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REVIEW ARTICLE: ANATOMICAL AND PHYSIOLOGICAL DEVELOPMENT OF REPRODUCTIVE SYSTEM IN FEMALE MURINE

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Abstract

The aim of this article is to address the challenges associated with the accurate and consistent staging of the estrous cycle in female laboratory animals, which is critical for reproductive research and preclinical safety evaluations of drugs and chemicals. Laboratory animals, particularly female murine models, are widely used in research as models for mammalian health and disease. However, many substances tested during preclinical investigations interfere with reproductive function, causing morphological changes in the reproductive tract or disruptions in the phases of the estrous cycle. Recognising these alterations requires a thorough understanding of the histological changes in the reproductive tract throughout the cycle. Existing literature, while comprehensive, often lacks precise criteria for defining the transitions between stages of the cycle, creating inconsistencies in staging. This review adopts a pragmatic and practical approach, synthesising personal experience and a century of literature to present a detailed examination of the anatomical and physiological development of the female murine reproductive system. The review emphasises the histological characteristics of the reproductive tract during different estrous phases, providing a framework for consistent and accurate staging. The findings highlight the reproductive traits that make female murine models ideal for reproductive research, including their well-documented physiology and reliable cycle patterns. This article serves as a valuable resource for toxicological pathologists and researchers, offering clarity on staging methods and reaffirming the relevance of murine models in reproductive studies.

Key terms: Embryonic development, estrus, gestation, mice, rats

1. Mice

1.1. Background

In mouse, the reproductive organs in both sexes are derived principally from embryonic mesoderm and the incorporation of primordial germ cells. There is a small contribution of ectoderm to the formation of external genitalia. Primordial germ cells, which are the indifferent precursors of sperm and ova, migrate from the yolk sac to the genital ridges early in embryonic development (De Felici, 2016). Occasional abnormal migration leads to abnormal deposition of these cells in extragonadal tissues, which can lead to extragonadal teratoma formation in adult animals. Differentiation of the gonads towards the appropriate genders specific organ is a complex process but is generally believed to be initiated by the presence of sex-determining factors such as *Sry* from the Y chromosome (Harley *et al.*, 2003; Roelen & Chuva de Sousa Lopes, 2022).

The tubular structures of the reproductive tract develop from the mesonephric or Wolffian duct and the paramesonephric or Mullerian duct. In the presence of developing testes, the production of testosterone and Mullerian inhibiting substance leads to the development of the Wolffian duct system into the seminal vesicles, epididymides and vas deferens from the urogenital sinus and the regression of the Mullerian duct. In the absence of testosterone, the Wollfian ducts regress and the Mullerian duct system develops into the uterus, oviduct and vagina. The caudal genital tract (prostate and ampullary glands) and external genitalia develop in both sexes from the urogenital sinus (Pritchett & Taft, 2007; Cunha *et al.*, 2019). At birth, the genital tubercle and the anogenital distance are larger in males than females, but the external genitalia are still relatively undifferentiated and do not complete development until day 10 (Hotchkiss & Vandenbergh, 2005). The penis and the clitoris derive from an ambivalent genital tubercle, with sex differentiation occurring from day 16 of gestation (Scudamore, 2014).

Puberty occurs at four to six weeks, depending on the mouse strain and environmental cues, and is defined by the onset of reproductive competence (Olson *et al.*, 2010). In females, the formation of the vaginal opening is a sign of the onset of puberty and generally marks the onset of cyclicity, although animals may not be able to sustain a pregnancy immediately. In males, puberty is defined by the production of mature sperm, although the actual ability to fertilise females may only occur several weeks after mature sperm are first observed (Borg *et al.*, 2010; Laffan *et al.*, 2018).

1.2. Anatomy

1.2.1. Ovary

The general anatomical structure of the mouse female reproductive tract is similar to most mammals that have multiple offspring and consists of paired ovaries, a duplex uterus with a relatively short body and long uterine horns, cervix and vagina (Scudamore, 2014). Significant variations in histological appearance occur with age and stage of the estrous cycle, and awareness of these changes is important when analysing the tissues. In mature (greater than 5–6 months), fertile mice the estrous cycle is generally around 4 days if the animal is not mated or pseudopregnant. Between puberty and full maturity, the cycle length tends to be longer, >5 days. From about 12 months (although there is considerable variation depending on the strain, nutrition and husbandry factors), the mouse starts to become reproductively senescent, with increases in cycle length and eventual loss of normal cyclicity (Nelson *et al.*, 1982; Dutta & Sengupta, 2016).

The ovary is contained within a thin-walled bursa (Figure 1), which originates from a mesovarium that, in turn, attaches the ovary to the peritoneum (Scudamore, 2014). The ovarian bursa is usually found embedded in fat, just caudal to the kidneys. The bursa is lined on both sides by flattened mesothelial cells and has a thin connective tissue core which contains scattered smooth muscle fibers. Cystic distension of the bursa is common in older mice and needs to be distinguished from cystadenoma. Hemorrhagic ovarian cysts are also common in ageing mice and can become very large (>2 cm in diameter). Rupture can be associated with significant blood loss, anaemia and even sudden death (Mara *et al.*, 2020).

