

3. MORPHOLOGICAL CHANGES DURING THE OESTROUS CYCLE

Introduction

3.1 The female reproductive tract is a highly dynamic organ system. It undergoes numerous sequential morphological changes over the course of oestrous cycle, driven by cyclic fluctuations in several reproductive hormones. Knowledge of the normal histological appearance of the reproductive tract at each stage of the oestrous cycle is essential when evaluating female reproductive tissues from TG 407 studies for evidence of endocrine disruption.

3.2 Thorough histopathological assessment and staging of the reproductive tract requires examination of individual organs, followed by an overall assessment of the system. In practice, because the vagina undergoes the most characteristic and consistent 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” (Li and Davies, 2007).

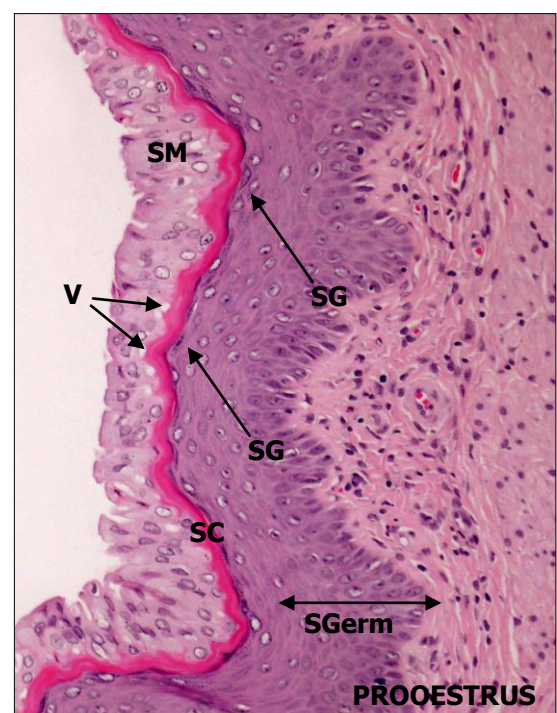
3.3 The histological changes occurring in the vagina do not occur uniformly along its length. Given this, the authors prefer to examine a transverse section of the mid vagina in order to ensure consistency when staging. Sampling from the caudal (posterior) one-third of the vagina should be avoided as the stratified squamous epithelium in this region is permanently keratinised. Care should also be taken not to incorporate the vulva or perineal skin in sections of the vagina (Figure 4.6, Section 4). If a longitudinal section is evaluated, this should bisect the vagina in the horizontal (dorsal) plane.

3.4 To facilitate the assessment of female reproductive tissues from TG 407 studies, the key morphological alterations associated with each stage of the rodent oestrous cycle are illustrated below, both at the organ (Figures 3.1 to 3.3) and system level (Figures 3.4 to 3.7). A summary of these histological changes is provided in Table 3.1.

Organ-specific morphological changes

A. Vagina

Figure 3.1a – Vagina: prooestrus (rat, H&E x20). A well developed stratum mucification (SM), composed of plump epithelial cells containing small mucin-filled vacuoles (V), is present overlying the stratum granulosum (SG) and stratum germinativum (SGerm). During prooestrus, flattening and increased eosinophilia of epithelial cells immediately above the stratum granulosum occurs forming an amorphous, intensely eosinophilic band – the stratum corneum (SC). This layer separates the stratum mucification from the underlying stratum granulosum and stratum germinativum. Only during mid to late prooestrus are all four layers of the vaginal epithelium apparent.



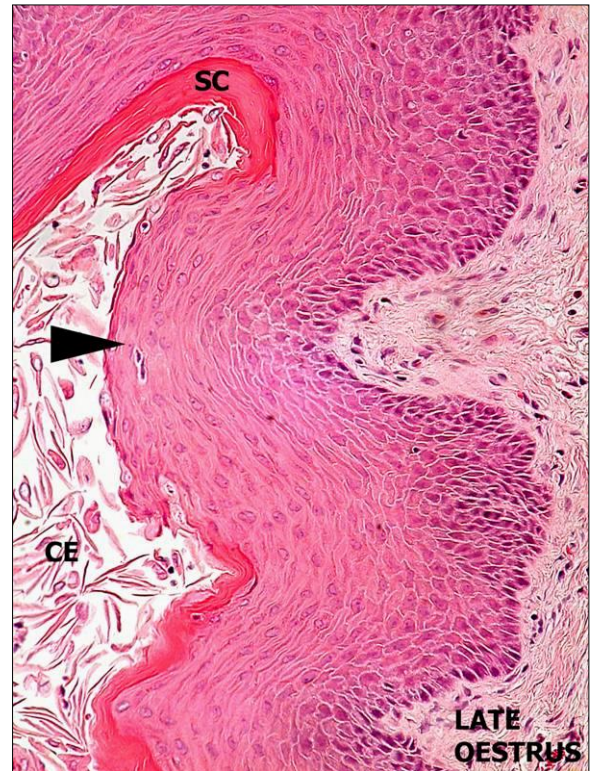
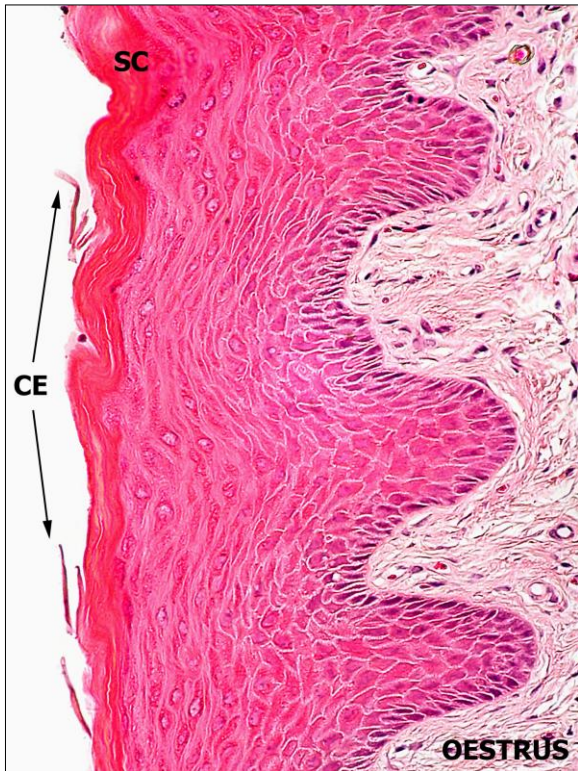


