PART 3: FEMALE REPRODUCTIVE SYSTEM

CONTENTS

INTRODUCTION

i. The female laboratory rat, like most placental mammals, demonstrates intrinsic reproductive cyclicity, characterised by the regular occurrence of an oestrous cycle. During this cycle numerous well defined and sequential alterations in reproductive tract histology, physiology and cytology occur, initiated and regulated by the hypothalamic-pituitary-ovarian (HPO) axis.

ii. The oestrous cycle consists of four stages: *prooestrus*, *oestrus*, *metoestrus* (or *dioestrus 1*) and *dioestrus* (or *dioestrus 2*). Because rats are *continuously polyoestrous* (i.e., cycle constantly throughout the year) dioestrus is immediately followed by the prooestrus phase of the next cycle. *Anoestrus*, a period of reproductive quiescence between oestrous cycles, is thus not usually observed in healthy, cycling female rats. Oestrous cyclicity only ceases during pseudopregnancy, pregnancy, and lactation, although a fertile postpartum oestrus does occur within 24 hours after birth.

iii. Sexual maturity in female rats usually occurs between 30 and 50 days of age. Kennedy and Mitra (1963) reported the mean age of puberty in female rats, based on the occurrence of vaginal opening (VO) and first oestrus, as 38 days. Recent studies evaluating the rodent pubertal female assay have recorded similar mean VO ages in control rats of 32 and 35 days (Kim *et al*, 2002; Goldman *et al*, 2000).

ix. The first oestrous cycle begins within approximately one week after vaginal opening and recurs regularly every 4 or 5 days for a variable proportion of the animal's lifespan, depending on the strain of rat. Sprague-Dawley rats can demonstrate oestrous cycle abnormalities as early as 4 to 6 months of age which may be detected in subchronic 90 day toxicity studies. In contrast, Fischer 344 rats show far greater reproductive robustness typically cycling normally until 15 to 18 months of age.

x. The length of each stage of the oestrous cycle, as reported by Long and Evans (1922), Astwood (1939), and Mandl (1951) is shown in Figure 1.1 below. Mandl (1951) found that within a given population of normally cycling rats, dioestrus showed the greatest variation in duration and thus exerted the most influence over oestrous cycle length.

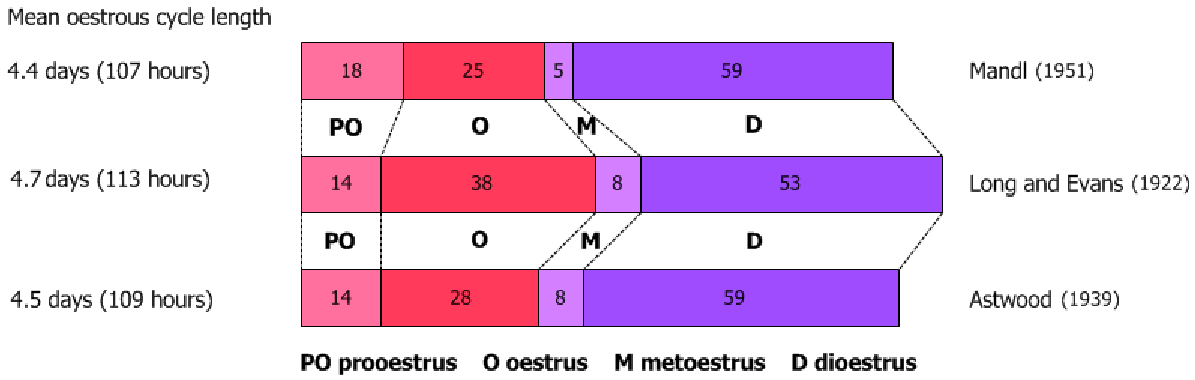

*Figure 1.1 – **Duration (in hours) of the four stages of the oestrous cycle**. Note the short duration of metoestrus.*

1. Normal Female Reproductive Tract Histology

Ovary

1.1 The paired ovaries of the rat are grape-like structures that vary in gross appearance and size, depending on the stage of the oestrous cycle. The subgross anatomy of the rodent ovary is shown in Figure 1.2. Covering its surface is a single layer of modified peritoneal mesothelium, the *ovarian surface epithelium (OSE)*, which is continuous with the broad ligament (mesovarium) that supports the ovary. The OSE of a single ovary can range from squamous to cuboidal, columnar or pseudostratified columnar in type; this regional variation in OSE morphology accompanies the cyclical changes that occur within the underlying ovarian parenchyma during the oestrous cycle (Figures 1.3a and 1.3b).

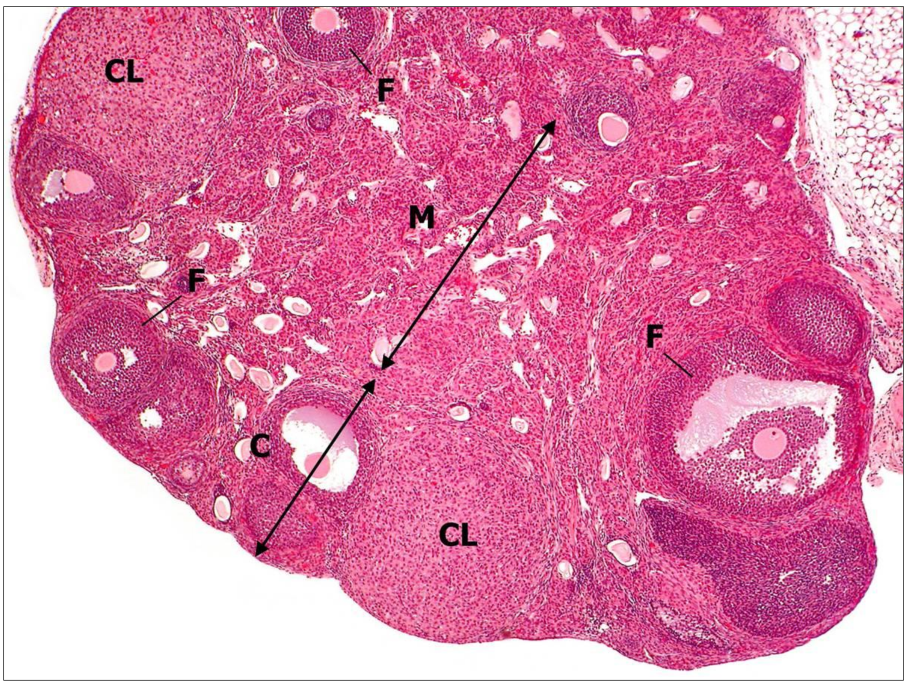

***Figure 1.2 – Subgross anatomy of the normal rodent ovary (mouse, H&E x4)**. The cortex (**C**) contains numerous follicles at various stages of maturation. The medulla (**M**), which is not always present in histological sections, contains lymphatics, nerves and numerous blood vessels.*

