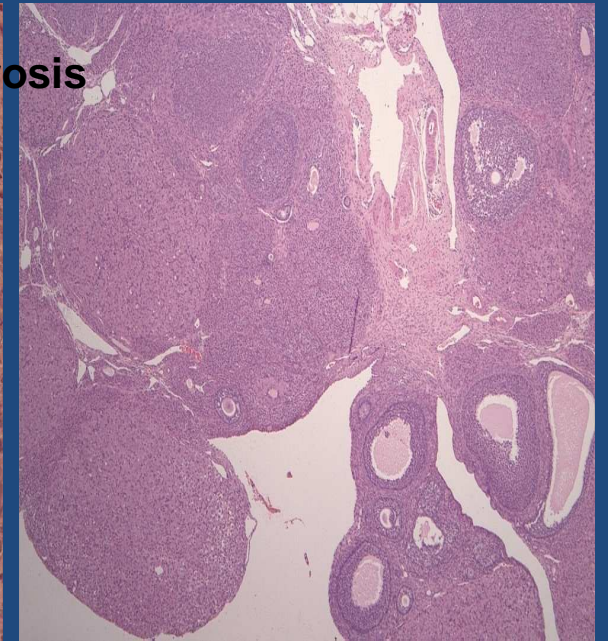
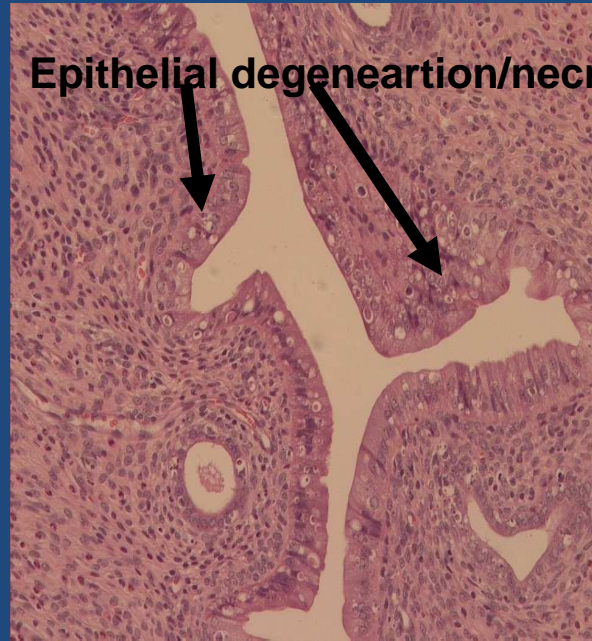
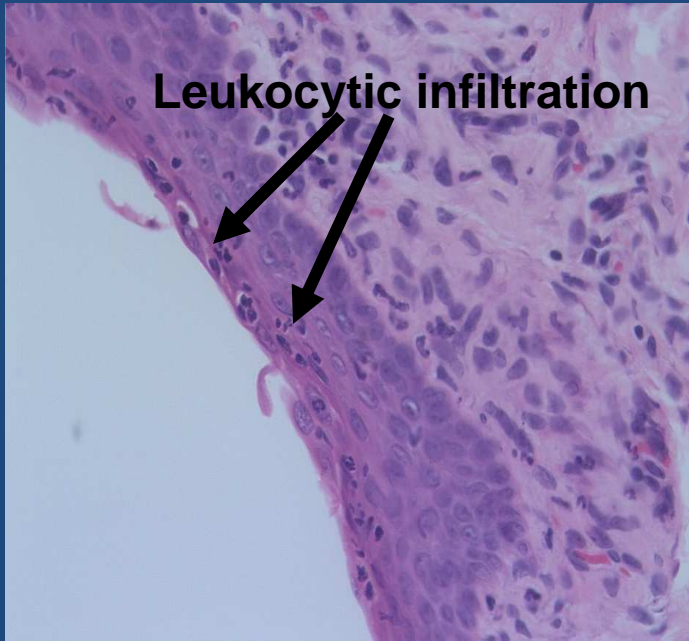


METESTRUS

Ovary

- Corpora lutea may still contain fluid cavity
- Smaller than at diestrus
- Slightly basophilic cells
- Generally devoid of fibrous tissue.