Figure 3.1b – Vagina: oestrus (rat, H&E x20). The stratum mucification has sloughed away to reveal the fully formed stratum corneum (SC). Large, anuclear cornified epithelial cells (CE) are present within the vaginal lumen. As oestrus progresses, dehiscence of the stratum corneum occurs; this exposes the underlying stratum granulosum (arrowhead) and releases large numbers of cornified epithelial cells into the vaginal lumen.

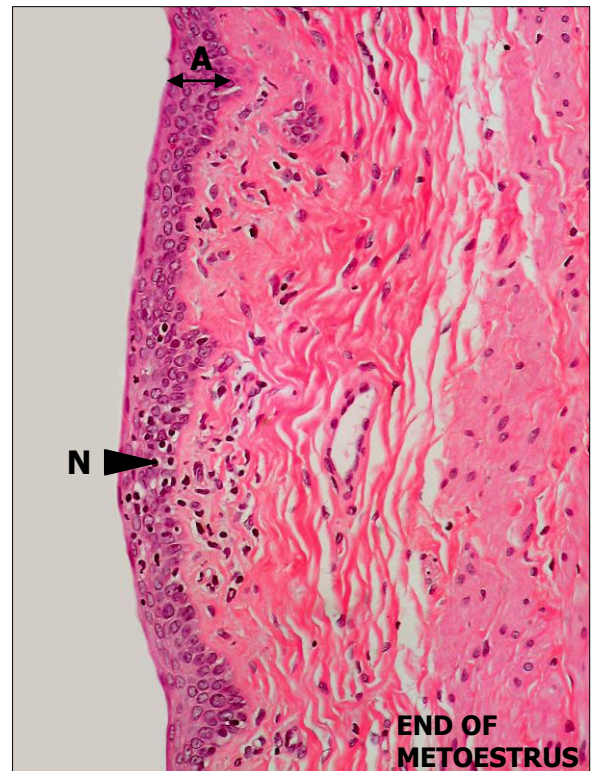
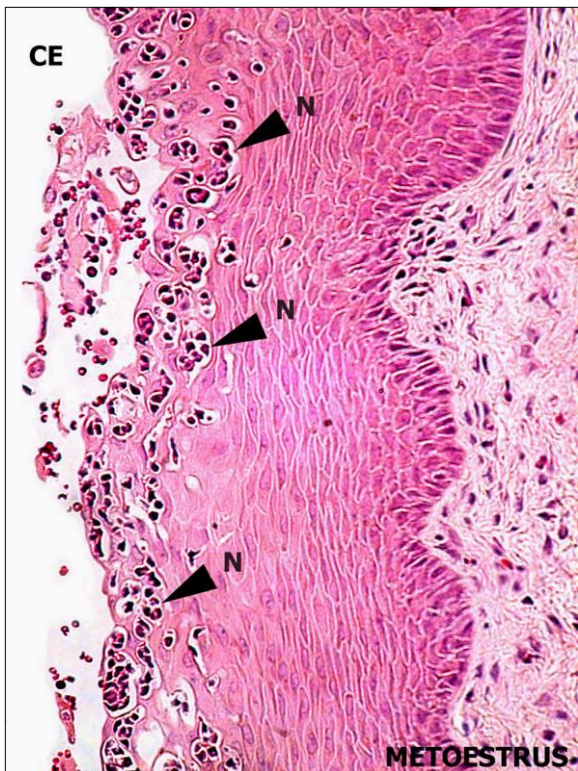


Figure 3.1c – Vagina: metoestrus (rat, H&E x20 and x10). Complete dehiscence of the stratum corneum has occurred and only a small number of cornified epithelial cells (CE) remain in the vaginal lumen. The stratum granulosum is absent – only the stratum germinativum remains. A prominent polymorphonuclear cell infiltration (N) is present in the superficial epithelial cell layers; variable numbers of polymorphonuclear cells are also present within the vaginal lumen. Note the attenuation of the stratum germinativum (A) and reduced numbers of polymorphonuclear cells towards the end of metoestrus.