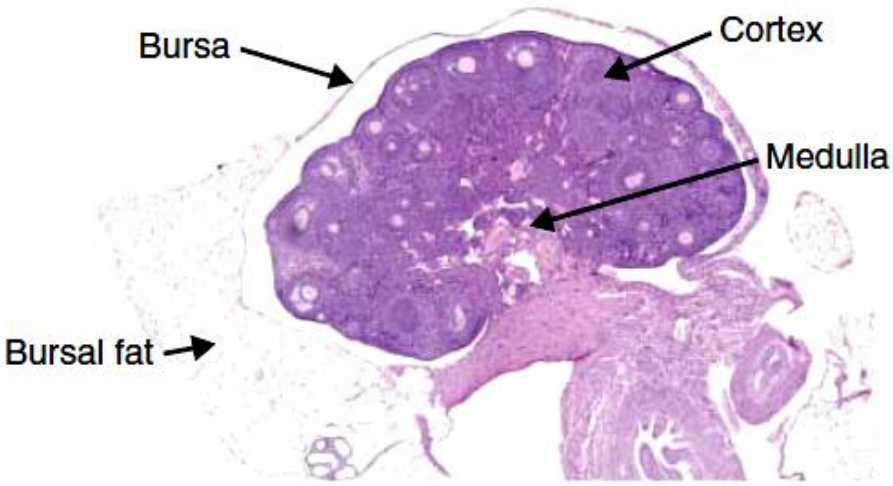

Figure 1: Overview of Ovary and Bursa (Scudamore, 2014)

The ovary has a surface epithelial layer composed of flattened to cuboidal epithelial cells derived from the peritoneal lining cells, which are attached to a thin basement membrane. The mouse ovary has an outer cortex that contains follicular structures and corpora lutea and a central medulla which contains stroma and blood vessels. The division between cortex and medulla is ill defined and, because of the small size of the ovary, the medulla may not be present in every section. The ovarian stroma contains spindle-shaped cells interspersed with collagen that support the follicles and corpora lutea. The collagen is denser in the area below the surface epithelium, and the region is sometimes called the tunica albuginea, although it is poorly defined and may be hard to distinguish in mice (Auersperg *et al.*, 2001; Barnett *et al.*, 2006).

Ovarian follicles are categorised based on the morphological appearance of the associated granulosa cell layers into primordial, primary, secondary, antral and atretic follicles (Myers *et al.*, 2004). Primordial follicles tend to be found towards the subcapsular region of the ovarian cortex and consist of an oocyte surrounded by a single layer of flattened granulosa cells. Polyovular follicles may be seen in young mice but are rare in mature animals. The growing oocytes are surrounded by a hyalinised layer of glycoproteins secreted by the oocyte called the zona pellucid (Alm *et al.*, 2010).

In primary follicles, the oocyte and zona pellucida are surrounded by a single layer of cuboidal granulosa cells and an outer layer of flattened cells (El-Mestrah *et al.*, 2002). Secondary follicles have multiple layers of cuboidal granulosa cells in close association with the oocyte, with no antral space. Antral follicles have multiple layers of granulosa cells and clearly visible fluid-filled antral space (or spaces). The numbers of large antral follicles increase during proestrus and decrease as a result of ovulation during estrus. In large

preovulatory antral follicles, the oocyte is separated from a single antral space by a surrounding layer of granulosa cells (the cumulus granulosa), which will be retained with the oocyte when it is released at ovulation (Diaz *et al.*, 2006; Da Silva-Buttkus *et al.*, 2008).

Most follicles do not reach the point of ovulation and either undergo attrition (primordial follicles) or atresia. Oocytes become atretic as a result of apoptosis, and so in early follicular atresia, fragmentation of the degenerate oocyte and granulosa cells may be seen (Tiwari *et al.*, 2015). Remnants of atretic follicles can be seen as shrivelled eosinophilic remnants of the zona pellucida, which stain PAS positive surrounded by theca interna cells. The remaining theca interna cells are sometimes referred to as 'interstitial glands' (Miyabayashi *et al.*, 2015).
The appearance of corpora lutea varies with the stage of the estrous cycle and whether they are from the current or previous cycles. When first forming following ovulation, the theca cells, which make up the corpora lutea, have basophilic cytoplasm and are a plump spindle shape; mitoses may be present (Young & McNeilly, 2010). The centre of a newly formed corpus luteum may have an irregular fluid-filled or hemorrhagic cavity. During metoestrus and dioestrus, the cells of the corpus luteum become plumper and may start to accumulate fine lipid vacuoles, reaching maximum size and vacuolation at dioestrus. Fibrous tissue may also be apparent in the centre of the corpus luteum at the diestrus, filling the hemorrhagic cavity (Prasad *et al.*, 2008). During proestrus, the corpora lutea starts to degenerate (with vacuolation of cytoplasm), and nuclear fragments and neutrophils may be present. Corpora lutea from up to three previous cycles (Greenwald) may be present and are identifiable by being more eosinophilic than the corpora lutea of the current cycle and becoming paler and smaller with time (Sandrock *et al.*, 2009; Bertolin & Murphy, 2014). The rete ovarii are tubular remnants of the mesonephric ducts, which can be found within the mesovarial fat or within the ovarian stroma at the hilus. The ducts may be single or multiple and lined by cuboidal to columnar epithelium, which may be ciliated. In ageing mice, it is common for the ducts to become dilated or cystic (Long, 2002).

1.2.2. Oviduct

The oviduct consists of four anatomical regions: intramuscular (uterotubular), isthmus, ampulla and infundibulum, lined by a folded mucosal surface (Figure 2). The light microscopic appearance of the oviductal mucosa does not vary significantly with the stage of the oestrous cycle (Stewart & Behringer, 2012). The intramuscular portion passes through the tip of the uterine horn and is lined by low columnar cells surrounded by a connective tissue lamina propria with scattered elastic fibres and smooth muscle cells. The remaining oviduct is lined by a single epithelial layer of pseudostratified cells with centrally located nuclei. The cells are a mixture of ciliated and secretory cells, with ciliated cells being more common in the infundibulum and ampulla and secretory cells in the isthmus (Yamanouchi *et al.*, 2010; Stewart & Behringer, 2012).