C	cortex
M	medulla
CL	corpus luteum
F	developing follicles

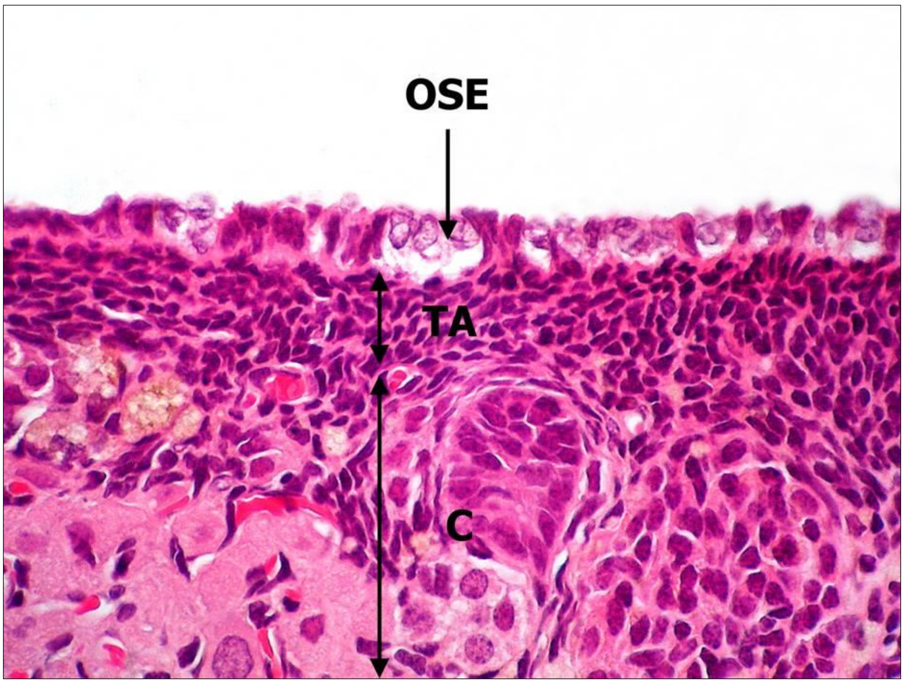

Figure 1.3a – Ovarian surface epithelium (OSE) – columnar type (rat, H&E x40).

OSE	ovarian surface epithelium
TA	tunica albuginea
C	cortex

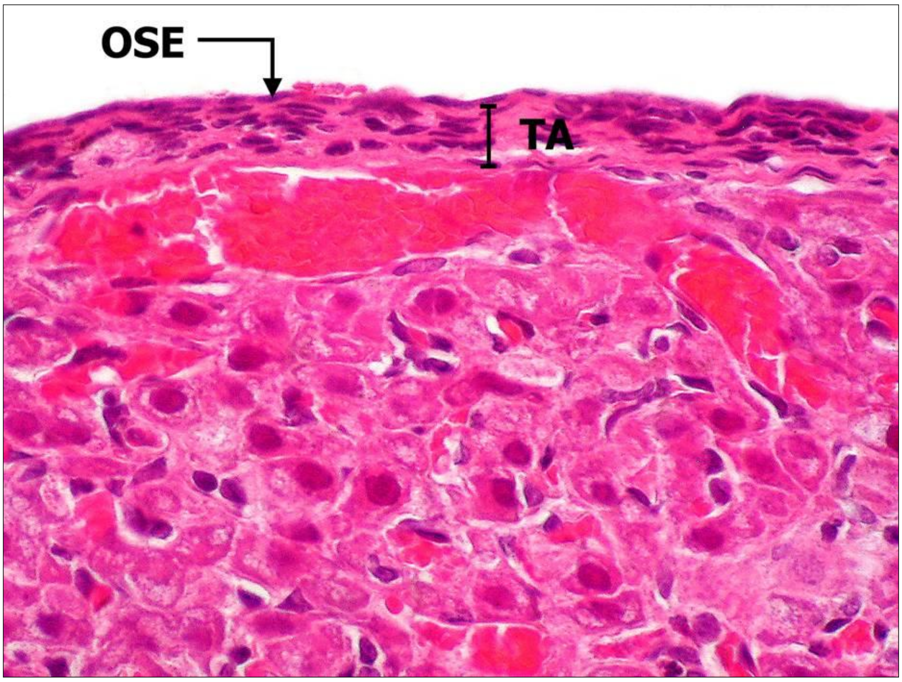

Figure 1.3b – Ovarian surface epithelium (OSE) – simple squamous type (rat, H&E x40).

1.2 The mesovarium completely encloses each ovary and oviduct within a single compartment, the *ovarian bursa* (Figure 1.3c). In rodents the ovarian bursa communicates with the peritoneal cavity via a small slit-like opening.

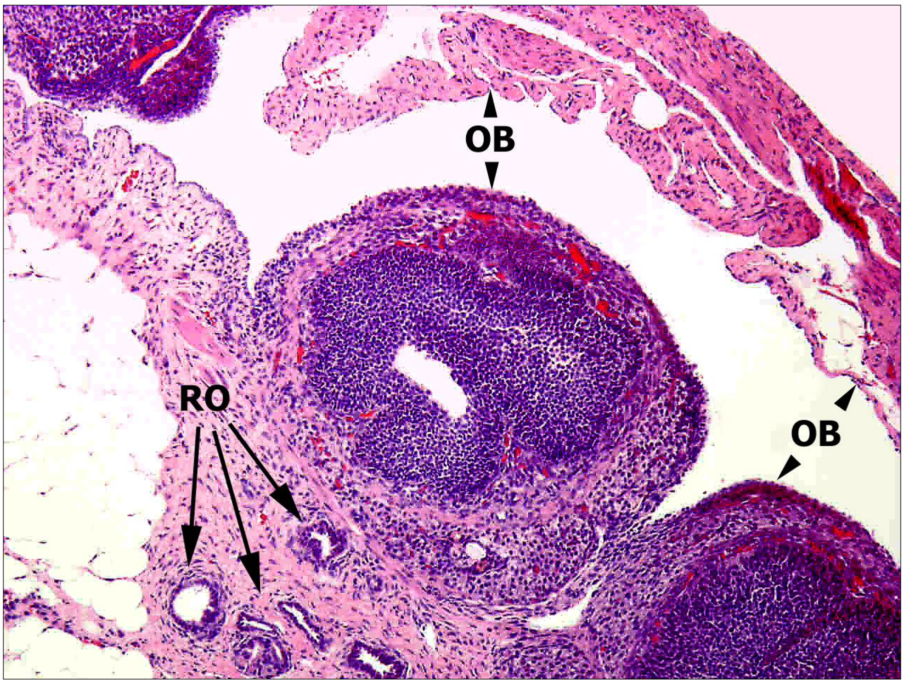

Figure 1.3c – Rete ovarii (RO) and ovarian bursa (OB) (rat, H&E x10).

1.3 The *ovarian stroma* forms the body of the ovary and is composed of spindle-shaped, fibroblast-like cells and delicate collagen fibres admixed with ground substance. The stroma directly beneath the OSE is dense and fibrous – it forms a narrow and variably distinct zone termed the *tunica albuginea.* The ovarian stroma beneath the tunica albuginea is divided into a peripheral cortex and central medulla (Figure 1.2), although the latter is not always visible in histological sections of ovary.

1.4 The *rete ovarii* may be observed histologically within the rodent ovary. This structure arises from cells of mesonephric origin which migrate into the developing gonad during embryogenesis. In the adult rat the rete ovarii is composed of several groups of anastomosing tubules embedded within the ovarian stroma and lined by a cuboidal or columnar epithelium (Figure 1.3c).