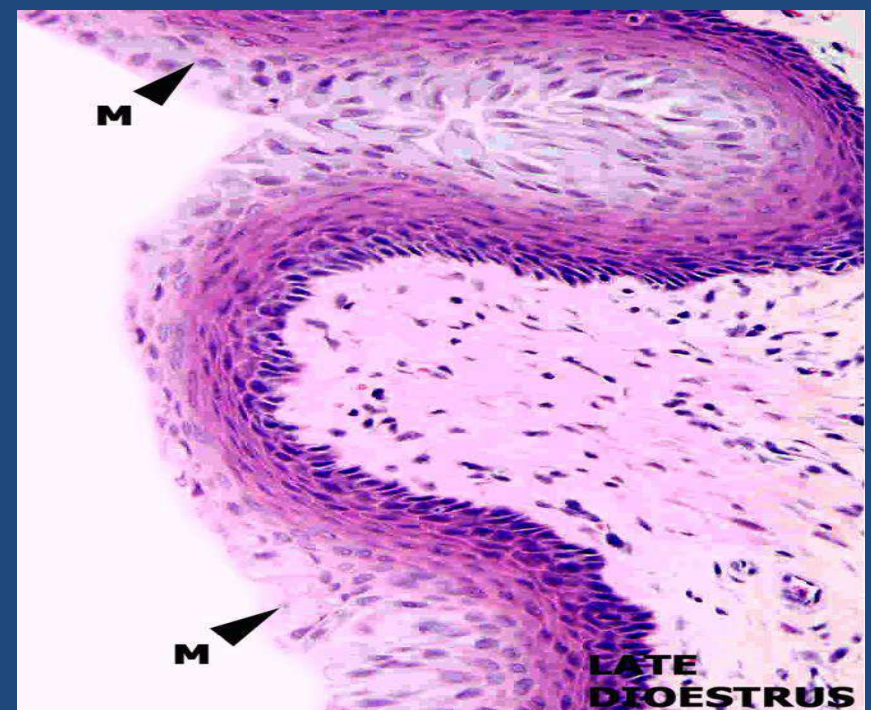
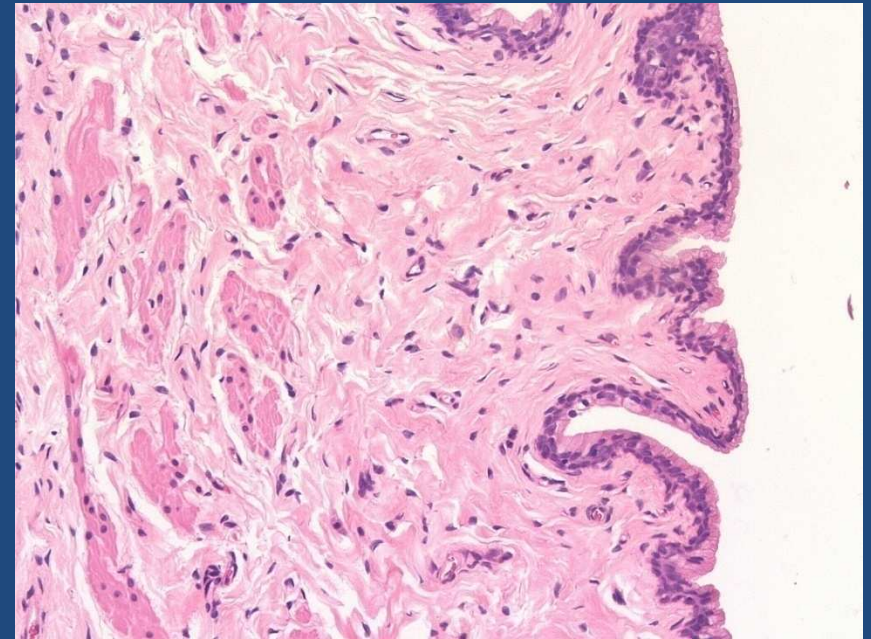