The ampulla and isthmus have an outer wall of smooth muscle. The isthmus has a thick muscular wall and, connects the intramuscular section to the ampulla and is highly coiled in the mouse, so it is common to see multiple cross-sections in standard preparations. The ampulla has an increased lumen diameter compared to the isthmus and connects to the infundibulum, which ends in the fimbria in the ovarian bursa (Stewart & Behringer, 2012; Wang & Larina, 2021).

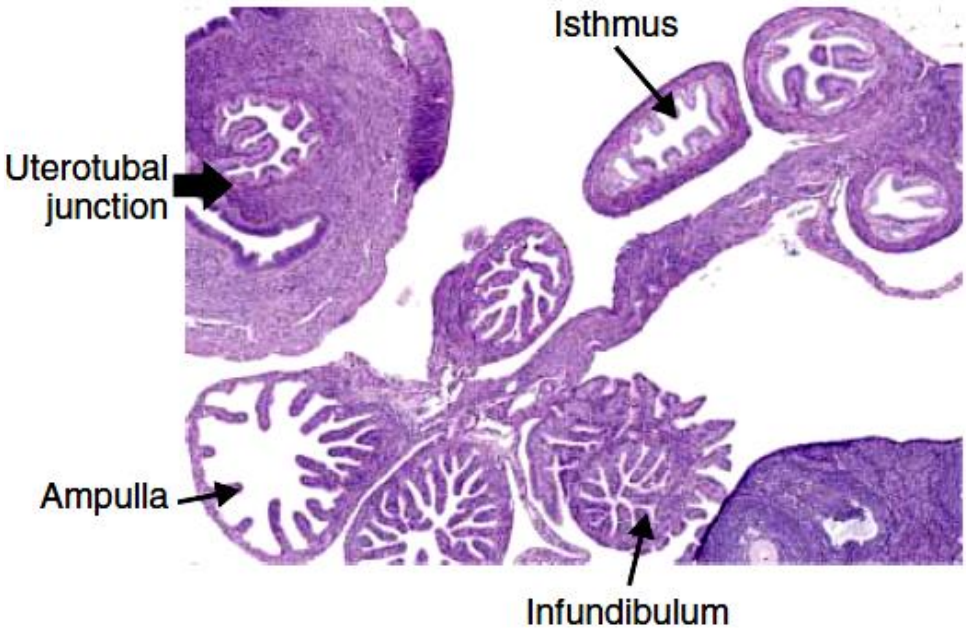

Figure 2: Regions of Oviduct (**Stewart & Behringer, 2012**)

1.2.3. Uterus and Cervix

The uterus consists of a body and two elongated horns. The cranial portion of the uterine body (or corpus) is divided by a midline septum; caudally, the body becomes one chamber, which is continuous with the cervix and the vagina (Deb *et al.*, 2006). The uterus is composed of an outer loose connective tissue serosal layer (perimetrium), two muscular layers (myometrium) and an inner mucosal layer (endometrium). The myometrium consists of an inner circular and an outer longitudinal layer of smooth muscle fibres separated by a thin vascular connective tissue layer (Kagami *et al.*, 2020; Rabie & Haibat, 2020). In the horns and divided portion of the uterine body, the endometrium and endometrial glands are lined by a single layer of columnar epithelial cells, the appearance of which varies with the stage of the cycle. The epithelial lining of the undivided portion of the uterine body and cervix is stratified squamous and continuous with the vaginal lining (Filant & Spencer, 2014; Rabie & Haibat, 2020).

At the start of estrus, the lumen of the uterus may be mildly distended with fluid, which gradually reduces with time (this normal appearance needs to be differentiated from fluid enlargement due to distal obstruction of the reproductive tract leading to gross distension due to mucometra or hydrometra (Pritchett & Taft, 2007). The glandular epithelium starts to degenerate, which can be seen by vacuolation and apoptosis of the epithelial cells, and there is an absence of mitoses; some infiltrating inflammatory cells may be present. In metoestrus, the lumen becomes reduced, and the lining epithelium continues to show signs of degeneration, although some mitoses may be present. In diestrus, the uterus is at its thinnest with a narrow slit-like lumen surrounded by a thin endometrium with condensed stromal tissue and low columnar lining cells with no evidence of degeneration, although occasional mitoses may be present. In proestrus, the uterine lumen becomes dilated by fluid, and the lining epithelial cells become tall and columnar with frequent mitoses, and the stroma may have an edematous appearance (Dixon *et al.*, 2014; Scudamore, 2014; Vidal, 2019).

1.3. Estrous cycle

The estrous cycle refers to the reproductive cycle in rodents. It is similar to the human reproductive cycle, commonly called the menstrual cycle (ovarian and uterine cycles) (Figure 3). The estrous cycle has four phases, namely proestrus, estrus, metestrus and diestrus and lasts for 4 to 5 days (Auta & Hassan, 2016). The reproductive period and estrous cycle of mice commences about the 26th day after birth with the opening of the vagina, which is about 10 days before vaginal cornification (Champlin *et al.*, 1973).