1.5 In sexually mature rats the cortex contains numerous follicles at various stages of development. Five stages of follicular maturation (folliculogenesis) are described:

i. ***Primordial follicle*** – This represents the earliest stage of follicular development. Primordial follicles form during early foetal development and are typically located within the peripheral cortex, just beneath the tunica albuginea. Each primordial follicle consists of a primary oocyte surrounded by a simple squamous follicular epithelium. Envelopment of the primary oocyte by follicular cells arrests development of the germ cell at the first meiotic division. During each oestrous cycle a cohort of "resting" primordial follicles starts to develop into primary follicles; this process occurs occurs independently of hormonal stimulation up until the formation of early tertiary follicles.

ii. ***Primary follicle*** **–** The squamous follicular cells surrounding the primordial follicle differentiate into a single layer of columnar cells, resulting in the formation of a primary follicle (Figures 1.4 and 1.5).

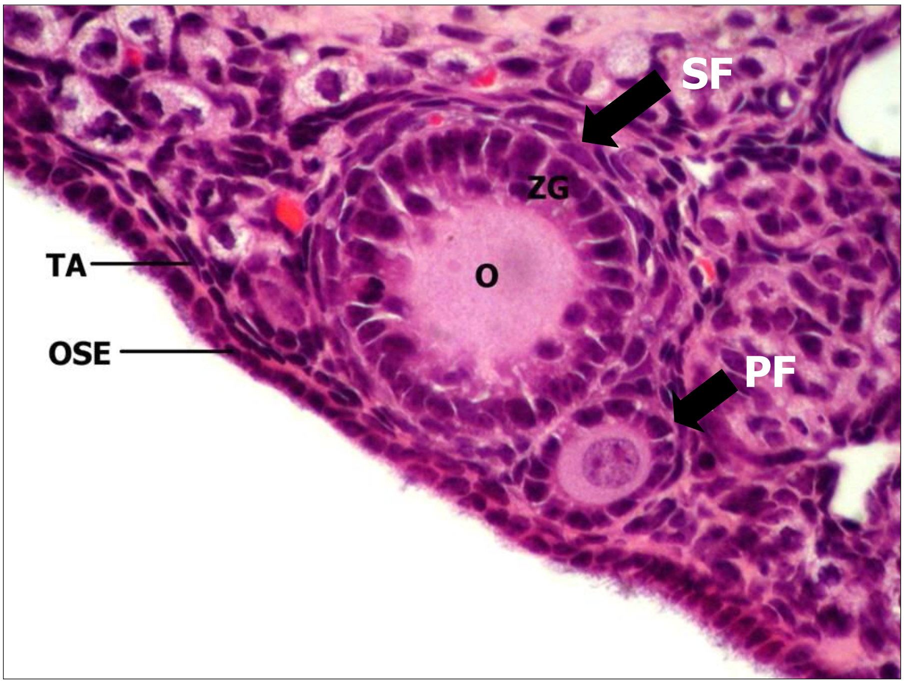

Figure 1.4 – Primary (PF) and early secondary (SF) follicle (rat, H&E x40).

O	primary oocyte	**TF**	theca folliculi
ZG	zona granulosa	**TA**	tunica albuginea
OSE	ovarian surface epithelium		

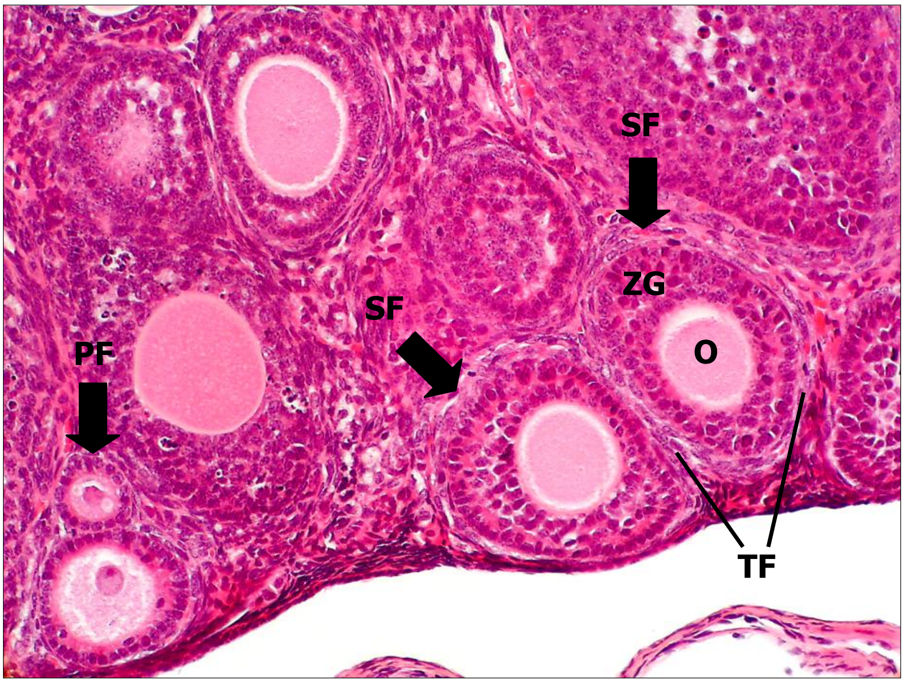

Figure 1.5 – Primary (PF) and secondary (SF) follicles (mouse, H&E x20).

iii. ***Secondary follicle*** – Proliferation of the columnar cell monolayer results in the formation of a multilayered zone of granulosa cells, the *zona granulosa*, around the oocyte (Figures 1.4 and 1.5). This is accompanied by the development of a thick glycoprotein and acid proteoglycan coat, the *zona pellucida*, between the oocyte and the zona granulosa (Figures 1.8 and 1.12). As the secondary follicle continues to grow, multiple fluid-filled spaces form within the zona granulosa – this stage is termed a *vesicular follicle*. Ovarian stromal cells surrounding the developing follicle become arranged into concentric layers and form the *theca folliculi*, or *theca* (Figures 1.5 and 1.6). This layer is separated from the zona granulosa by a basement membrane.

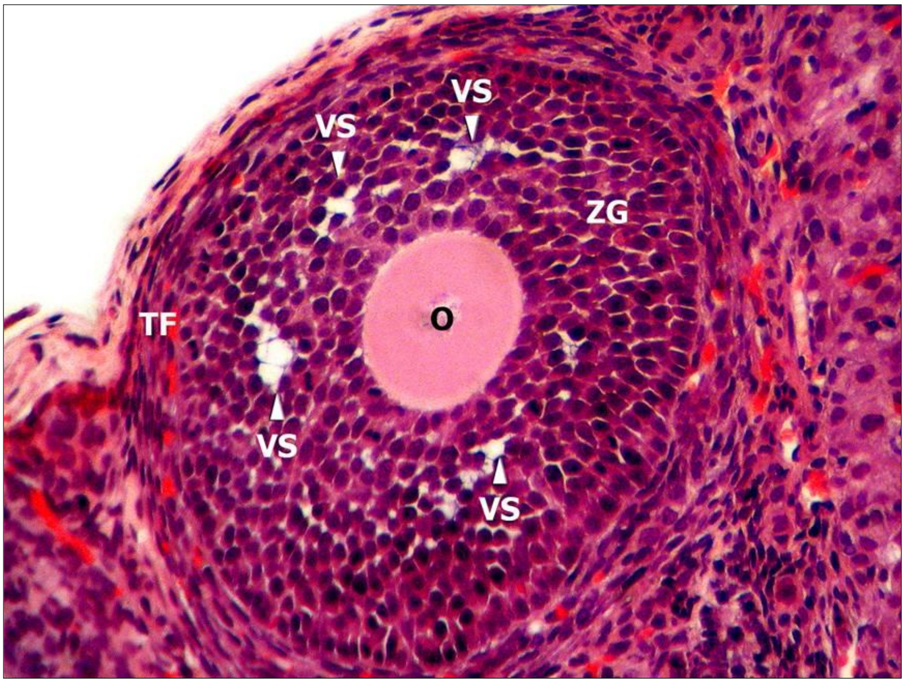

***Figure 1.6 – Vesicular follicle (mouse, H&E x20)**. Vesicular spaces (**VS**) are clearly visible within the zona granulosa (**ZG**).*