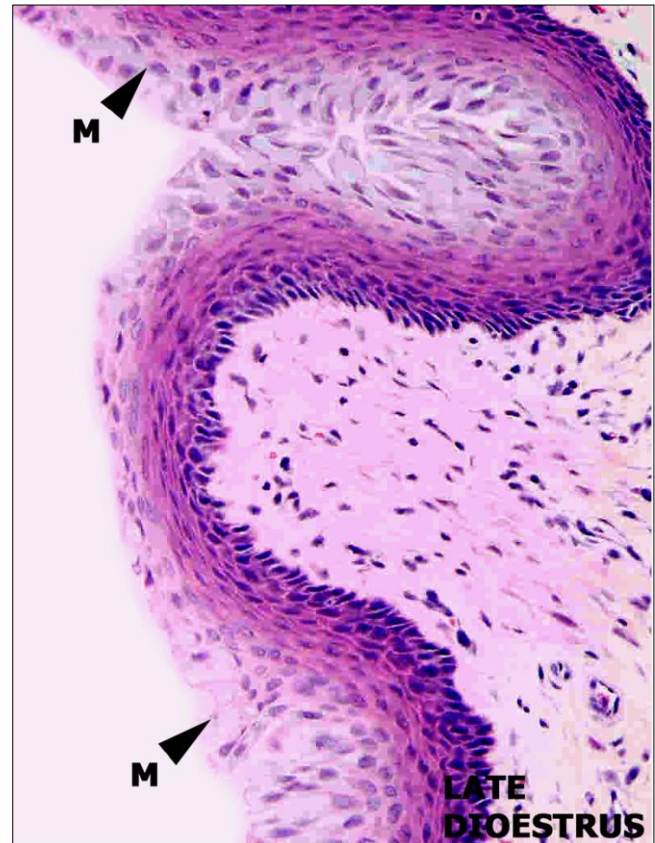
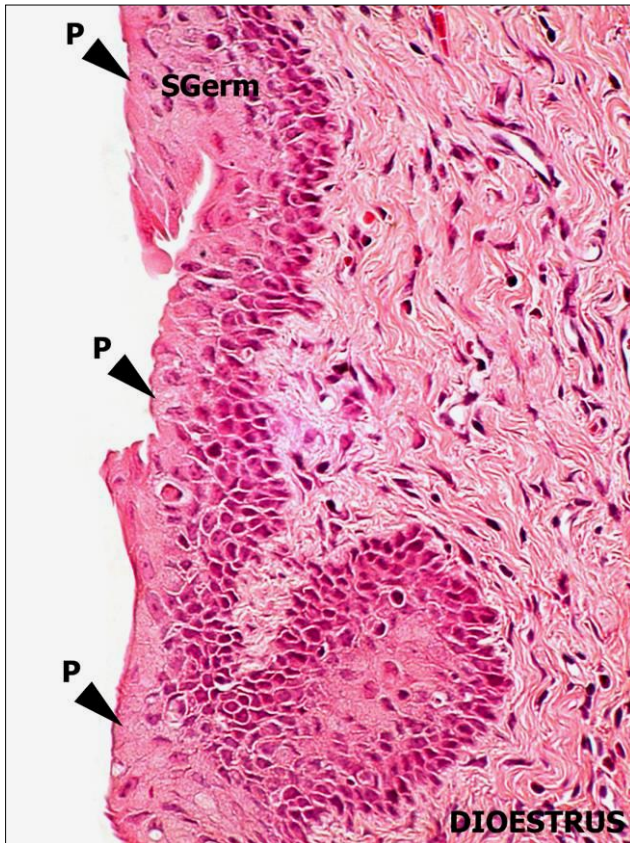


Figure 3.1d – Vagina: dioestrus (rat, H&E x20). The epithelial cells (P) in the superficial layers of the stratum germinativum (SGerm) become polygonal and plump, reflecting early mucification (M). During dioestrus, the attenuated epithelium of metoestrus increases in thickness from approximately 4-6 to 8-10 cell layers.

B. Uterus

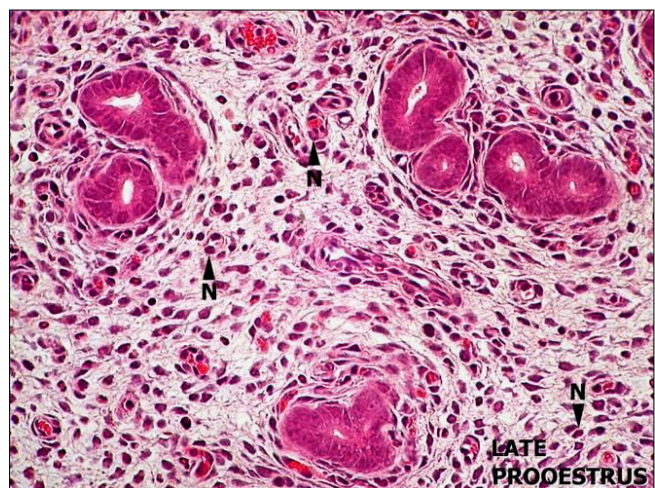
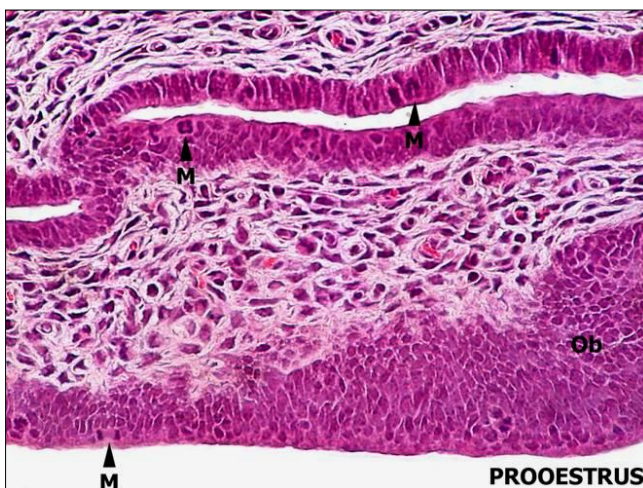


Figure 3.2a – Uterus: prooestrus (rat, H&E x20). The endometrial epithelium is composed of medium-sized, tall columnar cells. Low numbers of mitotic figures (M) may be observed in the luminal uterine epithelium. Polymorphonuclear cells (N) infiltrate the lamina propria, increasing in number during late prooestrus. Obliquely sectioned areas of normal endometrial epithelium (Ob) are frequently seen in histological sections of the uterus. Luminal dilatation is also commonly noted during this stage of the oestrous cycle.

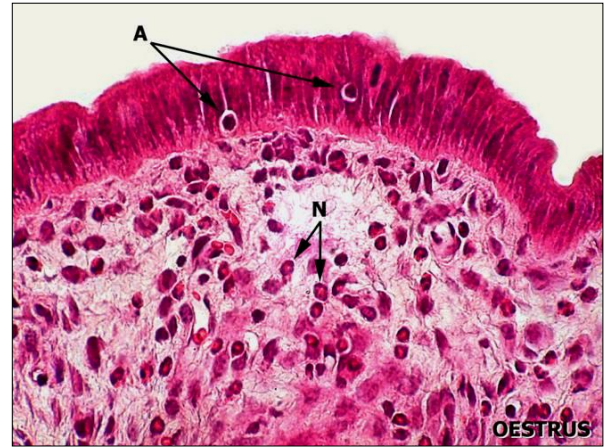


Figure 3.2b – Uterus: oestrus (rat, H&E x20 & x40). The endometrial epithelium is composed of very large, tall columnar cells. Vacuolar degeneration (D) and apoptosis (A) are frequently observed within the luminal and glandular epithelium, but mitotic figures are rare. Large numbers of polymorphonuclear cells (N) infiltrate the lamina propria and endometrial glands.