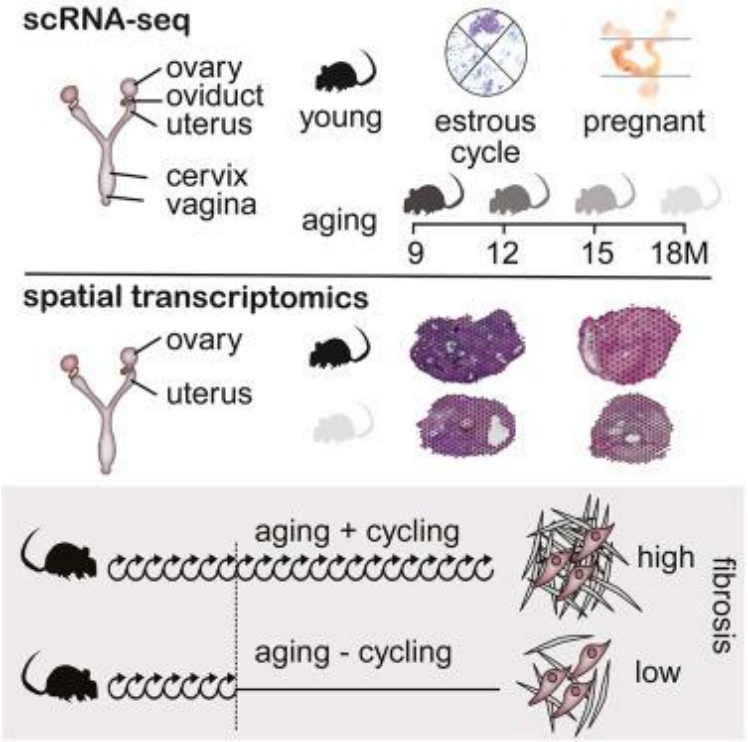

Figure 3: The Estrous Cycle Drives Organ-Specific Compositional Changes (**Winkler *et al.*, 2024**)

The apoptosis mediated vaginal opening is an essential secondary character in mice, which is used as a predictor of puberty (Cora *et al.*, 2015). The vaginal unfolding is associated with an increase in oestradiol concentration. In rats, a vaginal opening occurs during the first ovulation (Auta & Hassan, 2016). In female rats, puberty is preceded by the pulsatile release of luteinising hormone (LH) after the fourth postnatal week, approximately 30 days old (Ekambaram *et al.*, 2017). This period is the anestrus and occurs about 8 to 9 days before the first proestrus (Foitzik *et al.*, 2000). The first proestrus, estrus, metestrus, and diestrus follow. Metestrus only occurs in the absence of conception (Byers *et al.*, 2012).

On the other hand, in humans, there are three phases of the menstrual cycle: the menstrual, proliferative (follicular), and secretory (luteal) phases. This cycle begins at puberty. On average, it lasts about 28 days from the start of one menstrual period to the start of the next. At mid-cycle, between the proliferative and secretory phases, is the ovulatory phase, during which ovulation occurs following LH surge. The proliferative phase is primarily associated with high estrogen levels, while the secretory phase is associated with high progesterone levels (Me, 1994). The proestrus phase corresponds to the human follicular stage, which is associated with a rise in circulating estradiol concentrations and little surge in prolactin; this leads

to a rise in LH and Follicle Stimulating Hormone (FSH) release (Hussen *et al.*, 2024). The peak in FSH concentration with an associated rapid decline in estradiol levels correlates to ovulation and estrus phase. Metestrus and diestrus are homologous to human early and late secretory stages of the reproductive cycle, respectively, with high levels of progesterone (Heape, 1990).

2. Rat

2.1. Background

The laboratory rat belongs to the order Rodentia. Both rats and mice are in the family Muridae. The term *murine* refers to rats and mice. The word rodent originated from the Latin word *rodere*, which means "to gnaw" (Kusmeirczyk *et al.*, 2020). The laboratory rat is widely used in toxicological, nutritional, genetic, behavioural and environmental studies. The small size of rats and the ease of housing and caring for them have made them preferable as pets and research animals (Simpson & Kelly, 2011). The use of humans and food animals in experiments is restricted for ethical and economic reasons, respectively. Therefore, rats have long been used as models of mammalian health and disease. Rats have made valuable contributions to many fields, especially reproduction (Edrey *et al.*, 2011). This review provides the basic facts about female rat reproduction and highlights the reproductive characteristics which make the rat an appropriate animal model for research on human reproduction. The female reproductive system consists of the two ovaries and the female genital tract. The genital tract includes oviducts, uterus, cervix and vagina. The female genital tract in mammals arises from the Mullerian ducts, commencing with the ostium of the oviduct. In the rat, this ostium forms a complete capsule called the ovarian bursa, which envelops the ovary. The oviducts are small, highly coiled tubes. The uterus consists of two separated uterine horns, enabling the rat to have multiple offspring. The vagina of the rat opens directly to the exterior (Kent & Carr, 2001).

2.2. Anatomy

2.2.1. Ovary

The ovary undergoes considerable postnatal development in the rat. It becomes morphologically mature by 42 days, at which time there are corpora lutea representing at least two ovulations (Picut *et al.*, 2015). Generally, the first ovulation will occur in 35 days, but there is considerable individual animal variation, and the first ovulation has been reported to occur in rats as early as 28 days (Everett & Sawyer, 1949). Some landmark features in ovarian development include early antral follicles at 10 days, zona pellucida formation at 15 days, late antral follicles at 18 days, granulosa cell apoptosis, and interstitial glands at 21 days, and a wave of follicular atresia around 27 to 28 days (Hsueh *et al.*, 2000).