METESTRUS

DIESTRUS

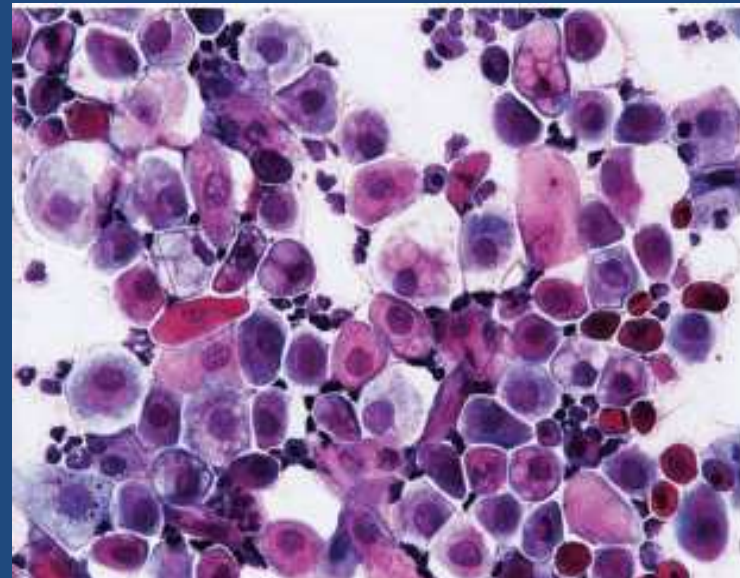
Ovary

- Start defined by epithelium at lowest level with variable leukocyte infiltration
- Subsequent epithelial proliferation and thickening (no clear stratum granulosum) with reduction in leukocyte infiltration.



Vaginal smear- Diestrus

- The vaginal smear is characterized by
 - Little mucus with some leukocytes,
 - Nucleated basophilic cells and
 - Occasional vacuolated cells.



DIESTRUS

Uterus

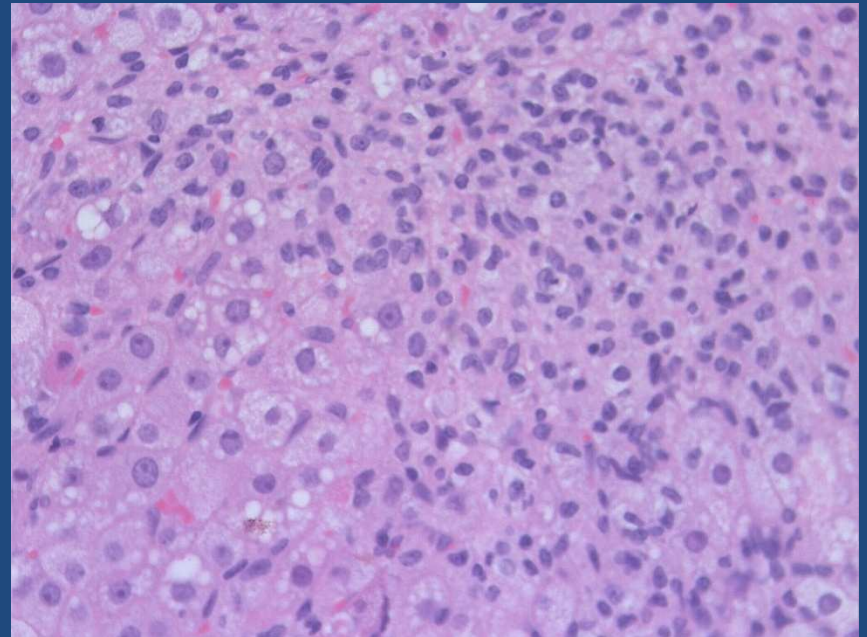
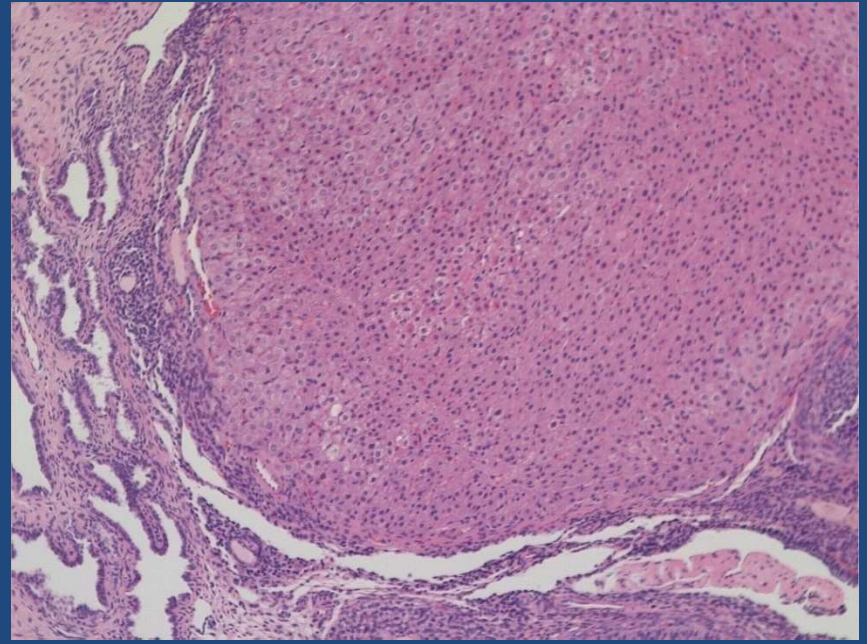
- Small, avascular, slit-like lumen
- Lined by low columnar epithelium. Initially few mitoses, but some increase during phase
- Only occasional degenerate cells
- Stromal edema at end of stage