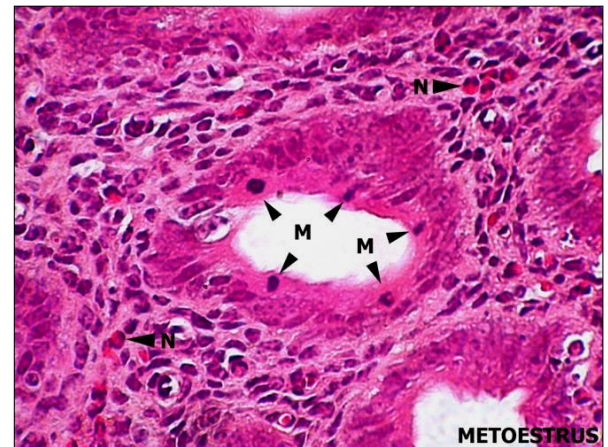
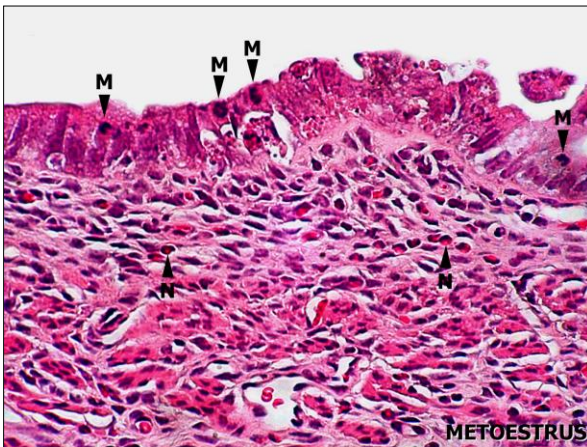


Figure 3.2c – Uterus: metoestrus (rat, H&E x20 & x40). The endometrial epithelium is reduced in height compared with oestrus. The stromal polymorphonuclear cell infiltration (N) and epithelial cell degeneration/apoptosis observed during oestrus persist during this stage. Epithelial regeneration, characterised by increased mitotic activity (M), begins during metoestrus.

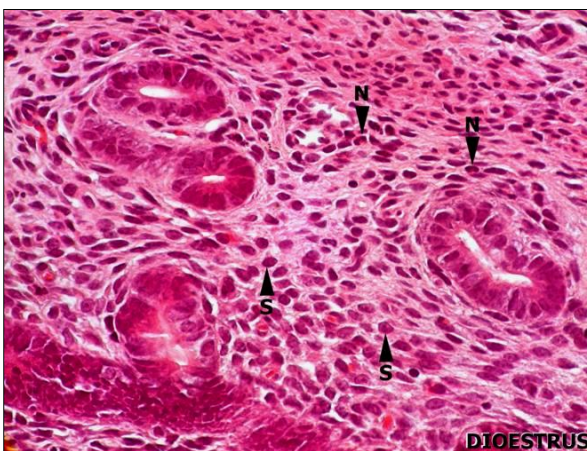


Figure 3.2d – Uterus: dioestrus (rat, H&E x40 & x20). The luminal and glandular epithelium are reduced in height; both are composed of small, low columnar cells. Occasional apoptotic/degenerate epithelial cells may be present and mitotic figures (M) are numerous. Low numbers of polymorphonuclear cells (N) remain within the lamina propria during this stage. Note the plump, round to oval stromal fibroblasts (S), activated by high levels of circulating progesterone.

C. Ovary

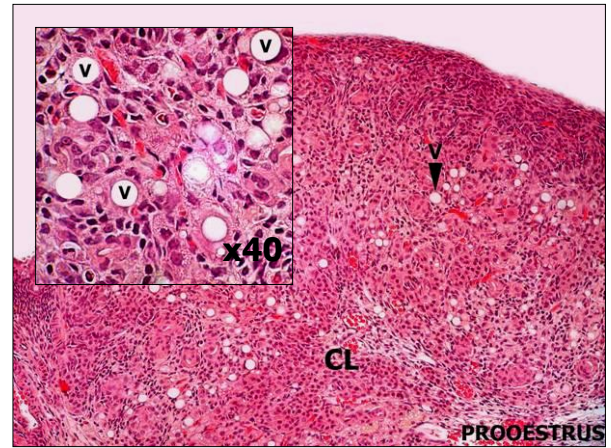


Figure 3.3a – Ovary: prooestrus (rat, H&E x10). Preovulatory (Graafian) follicles (GF) are observed during this stage; the absence of free-floating primary oocytes within the follicular lumina is a sectional artefact. The corpora lutea (CL) of preceding cycles are typically degenerate, characterised by luteal cell vacuolation (V, inset) and varying degrees of fibrous tissue proliferation.

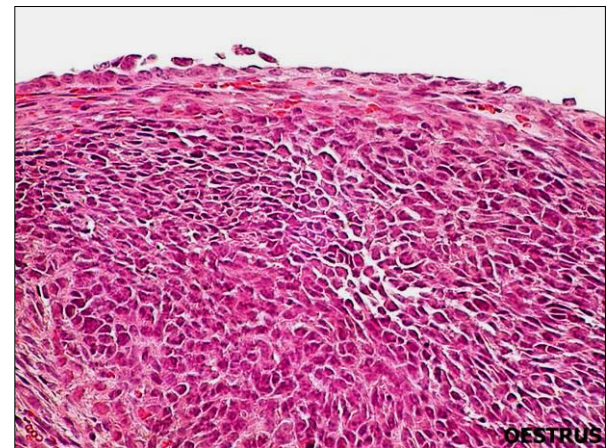


Figure 3.3b – Ovary: oestrus (rat, H&E x10 & x20). Newly formed corpora lutea (CL) are present during oestrus. These early postovulatory follicles are small in size and composed of small basophilic cells that are morphologically similar to the follicular granulosa cells from which they originate.