During the neonatal period, 0-7 days, the ovary is a solid parenchymal mass with no medulla, cortex, or hilus. It is comprised of ovigerous nests, which are packets of oogonia enveloped by undifferentiated mesenchyme (Dixon *et al.*, 2014; Mohamed *et al.*, 2017). Within the first few days, the ovigerous nests transform into primordial follicles (i.e., a primary oocyte surrounded by a single layer of flattened pre-granulosa cells) (Pepling, 2006). Follicular development starts in the core of the ovary, where these primordial follicles become primary follicles (primary oocyte surrounded by a single layer of cuboidal granulosa cells) and secondary follicles (primary oocyte surrounded by more than one layer of cuboidal granulosa cells), (Pepling, 2006; 2013). During this neonatal period of development, the ovary is independent of and unresponsive to pituitary gonadotropins LH (luteinising hormone) or FSH (follicle

stimulating hormone). Follicular development is under the control of local paracrine and autocrine factors (Hammond *et al.*, 1988).

The infantile period, 8-20 days, is the time when the granulosa cells of the ovary start responding to FSH and LH. Follicles develop an antrum (i.e., fluid-filled cavity), which is the hallmark feature of the effect of gonadotropins (Natraj & Richards, 1993). These early antral follicles are "atypical" because they have plump granulosa cells and thecal cells, with little discernment between these two layers. These atypical follicles have poor adhesion between the individual granulosa cells and no zona pellucida surrounding the oocyte (Hirshfield & DeSanti, 1995). As the infantile period proceeds into its second half (14-20 days), the antral follicles lose these atypical features: the thecal cells elongate, the plump granulosa cells become tightly packed, and a protective zona pellucida forms around the primary oocyte (Namlı Kalem *et al.*, 2023). Throughout the entire infantile period, follicle development proceeds from the central core of the ovary toward the periphery, with the more developed follicles in the core and the least developed follicles at the periphery. Often, the linear radiating arrangement of these follicles is apparent and reminiscent of the orientation of the ovigerous cysts during embryogenesis (Grive & Freiman, 2015).

An ovarian hilus is present at the start of the juvenile period (21 days). The most prominent features of the juvenile period (21-32 days) are apoptosis of granulosa cells and atresia of follicles with an increase in interstitial glands (Salvetti *et al.*, 2009). Apoptosis begins in clusters of pyknotic nuclei within the wall of secondary and tertiary follicles, and this proceeds to large-scale atresia of follicles in the core of the ovary around 26-28 days. This large-scale atresia is the event that gives rise to a medulla and cortex (Windsor, 2018). The atresia of follicles is caused by declining FSH levels and constitutes normal restructuring of the ovary and maturation of the hypothalamic-pituitary-gonadal (HPG) axis. From this time forward, the more advanced follicles will be present in the cortex rather than the medulla (Zakharova *et al.*, 2020).

The peri-pubertal and pubertal period (33-46 days) is the time frame in which the first ovulation occurs in the ovary. Ovulation corresponds to vaginal opening and is considered to be the day when puberty is reached for that individual animal (Elmaoğulları *et al.*, 2020). While there is considerable individual variation for the time of first ovulation, it generally occurs around 35 days. Ovulation is a complicated process that requires tertiary follicles of 0.9–1 mm in diameter and a mature HPG axis that can produce an LH mini-surge in response to rising estrogen levels (Wulff *et al.*, 2002). The presence of corpora lutea indicates ovulation has been successfully orchestrated. Corpora lutea, which develop from the ovulatory follicle, are irregularly round structures composed of large polygonal cells separated by well-vascularised stroma. Corpora lutea will persist for several estrous cycles (about 12 days), and therefore the ovary may contain corpora lutea of the current as well as prior estrous cycles (Fransen *et al.*, 1993; Haibet & Rabie, 2009). Current corpora lutea may be distinguished from old corpora lutea (of previous cycles) based on light microscopy and special stains. In general, current corpora lutea, especially those that are from the early part of the cycle, are composed of more basophilic luteal cells and contain less fibrovascular stroma compared to old corpora lutea (Mirsky *et al.*, 2011). Current corpora lutea, especially those at estrus, also stain more intensely with proliferating cell nuclear antigen immunohistochemical stain than old corpora lutea (or corpora lutea of prior cycles) (Yoshida *et al.*, 2009).

2.2.2. Oviduct

The oviduct is composed of three segments that reside outside of the uterus and one segment, known as the pars interstitialis, that resides within the wall of the uterus. The outer three segments of the oviduct are generally included in histologic sections with the ovary and include the infundibulum (at the ovarian end, having highly folded and branched mucosa), ampulla (more dilated, with less mucosal folds; middle section), and isthmus (heavy muscular wall with low folds). Each of these three segments features a pseudostratified columnar ciliated epithelium lining the mucosa (Jollie & Wynn, 2013). The epithelial cells include the ciliated epithelium with dark and light cells and darkly staining elongated peg cells. The peg cells are non-ciliated secretory cells that appear at 13-14 days. This epithelium is bordered adluminally by a basement membrane, a barely discernible mucosal stroma, and an underlying double layer of smooth muscle. The mucosal folds of the infundibulum have profuse branching toward the caudal segments. The smooth muscle layers are thin at the infundibulum but progressively thicken near the isthmus. The oviduct has a well-developed epithelium by 21 days; however, the muscular wall continues to mature and become more robust through 42 days (Hofstetter *et al.*, 2006; Nzalak, 2010).