O	primary oocyte	**ZG**	zona granulosa
TF	theca folliculi	**VS**	vesicular spaces

iv. ***Tertiary follicle*** **–** The cystic spaces within the zona granulosa coalesce and form a large central cavity, the *follicular antrum*. This cavity is filled with fluid, the *liquor folliculi*, and surrounded by the zona granulosa. The primary oocyte is eccentrically positioned within the tertiary follicle and resides within a mound of granulosa cells, called the *cumulus oophorus*, that protrudes into the antrum. The granulosa cells immediately surrounding the oocyte are termed the *corona radiata* (Figure 1.7).

The theca of the tertiary follicle is divisible into two zones: a *theca interna* and *theca externa*. The theca interna consists of polygonal cells with vacuolated cytoplasm and open faced, vesicular nuclei. These cells demonstrate the typical ultrastructural characteristics of steroid producing cells (e.g. numerous cytoplasmic lipid droplets, large numbers of mitochondria, and an extensive smooth endoplasmic reticulum), and are the main site of synthesis of androstenedione (a sex steroid intermediate). In contrast, the cells of the theca externa are spindle-shaped and merge with the surrounding ovarian stroma; they serve no endocrine function.

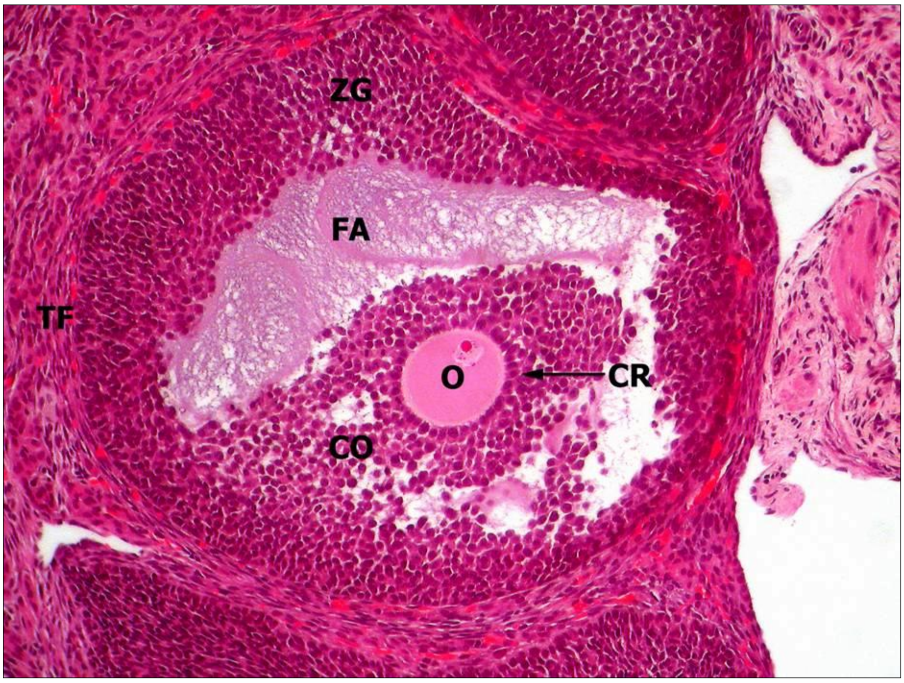

__Figure 1.7 – Tertiary follicle (rat, H&E x20)__. The large follicular antrum (__FA__) and eccentrically positioned primary oocyte (__O__) characterise this stage.

O	primary oocyte	**CO**	cumulus oophorus
CR	corona radiata	**FA**	follicular antrum
ZG	zona granulosa	**TF**	theca folliculi

v. ***Preovulatory (Graafian) follicle*** – A small number of tertiary follicles enter a preovulatory stage and undergo further morphological changes. The follicular antrum continues to enlarge causing attenuation of the surrounding zona granulosa. Degeneration of the granulosa cells of the cumulus oophorus occurs; this causes the primary oocyte to detach from the zona granulosa and float freely within the follicular antrum (Figure 1.8). The primary oocyte completes the first meiotic division just prior to ovulation and forms the secondary oocyte.

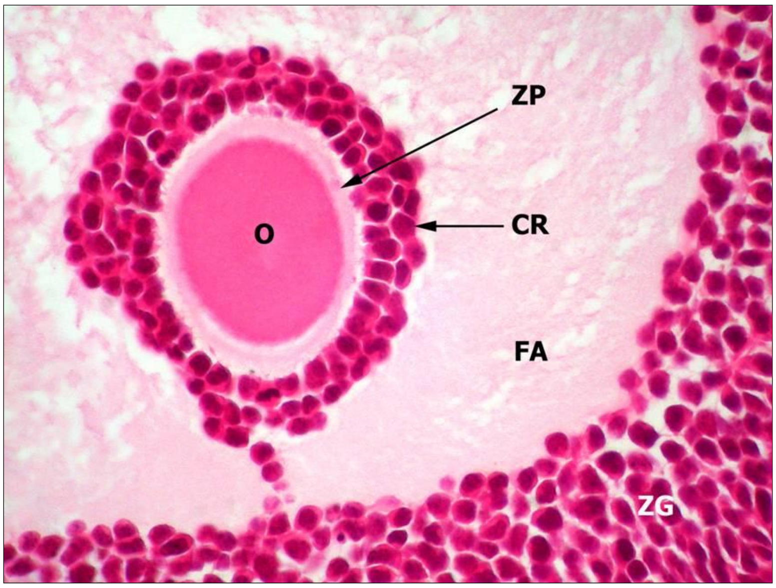

__Figure 1.8 – Preovulatory (Graafian) follicle (rat, H&E x40)__. The primary oocyte (__O__) floats freely within the follicular antrum (__FA__).

O	primary oocyte
ZP	zona pellucida
CR	corona radiata
FA	follicular antrum
ZG	zona granulosa

1.6 Following extrusion of the secondary oocyte from the Graafian follicle, the granulosa and thecal cells of the follicle remnant undergo hypertrophy and, to a lesser extent, hyperplasia (Figure 1.9). This process, termed *luteinisation*, occurs under the influence of luteinising hormone (LH) and prolactin, the two major luteotrophic hormones in rodents. Luteinisation is accompanied by degeneration of the basement membrane separating the theca interna and zona granulosa, and infiltration of the postovulatory follicle by blood vessels from the theca interna. The resulting mature *corpus luteum* ("yellow body") is a large eosinophilic structure that may bulge out from the ovarian surface or obscure the ovarian corticomedullary junction, depending on its location (Figure 1.10). Note that prior to luteinisation immature corpora lutea typically appear more basophilic (Figure 3.3b, Section 3).