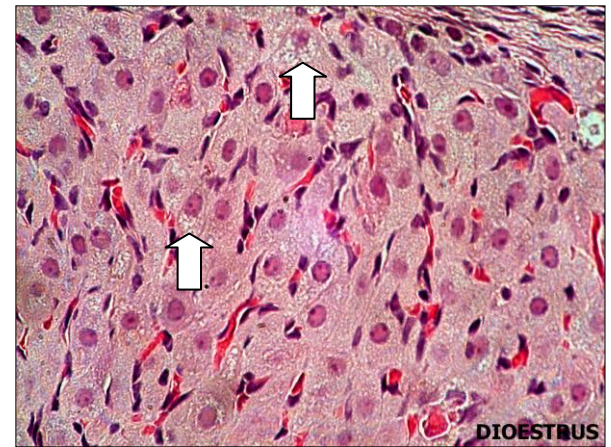
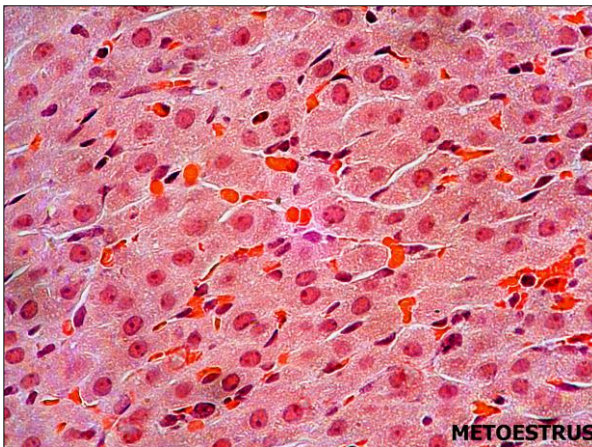


Figure 3.3c – Ovary: metoestrus and dioestrus (rat, H&E x40). In metoestrus, the luteal cells of the recently formed corpora lutea are large and plump, and contain moderate amounts of foamy eosinophilic cytoplasm. Note the absence of cytoplasmic vacuolation and fibrous tissue proliferation. During dioestrus, small cytoplasmic vacuoles (white arrows) may form within the luteal cells. Early fibrous tissue proliferation is often evident during this stage.

Stage-specific reproductive tract morphological changes

Prooestrus

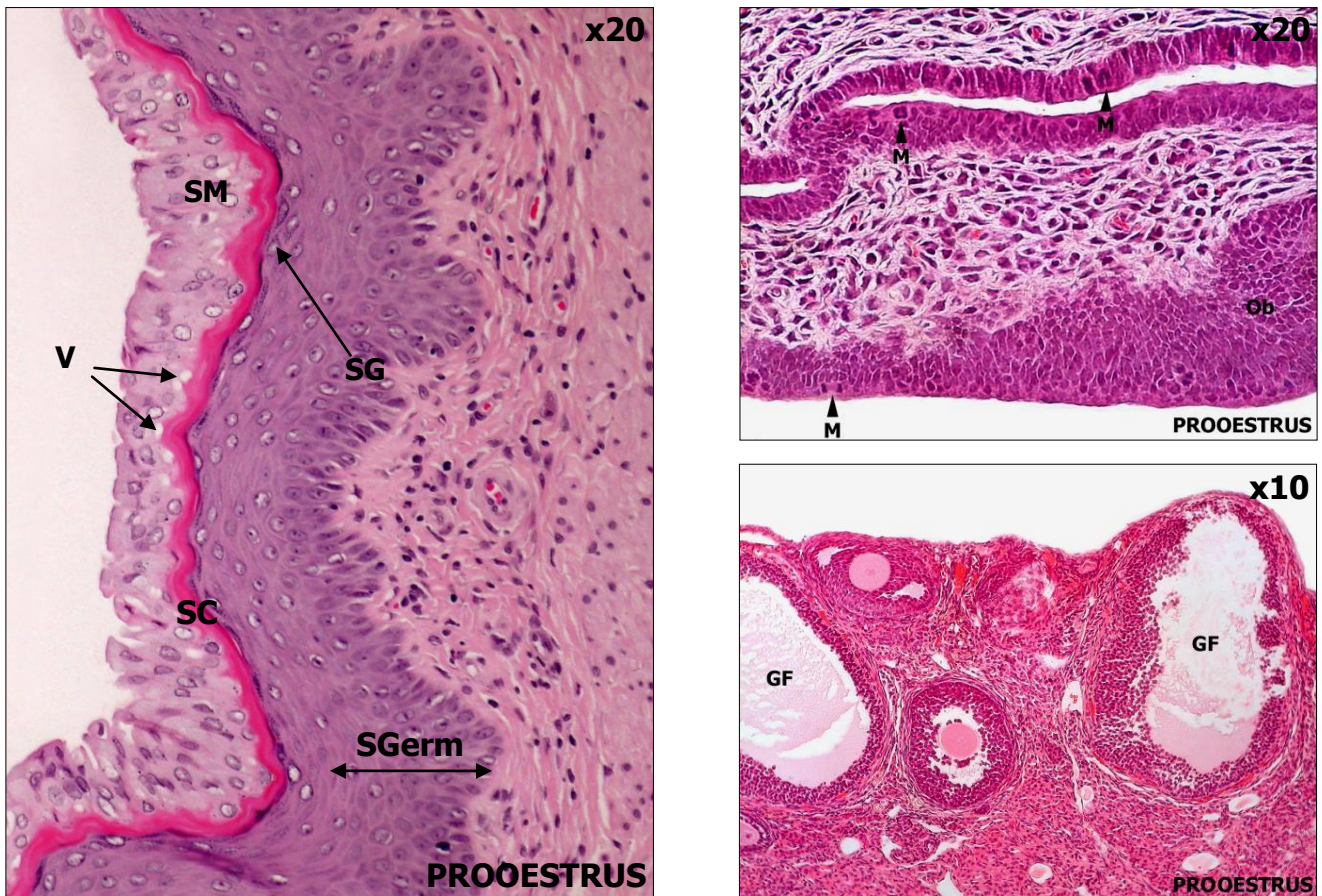


Figure 3.4 – clockwise from left: vagina, uterus and ovary during prooestrus (rat, H&E).