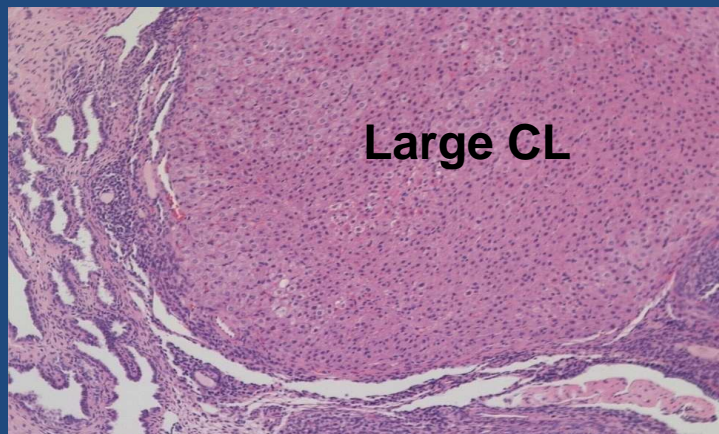
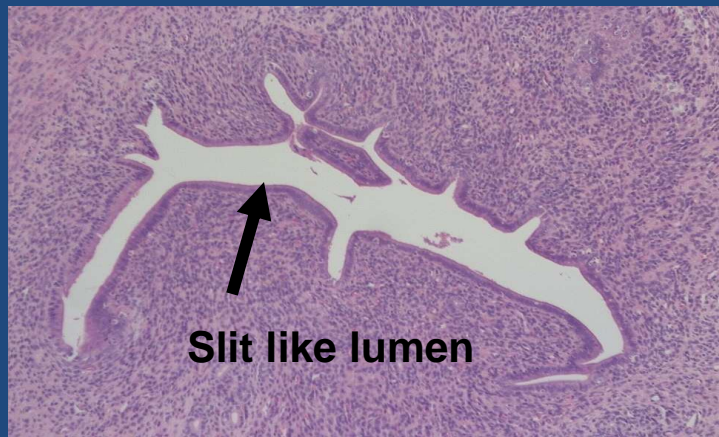
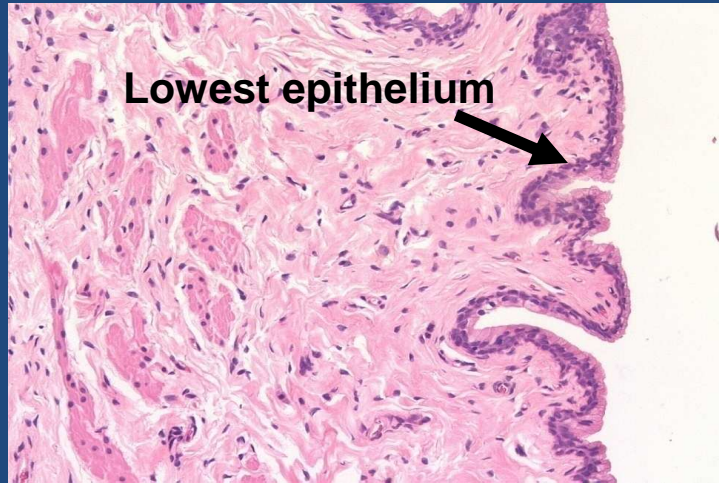
DIESTRUS