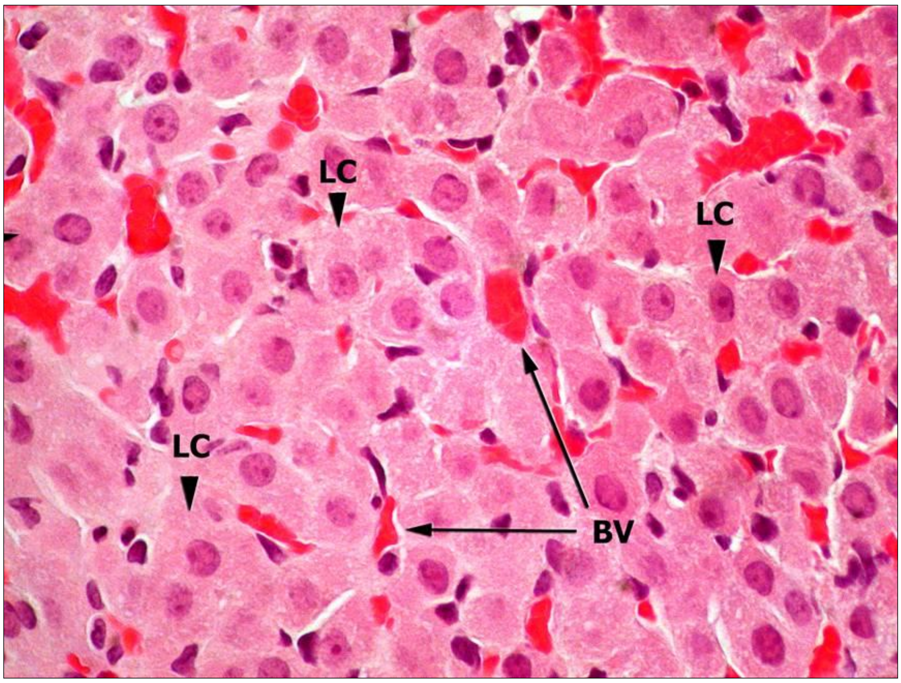

***Figure 1.9 – Corpus luteum (rat, H&E x40)**. The luteal cells (**LC**) comprising the corpus luteum are plump and polygonal; they contain large nuclei and moderate amounts of eosinophilic cytoplasm. Cytoplasmic vacuoles form within luteal cells as the corpus luteum matures and subsequently degenerates. Numerous blood vessels (**BV**) are present, consistent with this structure's function as a temporary endocrine gland.*

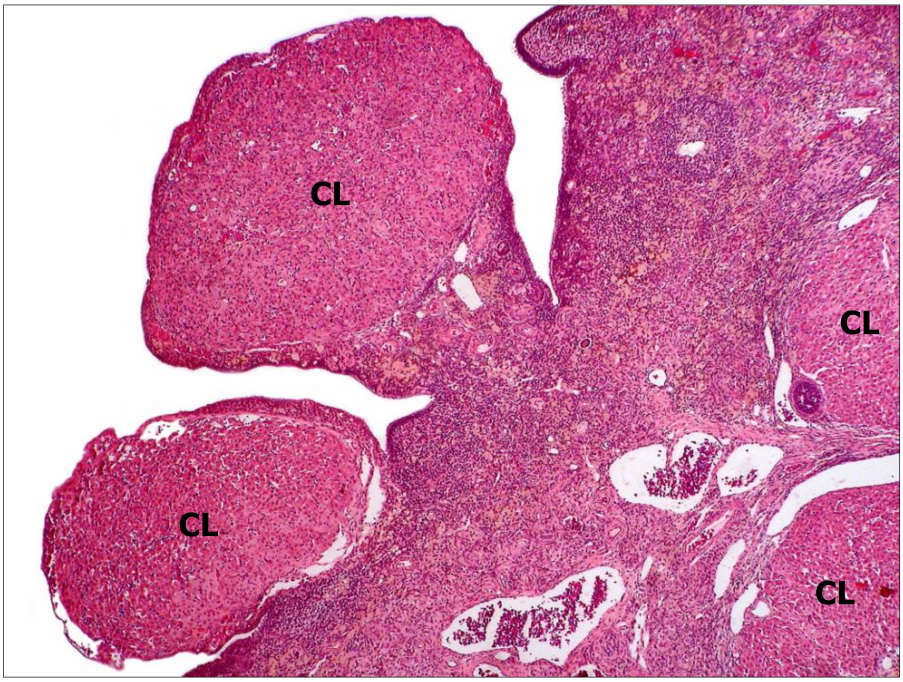

***Figure 1.10 – Corpora lutea (CL) (rat, H&E x10)**. Note the marked protrusion of these large postovulatory follicles beyond the surface of the ovary. Another pair of corpora lutea are present within the body of the ovary.*

1.7 Each corpus luteum matures during the oestrous cycle in which it is formed before regressing over the course of several subsequent cycles. Consequently, at least three sets of corpora lutea are present within the ovaries of normally cycling rats. Degenerating corpora lutea progressively shrink in size and are characterised by increased amounts of fibrous tissue and yellow-brown lipofuscin pigment (Figure 1.11). The fibrous tissue mass that constitutes the corpus luteum during the final stages of regression is termed the *corpus albicans* ("white body"). In the rat this structure undergoes complete regression and leaves no fibrous tissue remnant within the ovary.

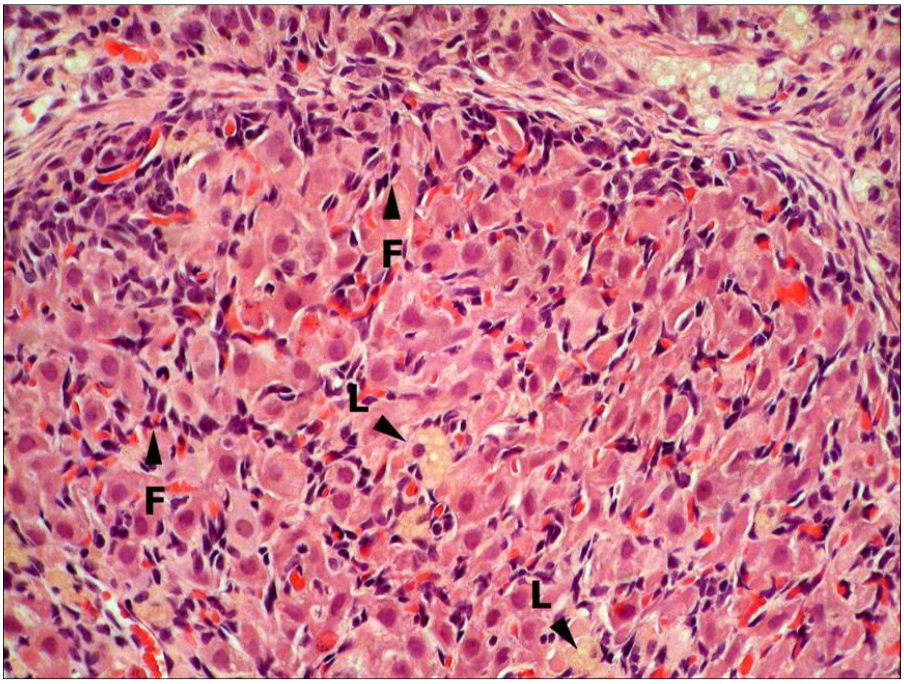

Figure 1.11 – Degenerating corpus luteum (rat, H&E x20)*. Note the invading fibroblasts (****F****) and yellow-brown pigment (lipofuscin) (****L****).*

1.8 Only a small number of primordial follicles within the cohort progress through folliculogenesis to form Graafian follicles and ovulate. The remainder undergo follicular degeneration, or atresia, at various stages during follicular maturation (Figures 1.12 to 1.14).