Key histological features – prooestrus

- Stratum mucification and stratum granulosum present throughout prooestrus (vagina)
- Stratum corneum present during mid to late prooestrus (vagina)
- Medium-sized tall columnar endometrial epithelial cells lining dilated lumen (uterus)
- No epithelial degeneration/apoptosis present but mitotic figures observed (uterus)
- Preovulatory (Graafian) follicles (ovary)

SM	stratum mucification
SC	stratum corneum
SG	stratum granulosum
SGerm	stratum germinativum
V	mucin-filled cytoplasmic vacuole
M	mitotic figure
Ob	oblique section through luminal endometrial epithelium
GF	preovulatory (Graafian) follicle

Oestrus

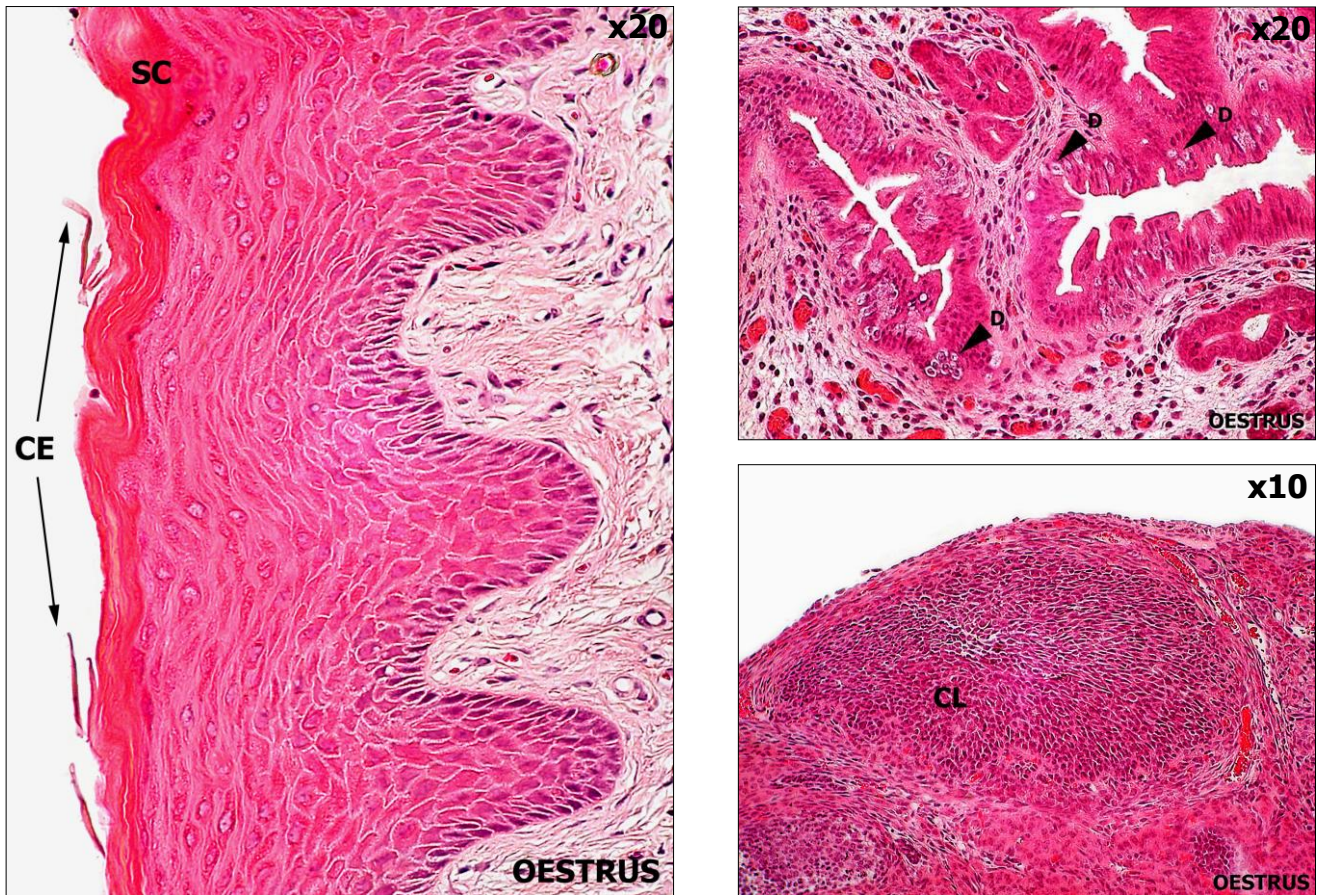


Figure 3.5 – clockwise from left: vagina, uterus and ovary during oestrus (rat, H&E).