2.2.3. Uterus and Cervix

The bicornuate uterus is poorly developed at birth, consisting of a simple tube lined by low cuboidal epithelium embedded in undifferentiated mesenchymal stroma. On 21 days, the uterus is only 1.0 mm in diameter and grossly appears like a mucus thread (Polenz *et al.*, 2024). By 28 days, inflammatory cells first appear within the endometrial stroma; vacuolation of epithelial cells can be observed, and the diameter of the uterus is 1.25 mm. By 36 days, inflammatory cells are routinely found in the uterine stroma in response to hormonal influence associated with the onset of puberty (Gan *et al.*, 2017). The uterus matures by 42 days, with a well-developed muscularis that is composed of a thick inner circular layer, an intervening layer of smooth muscle containing large blood vessels, and a thinner outer longitudinal layer. The diameter of the uterus is approximately 1.75 mm by this time. Each of the two uterine horns communicates with the vagina through a separate cervical os. Each os has two histological regions: (1) a lower (posterior) segment that is like the vagina and (2) an upper (anterior) segment that is transitional between the lower cervix and the uterus (Porto *et al.*, 2010; Hamid & Zakaria, 2013).

2.2.4. Vagina

The vagina forms from the caudal end of the Müllerian duct system. The distal ends remain incompletely formed until the onset of puberty, at which time the tract becomes complete with a vaginal opening (Cai, 2009). The vaginal epithelium matures and commences cycling before the ovary, and estrous staging of vaginal epithelium can be accomplished several days prior to the first ovulation. The vagina at 20 days is lined by a stratum germinitivum with the early stages of stratum granulosum. By 30 days (prior to the first ovulation), a hormonally responsive epithelial lining has fully developed, including a stratum corneum characteristic of estrus, and/or a stratum corneum and stratum mucification compatible with proestrus (Cason, 2012; Hamid & Zakaria, 2013; Ajayi & Akhigbe, 2020).

2.3. Estrous Cycle

The rat estrous cycle is short, lasting four to five days. It occurs throughout the year with no seasonal effect. The first regular estrous cycle occurs about one week after the opening of the vaginal orifice, usually 33 to 42 days after birth (Maeda *et al.*, 2000). The cycle length increases slightly with age and lasts about 6

days near the end of the reproductive life span (Lu *et al.*, 1979). The estrous cycle in rats consists of four stages: proestrus, estrus, metestrus, and diestrus. Proestrus lasts approximately 12 h; estrus, 9 to 15 h; metestrus, 21 h; and diestrus (the longest phase), over 57 h (Lohmiller & Swing, 2006). Hormones play critical roles in the estrous cycle. Gonadotrophins, which are secreted by the anterior pituitary, regulate the estrous cycle through luteinising hormone (LH) and follicle-stimulating hormone (FSH). Hormonal fluctuations result in ovarian and follicular changes, as well as changes in vaginal cytology. FSH stimulates follicle growth, while LH stimulates the follicles to ovulate and form the corpus luteum. Progesterone is secreted by the corpus luteum during metestrus and declines during diestrus. During follicular development, the level of estradiol-17β increases. The cycle ends when estrogen peaks during proestrus, stimulating gonadotropin release to trigger ovulation (Freeman, 1988).

3. Identification of Estrous Cycle Stages

Phases of the estrous cycle can be detected by observing behavioural changes or examining vaginal cytology (Lohmiller & Swing, 2006). The latter method is widely used and considered a rapid and practical way to determine the phases of the estrous cycle (Marcondes *et al.*, 2002). Accurate phase identification depends on smears taken at fixed times in the day, as the cell populations vary throughout a 24-h period, behaviour and vaginal smear morphology during the different phases of estrous cycle as well as the duration of each phase. Estrus is defined as the period when the female accepts the male and allows copulation. Many behavioural changes occur during this phase, including increased running activity, lordosis, and ear quivering. During estrus, dry vaginal walls and a swollen vulva can also be observed (Baker, 1979). The female accepts the male at the end of proestrus, while during metestrus and diestrus, the female does not accept the male (Lohmiller & Swing, 2006). Vaginal cytology in estrus reveals cornified cells and nucleated cells. In metestrus, leukocytes, nucleated cells and cornified cells are seen. In diestrus, which is the longest phase, vaginal cytology principally reveals leukocytes. Nucleated cells are predominant in vaginal smears during proestrus (Lohmiller & Swing, 2006).

4. Environmental Factors Affecting the Estrous Cycle

The estrous cycle can be affected by various environmental factors, such as temperature, photoperiod, noise, restraint, immobilisation, handling and research procedures. High ambient temperatures (35°C) increase the duration of the cycle and thus reduce the number of estrous cycles occurring in a given period of time (Sod-Moriah, 1971). Changes in photoperiod can also alter estrous-cycle length. Extending the light period from 12 to 16 h a day increased the estrous cycle from four days to five days (Clough, 1982), but constant light disturbed the estrous cycle in female rats (Hardy, 1970) and resulted in persistent estrus. Noise also influences the estrous cycle in rats. Exposure of female rats to ultrasound in the sensitive hearing range of the rats (range near 40 Hz) alters the estrous cycle (Clough, 1982). Euker and Riegle (1973) observed the delay of the estrus phase and mating after restraint stress during diestrus. They concluded that restraint stress during diestrus can block the cyclic release of gonadotrophins that are necessary for estrogen secretion and ovulation. Acute stress or immobilisation of female rats suppresses LH pulses independent of estrogen (Maeda *et al.*, 2000). Handling and research procedures (that is, restraint and subcutaneous (SC) and tail intravenous (IV) injection) can induce stress but have little effect on the estrous cycle, regardless of the stage (Sharp *et al.*, 2002).