Ovary

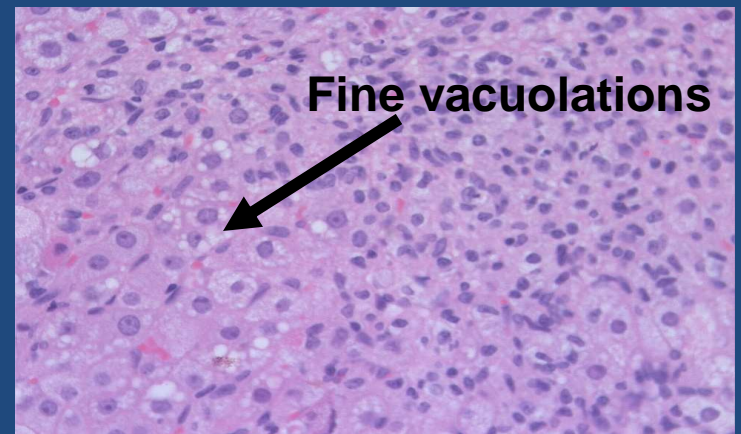
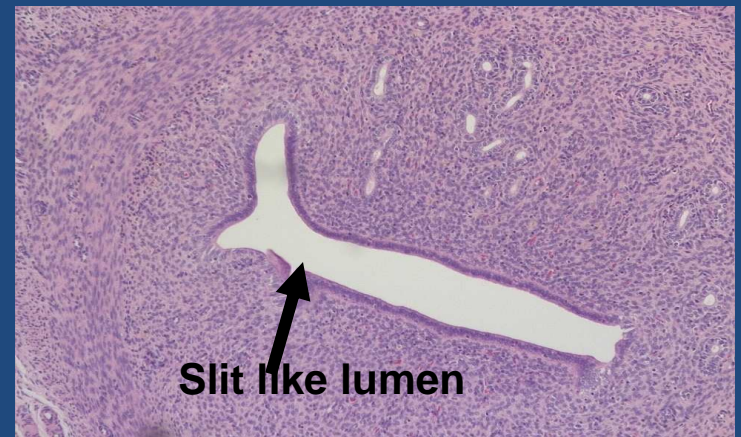
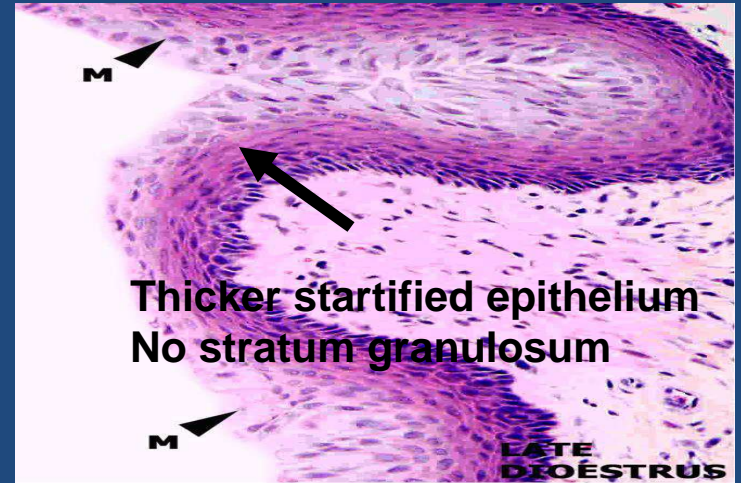
- Large corpora lutea
- May be finely vacuolated
- Fibrous tissue formation in central cavity.