Key histological features – oestrus

- Stratum mucification absent (vagina)
- Well developed stratum corneum (vagina)
- Cornified epithelial cells typically present in lumen (vagina)
- Very large, tall columnar endometrial epithelial cells (uterus)
- Marked epithelial degeneration/apoptosis (uterus)
- Immature, basophilic corpora lutea (ovary)

SC	stratum corneum
CE	cornified epithelial cell
D	vacuolar degeneration
CL	immature corpus luteum

Metoestrus

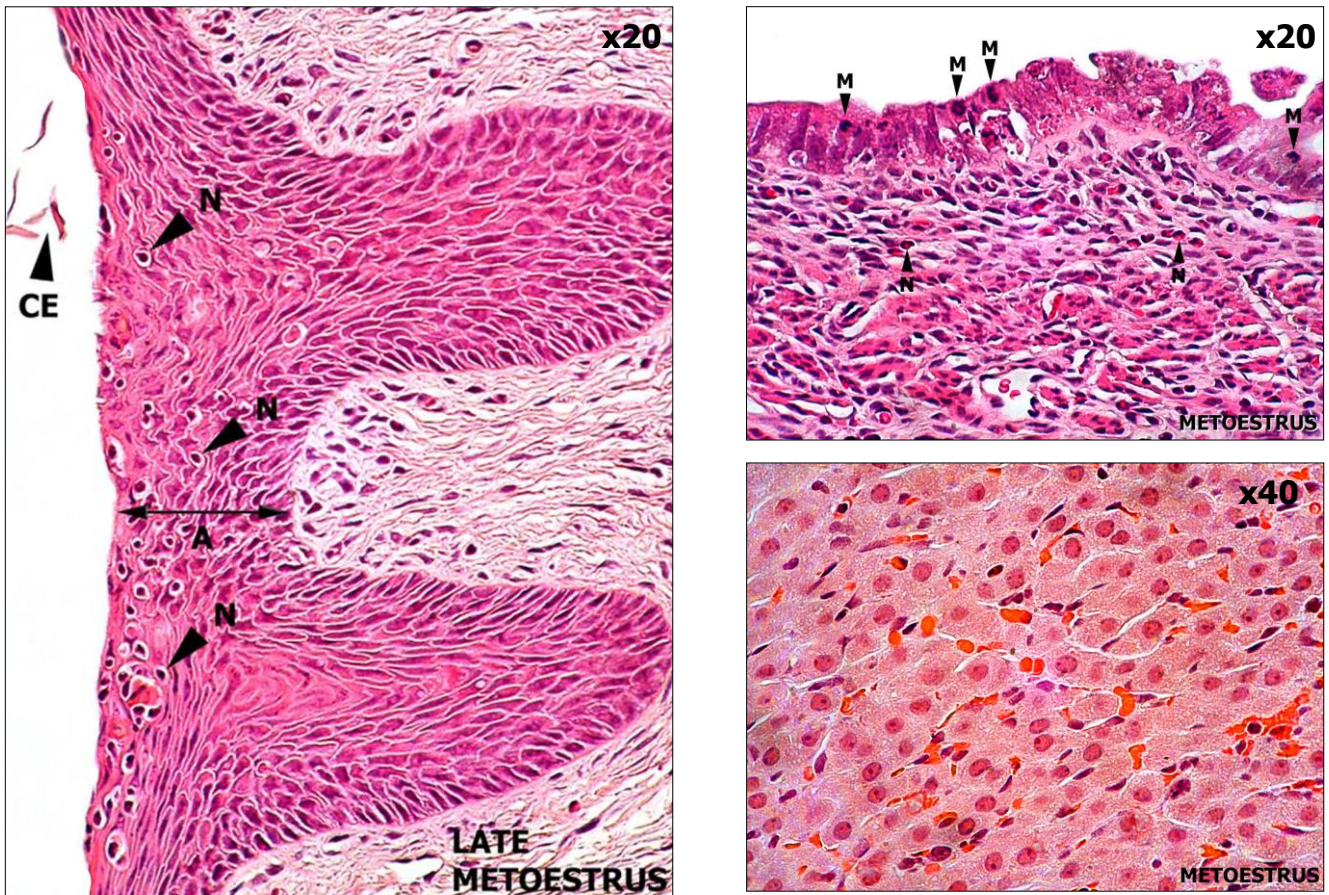


Figure 3.6 – clockwise from left: vagina, uterus and ovary during metoestrus (rat, H&E).

Key histological features – metoestrus

- Stratum mucification and stratum corneum absent (vagina)
- Epithelial polymorphonuclear cell infiltration (vagina)
- Progressive attenuation of stratum germinativum (vagina)
- Large, tall columnar endometrial epithelial cells (uterus)
- Epithelial degeneration/apoptosis and mitotic figures present (uterus)
- No fibrous tissue proliferation within most recently formed corpora lutea (ovary)
- Plump luteal cells with foamy eosinophilic cytoplasm (ovary)

A	attenuated stratum germinativum
CE	cornified epithelial cell
N	polymorphonuclear cell
M	mitotic figure