5. Mating and Reproductive Behavior

Mating behaviour in females is controlled by both estrogen and progesterone; in males, it is controlled by testosterone (Meisel & Sachs, 1994; Maeda *et al.*, 2000). Lordosis is a characteristic mating behaviour of female rats, whereas auditory stimuli play crucial roles in the reproductive behaviour of both male and female rats (Barfield & Thomas, 1986; Maeda *et al.*, 2000). In addition, olfactory cues from pheromones are very important to the sexual behaviour of the male (Nelson, 1995). Copulation in rats mostly occurs during the last third of the dark cycle (Mercier *et al.*, 1987).

6. Pregnancy Detection

The presence of sperm in the vaginal smear or observation of a vaginal plug indicates the occurrence of mating. In rats, the vaginal plug does not persist as long as in mice; thus, the absence of the vaginal plug is not a reliable indicator that copulation did not occur (Hamid & Zakaria, 2013). On the other hand, the detection of sperm in a vaginal smear is an excellent predictor of pregnancy in rats (Baker, 1979). The day that sperm is detected in the vaginal smear is designated as day 1 of gestation. After 10 days of gestation, the fetuses can be palpated, but palpation is more accurate after day 12. By day 13 of gestation, the abdominal enlargement is visible, and mammary development and nipple enlargement can be observed on day 14 of gestation.

7. Fertilisation and Early Embryonic Development

Fertilisation in mammals takes place in the oviduct. Successful fertilisation requires complex spermatozoa-ova interactions. Fertilisation involves many sequential steps, beginning with the binding of spermatozoa to the zona pellucida, followed by the acrosome reaction and penetration of spermatozoa through the zona pellucida, then the spermatozoa bind to and fuse with the egg, leading to egg activation (Yanagimachi, 1994). Sperm migration through the oviduct depends on both estradiol and progesterone (Orihuela *et al.*, 1999). Fertilisation steps in mammals are thought to be regulated by proteins located in the acrosome of the spermatozoa. Fertilisation in rats is regulated by epididymal 37 kDa protein (DE) (Cohen *et al.*, 2000). After fertilisation, a single-cell embryo (zygote) doubles into two cells and then undergoes a series of mitotic divisions into four cells, eight cells, and a morula. Several more rounds of mitotic division form the blastocyst. The blastocyst is composed of differentiated tissues: a layer of trophectoderm cells, which give rise to the placenta, and the inner cell mass (ICM), which gives rise to the embryo. The blastocyst becomes competent for implantation after shedding the zona pellucida (Lee & DeMay, 2004). In rats, fertilisation takes place in the morning at 4-5h. The zygote develops into two and four cells on the first day, to eight cells on the second day, and to a sixteen-cell embryo on the third day after fertilisation. The embryo develops to the morula stage on day four and to the blastocyst stage on day five of pregnancy (Agca & Critser, 2006). Pre-implantation embryos can be collected from the female reproductive tract and used in basic research, embryo culture studies, genome banking, and the establishment of stem cells (Jiang *et al.*, 1999; Agca & Critser, 2006). However, the time of embryo collection depends on the embryo stage needed.

8. Maternal Recognition of Pregnancy

The establishment of pregnancy requires the presence of a functional corpus luteum that is able to produce sufficient progesterone. A viable conceptus can send specific signals to a "pregnancy-ready" uterus; these signals rescue the corpus luteum from luteolysis. This process is called maternal recognition

of pregnancy (Accialini *et al.*, 2015). Maternal recognition of pregnancy in rodents involves the activation of the non-functional corpus luteum of the estrous cycle into the functional corpus luteum of pregnancy. This functional corpus luteum must be maintained until day 17 (Hamid & Zakaria, 2013).

The formation and maintenance of the corpus luteum and the production of progesterone require two events. First, mating induces the release of prolactin (PRL) from the anterior pituitary, which increases LH receptors on luteal cells to form the corpus luteum and suppress 20α-hydroxysteroid dehydrogenase activity; this transition prevents the conversion of progesterone to 20α-hydroxyprogesterone, which will not support pregnancy. Second, the lactogenic hormones that are produced by the uterine decidua and placenta act through prolactin receptors on the luteal cells to maintain their function and the production of progesterone throughout gestation. Thus, PRL is the initial luteotrophic signal for corpus luteum formation and progesterone production (Soares, 2004; Hamid & Zakaria, 2013; Accialini *et al.*, 2015).

9. Embryo Implantation

Implantation is divided into three stages: apposition, adhesion (attachment), and invasion (Enders & Schlafke, 1967). In rodents, the embryo that enters the uterus attaches to the uterine epithelium immediately. After the loss of the zona pellucida, the closure of the uterine lumen brings the blastocyst into close apposition to the luminal epithelium (Parr & Parr, 1989). The blastocyst attaches to the anti-mesometrial side of the endometrium, and the inner cell mass is directed to the mesometrial side. The epithelial cells in contact with the blastocyst undergo apoptosis and are phagocytised by the polytene cells (that is, cells from the wall of the blastocyst), facilitating penetration of the epithelium. Rodents demonstrate rapid implantation, as apposition, attachment and invagination of the uterine epithelium occur within 6 h (Lee & DeMay, 2004). Invasion or penetration in rats occurs when the trophoblast cells displace the underlying uterine epithelium and penetrate the epithelial basal lamina and stroma. The trophoblast migrates into the endometrial stroma and penetrates the superficial endometrial vessels. This mode of penetration is known as displacement penetration (Schlafke & Enders, 1975; Bowen & Burghardt, 2000).