EARLY DIESTRUS



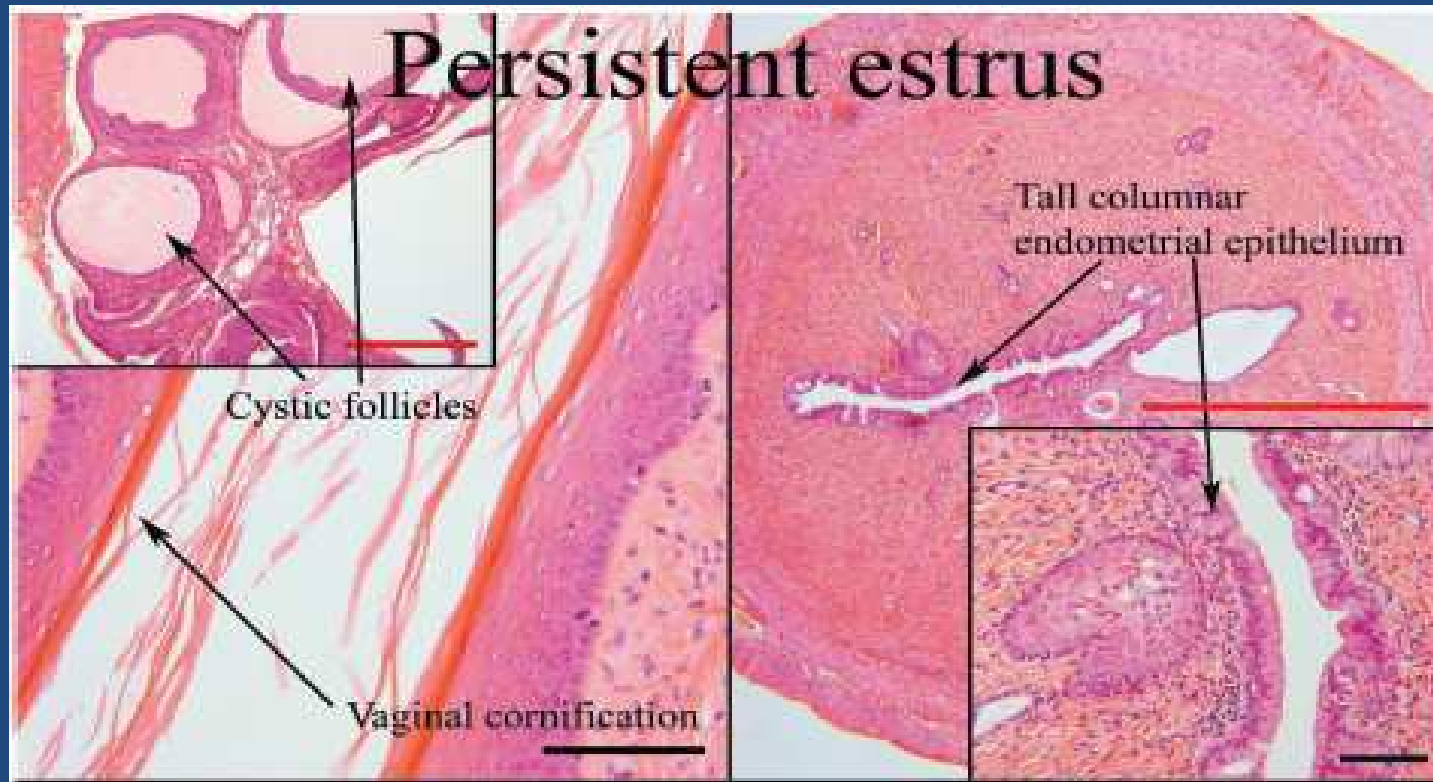
LATE DIESTRUS





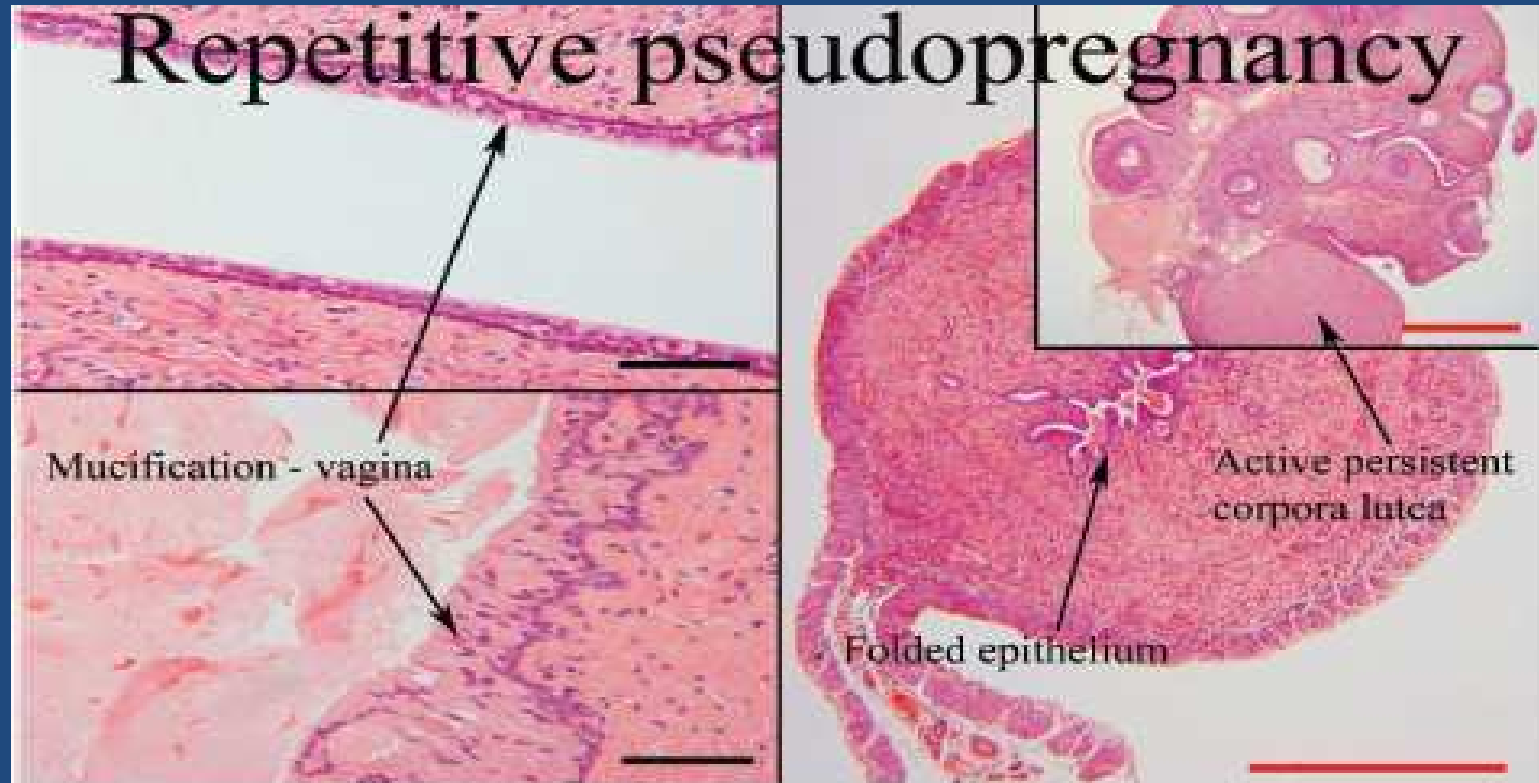
Persistent Estrus

- Most aging females between 6 and 8 months of age exhibit periods of persistent estrus, with constant sexual receptivity
- This state results from **estradiol levels being maintained at a tonic level** reflecting the presence of numbers of ovarian follicular cysts and a tall columnar endometrial epithelium.
- The tonic estrogen secretion stimulates cornification of the vaginal epithelium ---- **Persistent vaginal cornification**
- Low levels of progesterone during this period are considered responsible for the maintenance of this state and are associated with an absence of corpora lutea



Repetitive Pseudopregnancy

- In young adult female rats, whether sterile or fertile, for the first nine or ten days after mating, **prolactin secreted by the pituitary maintains the corpora luteum**
- If mating is unsuccessful, the corpora luteum degenerate by Day 12 or 13.
- States of pseudopregnancy is **characterized by maintenance of corpora lutea beyond the normal two-day life span** and is associated with high progesterone levels
- The vagina during this period shows a low stratum germinativum, but variable overlying mucification.
- The uterus commonly exhibits a folded endometrial epithelium