1.9 In rodents degenerating tertiary follicles eventually give rise to *interstitial glands* within the ovarian stroma. These glands are composed of aggregates of theca interna cells, often arranged around remnants of degenerate zonae pellucida (Figure 1.15). They are transitory structures, eventually breaking up into small clusters of interstitial cells that become scattered throughout the medulla.

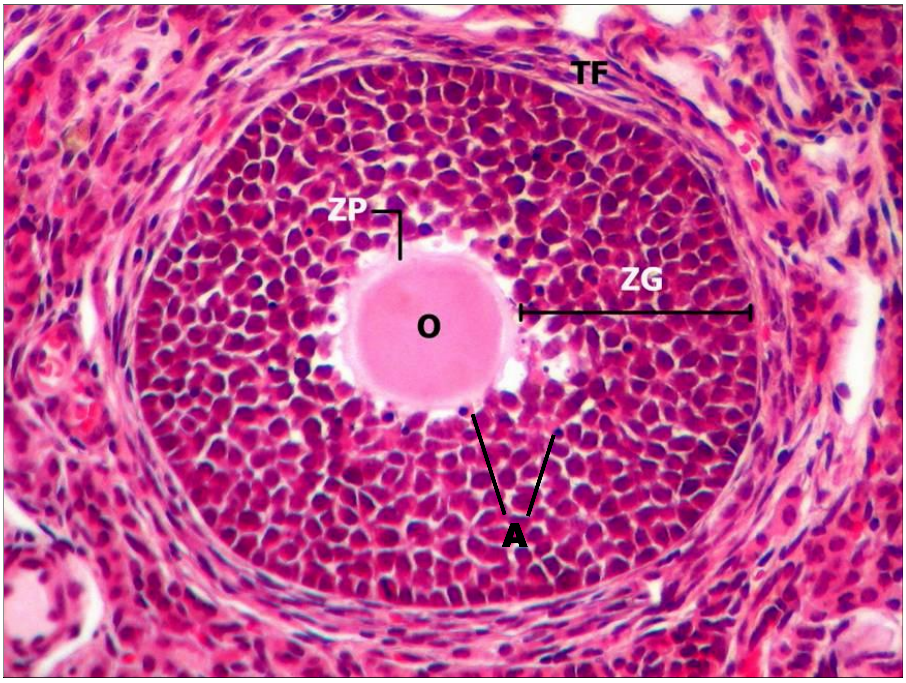

***Figure 1.12 – Atretic secondary follicle (mouse, H&E x20)**. Apoptosis (**A**) is visible in the zona granulosa (**ZG**) of this degenerating secondary follicle.*

O	primary oocyte	**ZG**	zona granulosa
ZP	zona pellucida	**TF**	theca folliculi
VS	vesicular spaces		

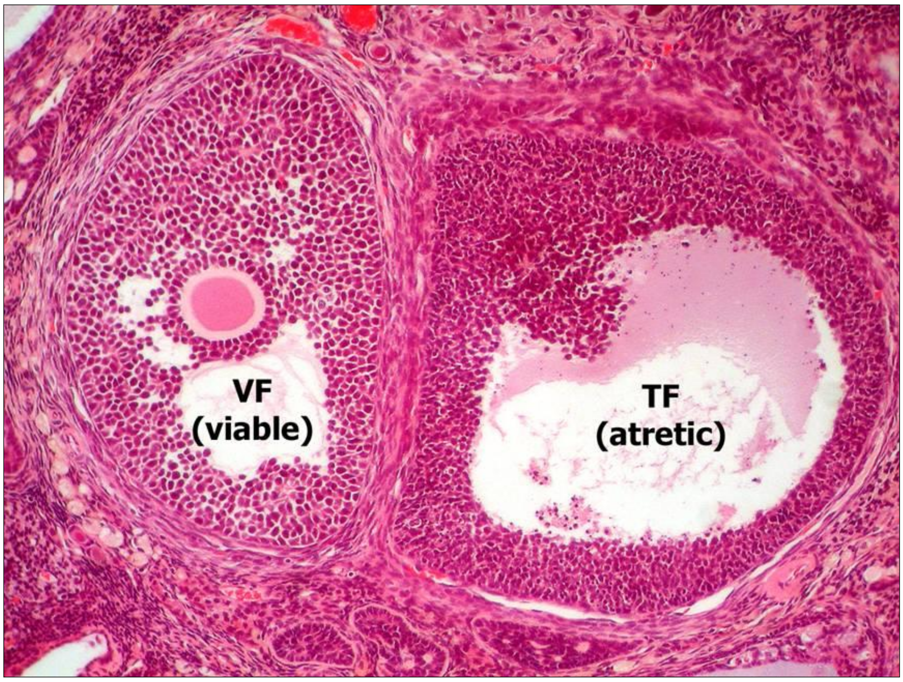

***Figure 1.13 – Degenerate (atretic) tertiary follicle (TF) (rat, H&E x10).** A viable vesicular follicle (**VF**) is also present.*

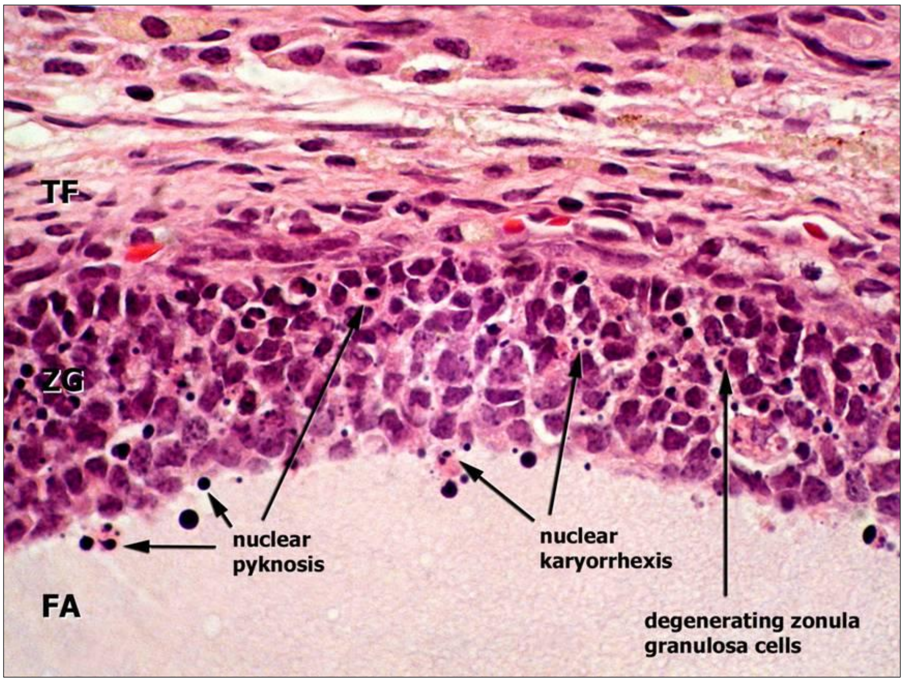

*Figure 1.14 – **Degenerate (atretic) tertiary follicle (rat, H&E x40)**. Pyknotic nuclei and karyorrhectic nuclear debris are scattered throughout the degenerate zonular granulosa (**ZG**).*