Dioestrus

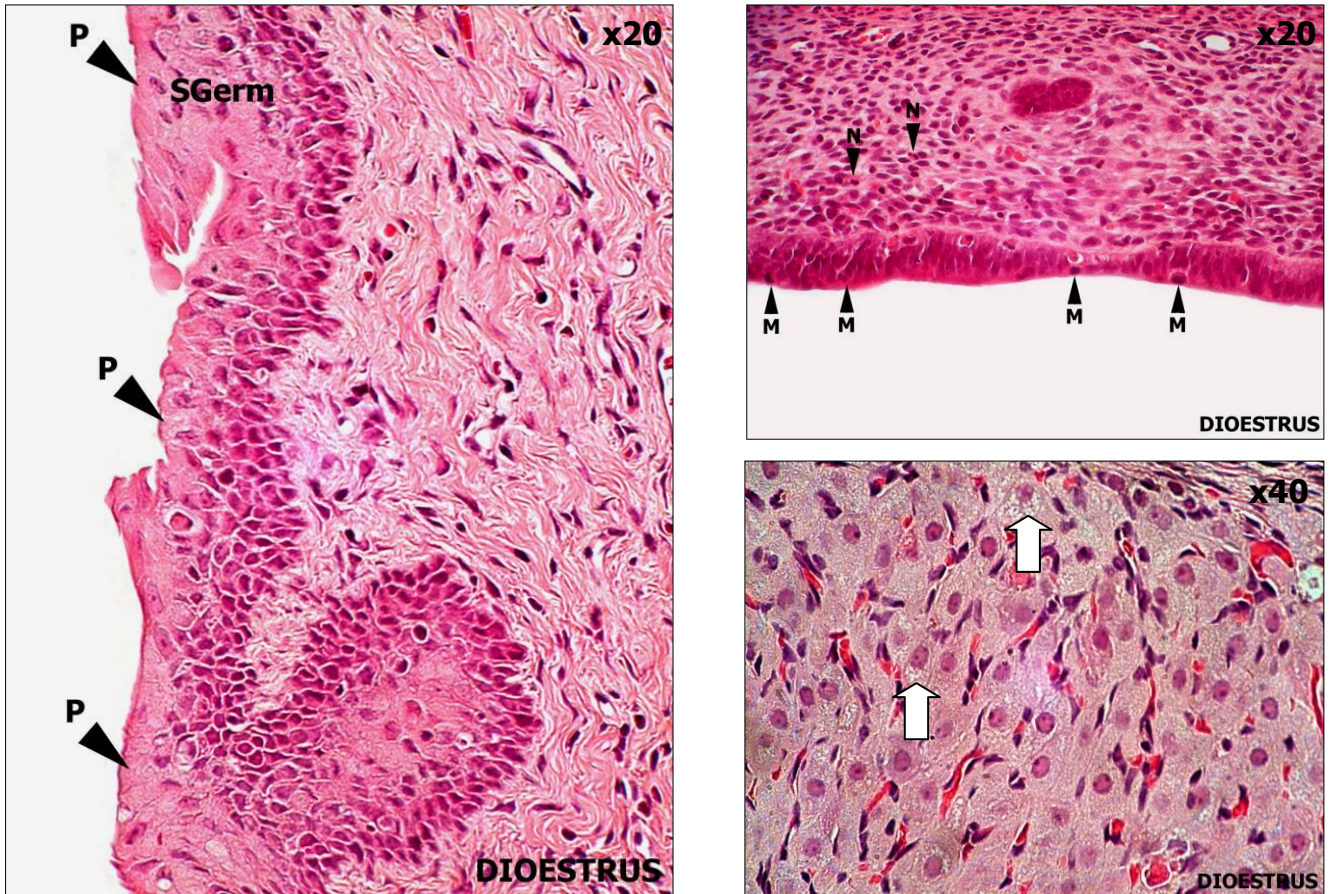


Figure 3.7 – clockwise from left: vagina, uterus and ovary during dioestrus (rat, H&E).

Key histological features – dioestrus

- Stratum mucification, stratum corneum and stratum granulosum absent (vagina)
- Early mucification of stratum germinativum (vagina)
- Small, low columnar endometrial epithelial cells with numerous mitotic figures (uterus)
- Early fibrous tissue proliferation within most recently formed corpora lutea (ovary)

SGerm	stratum germinativum
P	plump superficial epithelial cell (early mucification)
N	neutrophil
M	mitotic figure
↑	early lipid vacuolation

Table 3.1 – Typical histological changes observed in the female rat reproductive tract during the oestrous cycle.

ORGAN	PROOESTRUS	OESTRUS	METOESTRUS	DIOESTRUS
VAGINA	<p>Stratum mucification and stratum granulosum present throughout</p> <p>Stratum corneum present mid to late prooestrus</p> <p>Occasional polymorphonuclear cells may be present in epithelium</p>	<p>Stratum mucification absent</p> <p>Well developed stratum corneum with cornified epithelial cells in lumen</p> <p>Few polymorphonuclear cells present in epithelium at start of oestrus but numbers progressively increase</p>	<p>Stratum mucification, stratum granulosum and stratum corneum all absent; cornified epithelial cells may be present in lumen</p> <p>Attenuation of stratum germinativum to 4-6 cell layers thick marks end of metoestrus</p> <p>Marked epithelial polymorphonuclear cell infiltration at start of metoestrus but progressively decreases</p>	<p>Early mucification of stratum germinativum (plump and polygonal superficial cells)</p> <p>Attenuated stratum germinativum increases in thickness from 4-6 to 8-10 cell layers</p> <p>Few polymorphonuclear cells may remain scattered in epithelium</p>
UTERUS	<p>Medium-sized, tall columnar endometrial epithelial cells lining dilated lumen</p> <p>Vacuolar degeneration/apoptosis absent</p> <p>Frequent epithelial mitotic figures but numbers progressively decrease</p> <p>Minimal polymorphonuclear cell infiltration of lamina propria but progressively increases</p>	<p>Very large, tall columnar endometrial epithelial cells</p> <p>Marked vacuolar degeneration/apoptosis of endometrial epithelium</p> <p>Rare epithelial mitotic figures</p> <p>Marked polymorphonuclear cell infiltration of lamina propria & endometrial glands</p>	<p>Large, tall columnar endometrial epithelial cells</p> <p>Epithelial vacuolar degeneration/apoptosis persists</p> <p>Frequent epithelial mitotic figures</p> <p>Moderate polymorphonuclear cell infiltration of lamina propria & endometrial glands but progressively decreases</p>	<p>Small, low columnar endometrial epithelial cells</p> <p>Scant vacuolar degeneration/apoptosis of endometrial epithelium</p> <p>High numbers of epithelial mitotic figures</p> <p>Minimal polymorphonuclear cell infiltration of lamina propria</p>
OVARY	<p>Preovulatory (Graafian) follicles</p> <p>Degenerate corpora lutea of preceding cycles, characterised by luteal cell vacuolation and varying degrees of fibrous tissue proliferation, are present</p>	<p>Immature, basophilic corpora lutea</p> <p>Central cavity/haemorrhage may be present</p>	<p>Recently formed corpora lutea:</p> <p>Plump luteal cells with foamy eosinophilic cytoplasm</p> <p>No fibrous tissue proliferation</p>	<p>Recently formed corpora lutea:</p> <p>Small lipid vacuoles within plump luteal cells may be present</p> <p>Early fibrous tissue proliferation</p>