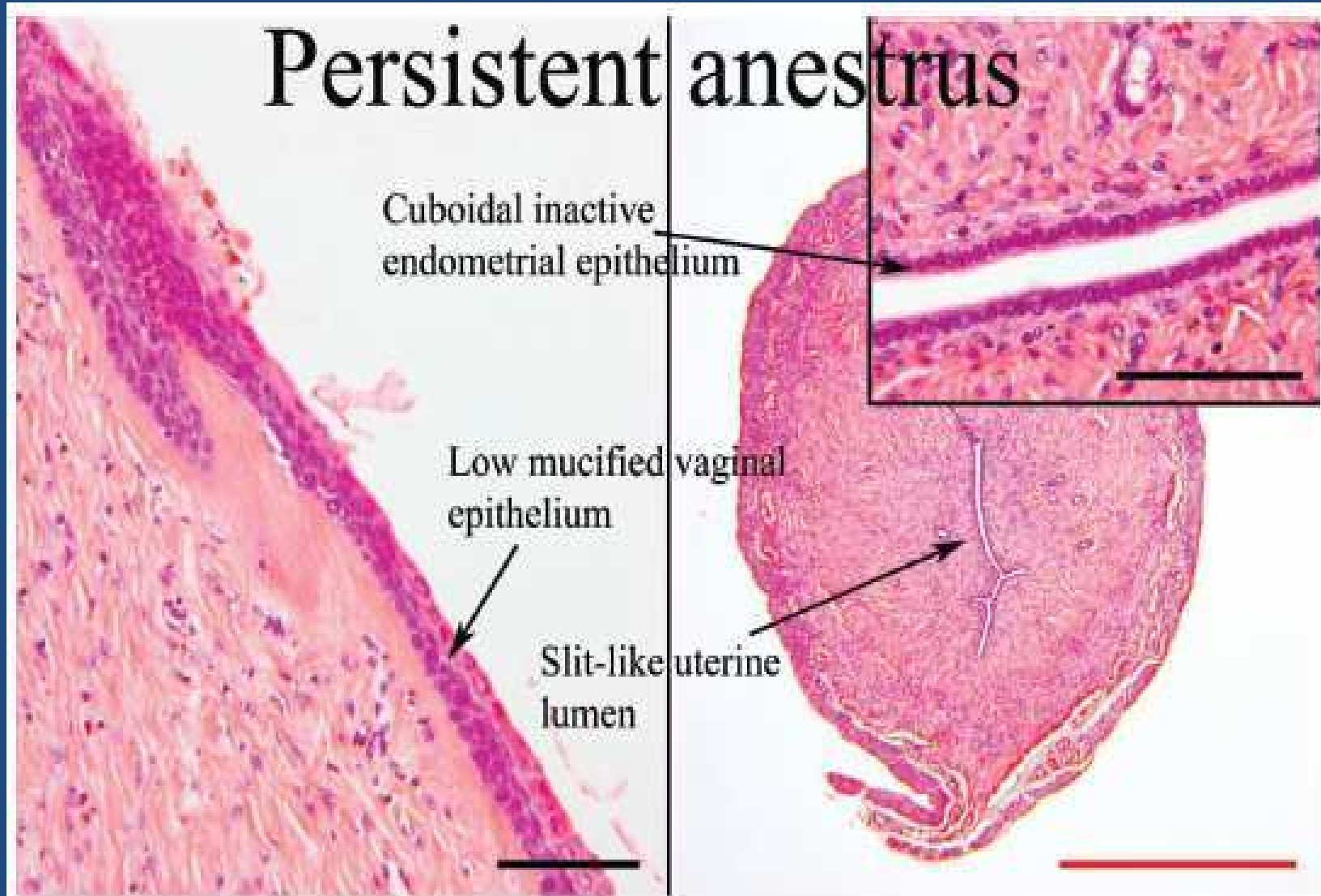


Persistent Anestrus



- Old rats eventually reach a final stage of reproductive senescence
- This is termed persistent anestrus, or persistent diestrus
- After the cessation of ovulation and entry into persistent estrus, there is still some maturation of follicles, followed by atresia without ovulation, culminating in complete follicular depletion
- Morphological features of the reproductive tract are characterized by a low, somewhat mucified vaginal epithelium and a low inactive endometrial epithelium
- There are low progesterone levels, reflecting the absence of corpora lutea

Persistent anestrus



THANK YOU.....