TF	theca folliculi
FA	follicular antrum
ZG	degenerating zona granulosa
CL	corpus luteum

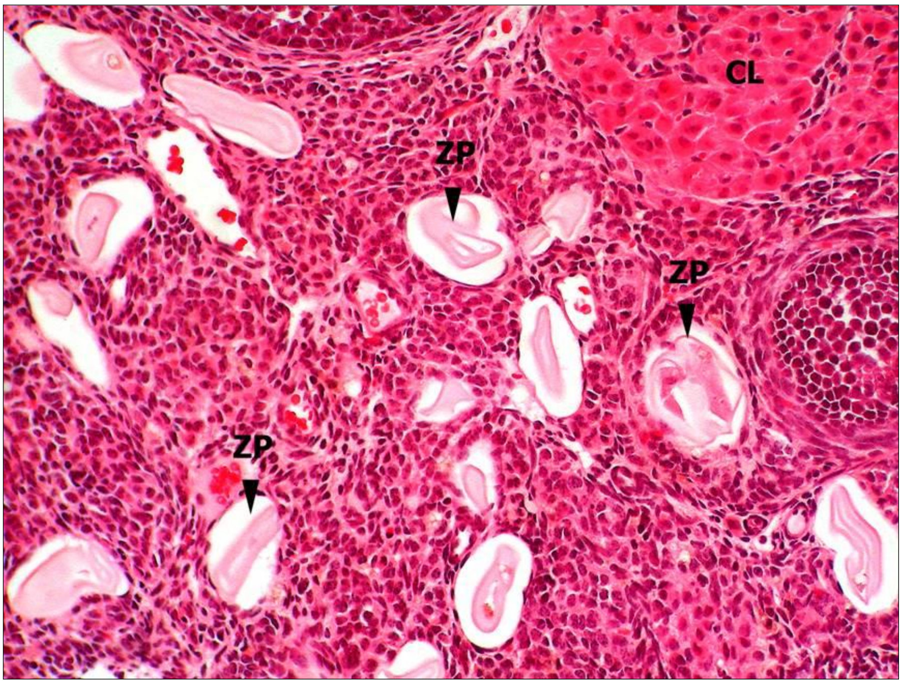

*Figure 1.15 – **Interstitial glands (mouse, H&E x20)**. Theca interna cells surround remnants of degenerate zonae pellucida (**ZP**).*

Uterus

1.10 The rat uterus is duplex, i.e. it comprises two uterine horns that join together and open into the vagina via two separate cervices. The basic histological organisation of the uterus is shown below:

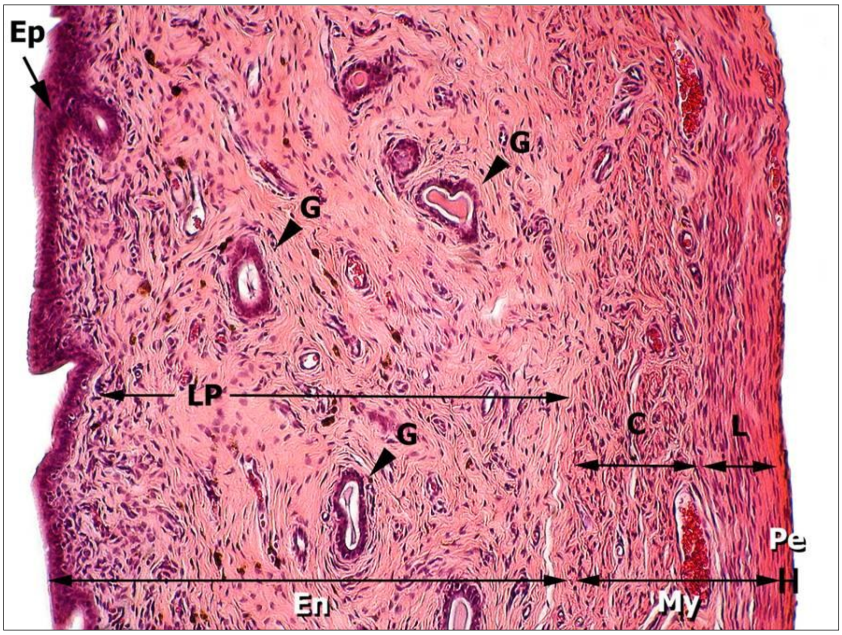

Figure 1.16 – Uterine horn (rat, H&E x10, longitudinal section).

En	**endometrium**
Ep	surface epithelium
LP	lamina propria
G	endometrial gland
My	**myometrium**
C	circular layer (inner)
L	longitudinal layer (outer)
Pe	**perimetrium**

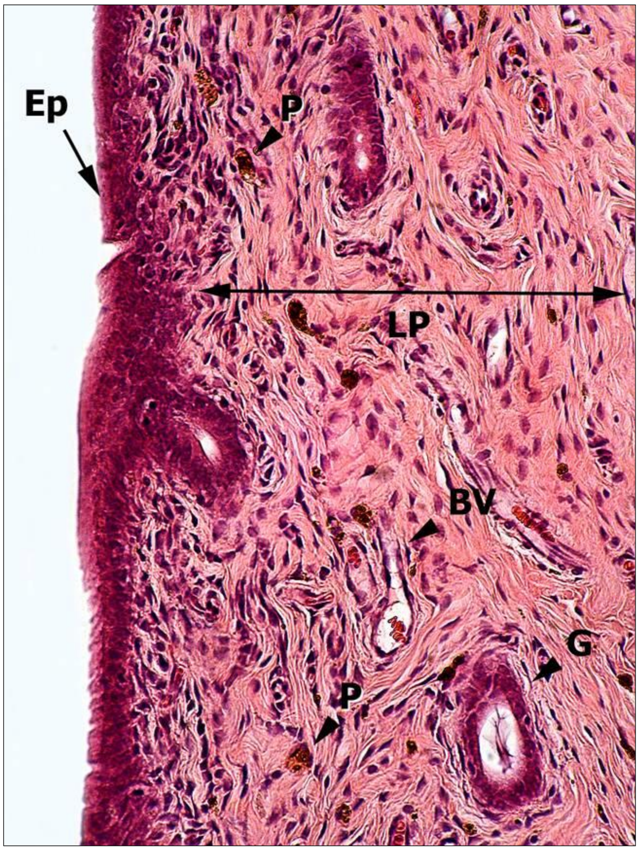

Figure 1.17 – Endometrium (rat, H&E x20). *The variation in surface epithelium height is an artefact of sectioning.*

Ep	surface epithelium	BV	blood vessel
LP	lamina propria	P	pigment
G	endometrial gland		

The inner mucosa, or *endometrium*, consists of a surface columnar epithelium overlying a thick lamina propria containing numerous blood vessels and endometrial glands. The middle muscular layer, or *myometrium*, is composed of an inner circular and outer longitudinal smooth muscle layer. The myometrium is covered by the *perimetrium*, a thin connective tissue layer overlain by a simple serosa. Variable numbers of lymphocytes and other leucocytes are present within the superficial lamina propria of the endometrium. Pigmented stromal cells, containing fine to coarse green-brown granules composed of ferritin, haemosiderin and lipofuscin, may also be observed within the lamina propria of postpartum and mature rodents (Figure 1.17).

Vagina

1.11 The basic trilaminar arrangement of tissues observed in the uterus is conserved in the vagina which consists of an inner mucosa, middle muscularis and outer adventitia (Figures 1.18 and 1.19). The vaginal mucosa comprises a lamina propria covered by a stratified squamous epithelium (Figures 1.19 and 1.20). The mucosa undergoes profound cyclic changes over the course of the oestrous cycle; these alterations are discussed in more detail in Section 3. The smooth muscle bundles that form the muscularis are also disposed in inner circular and outer longitudinal layers, although these are ill-defined in comparison with those forming the myometrium. The outer longitudinal layer of the muscularis merges with the adventitia, a thin outer connective tissue layer.

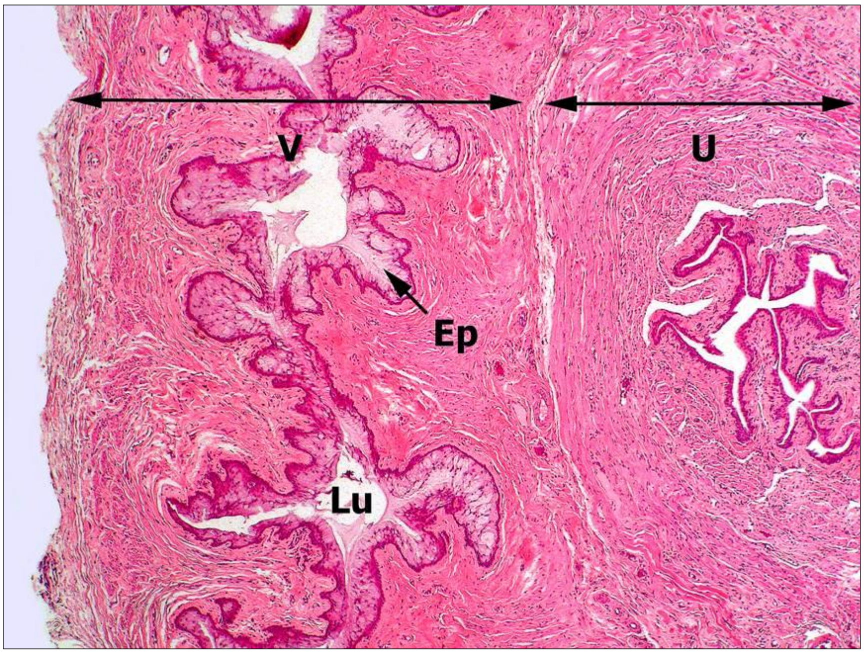

Figure 1.18 – Transverse section through vagina and urethra (rat, H&E x4).

V	vagina
U	urethra
Lu	vaginal lumen
Ep	surface epithelium

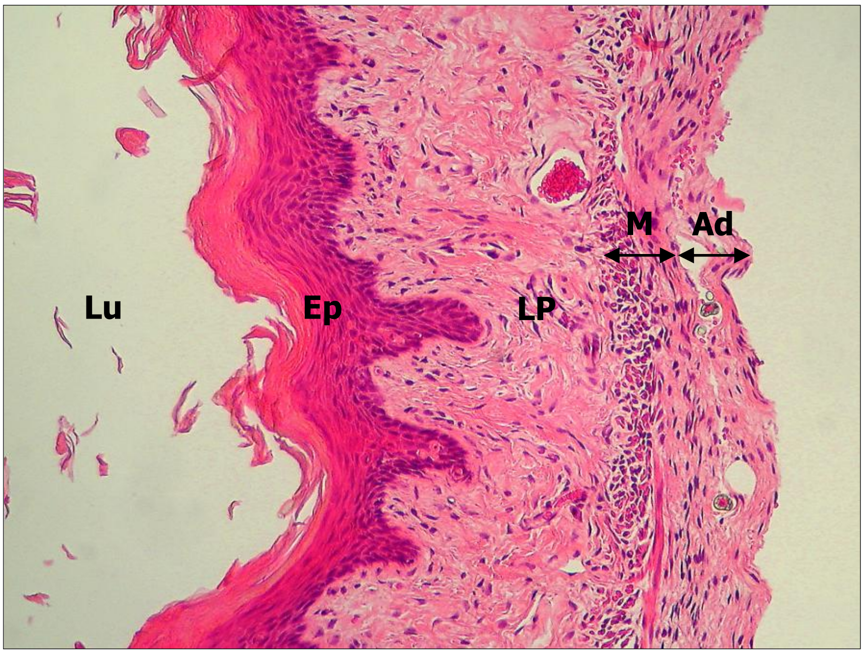

Figure 1.19 – Transverse section through vagina (rat, H&E x10).

Lu	vaginal lumen
Ep	surface epithelium
LP	lamina propria
M	muscularis
Ad	adventitia

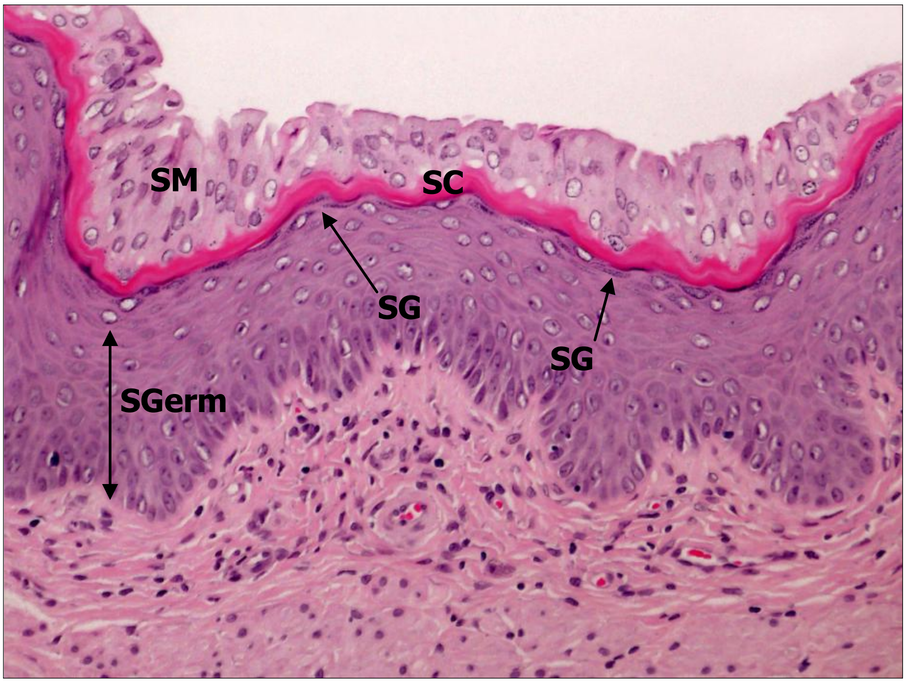

***Figure 1.20 – Transverse section through prooestrus vagina (rat, H&E x20)**. During prooestrus, the vaginal epithelium consists of four layers (**Table 3.1, Section 3**). The stratum germinativum (**SGerm**) is the only layer that is present throughout the oestrous cycle. The stratum granulosum (**SG**) comprises the superficial 2-3 cell layers immediately beneath the stratum corneum (**SC**), overlying which is the stratum mucification (**SM**).*

SM	stratum mucification
SC	stratum corneum
SG	stratum granulosum
SGerm	stratum germinativum