Implantation may also be divided into three categories based on the type of blastocyst–uterine cell interaction: centric, eccentric and interstitial (Wimsatt, 1975; Bazer *et al.*, 2010). Implantation in rats is eccentric; the luminal epithelium forms an invagination to surround the trophoblast. After the trophoblast invades the endometrial stroma, the stromal cells undergo extensive differentiation to form the decidua (Johnson *et al.*, 2003). In rats, decidualisation requires both estrogen and progesterone. These hormones exert their effects on the endometrium via nuclear estrogen (ER) and progesterone (PR) receptors (Wang & Dey, 2006). The first sign of implantation is the increase in uterine vascular permeability at the site of blastocyst apposition (Psychoyos, 1986). In mice and rats, an intravenous injection of macromolecular blue dye solution can show the implantation sites as blue bands along the uterus. Increases in vascular permeability coincide with the attachment reaction between the blastocyst and uterine epithelium (Psychoyos, 1986). Implantation in rats is initiated on day 5 and completed by day 7 of pregnancy (Hamid *et al.*, 2012).

10. Gestation, Parturition and Weaning

Gestation in rats takes 21 to 23 days from copulation to parturition. Placentation is discoidal and hemochorial (Kaufmann & Burton, 1994). That is, the fetal and maternal tissue attaches at a circular area (discoid placentation), and the fetal trophoblasts invade the maternal vessels and contact directly with the maternal blood (hemochorial placentation). Delivery in rats takes from 55 min to 4 hours, depending on the litter size, with an average of 1.5 hours (Baker, 1979). Weaning in rats occurs at around 21 days of age. At this age, pups are able to eat and drink.

CONCLUSION AND RECOMMENDATION

Conclusion: Mice and rats have been used in research for almost two centuries. Short estrous cycles and gestation periods make the mice and rats ideal animals for research on reproduction. In view of the mode of implantation and hemochorial placentation, studies in rats can provide insights into the cellular and molecular basis of human implantation. Therefore, mice and rats are good models for the studies of human embryo implantation and early pregnancy disorders. Summing up, it is worth knowing that various techniques can determine the phases of estrous cycles. Careful selection of the method to employ is essential. Vaginal smear/cytology remains the gold standard in life animals upon which other methods are verified. Although histological examinations of reproductive organs are as specific and reliable as vaginal smear/cytology, it is not useful in live animals. Improvement of the available techniques to enhance their reliability and specificity is pertinent. Studies to achieve benchmark values for vaginal wall impedance and urine biochemical parameters at different phases of estrous in commonly used experimental animals are also essential.

Recommendations: To enhance the accuracy and consistency of estrous cycle staging in female murine models, it is recommended that researchers adopt a comprehensive approach combining multiple assessment methods. Utilising vaginal cytology alongside visual evaluation of the external genitalia can provide a more precise determination of the cycle's phases. Vaginal cytology, recognised as a reliable method, involves analysing cell types present in vaginal smears to identify specific stages of the estrous cycle. This technique, while accurate, can be labour-intensive and requires expertise in microscopic examination. In addition to cytology, visual observation of the vaginal opening offers a quicker, less invasive method to assess the estrous stage. This approach evaluates changes in the appearance of the vaginal orifice, which correlate with different cycle phases. When performed by trained personnel, visual assessment can efficiently identify animals in proestrus or estrus, facilitating timed mating and other reproductive studies. Furthermore, integrating advanced technologies such as deep learning algorithms can automate and enhance the accuracy of estrous cycle classification. Recent studies have demonstrated the potential of models like EfficientNet in automating the recognition of estrous stages in rodents, reducing observer bias and improving consistency in cycle staging. By combining traditional methods with technological advancements, researchers can achieve a more accurate and consistent staging of the estrous cycle in female murine models, thereby improving the reliability of reproductive research and preclinical evaluations.

REFERENCES

1. Accialini, P., Hernández, S. F., Bas, D., Pazos, M. C., Irusta, G., Abramovich, D., & Tesone, M. (2015). A link between Notch and progesterone maintains the functionality of the rat corpus luteum. Reproduction, 149(1), 1–10. https://doi.org/10.1530/REP-14-0449
2. Agca, Y., & Critser, J.K. (2006). Assisted Reproductive Technologies and Genetic Modification in Rats: In M.A., Suckow, S.H., Weisbroth, and C.L., Franklin. The Laboratory Rat. (pp 165-189).
3. Ajayi, A.F., Akhigbe, R.E. Staging of the estrous cycle and induction of estrus in experimental rodents: an update. Fertil Res and Pract 6(5). https://doi.org/10.1186/s40738-020-00074-3
4. Alm, H., Kuhlmann, S., Langhammer, M., Tuchscherer, A., Torner, H., & Reinsch, N. (2010). Occurrence of polyovular follicles in mouse lines selected for high fecundity. The Journal of reproduction and development, 56(4), 449–453. https://doi.org/10.1262/jrd.09-224h.
5. Auersperg, N., Wong, A. S., Choi, K. C., Kang, S. K., & Leung, P. C. (2001). Ovarian surface epithelium: biology, endocrinology, and pathology. Endocrine reviews, 22(2), 255–288. https://doi.org/10.1210/edrv.22.2.0422
6. Auta, T., and Hassan, A.T. (2016). Alteration in oestrus cycle and implantation in Mus musculus administered aqueous wood ash extract of Azadirachta indica (neem). Asian Pacific Journal of Reproduction, 5(3), 188-192.
7. Baker, D.E.J. (1979). Reproduction and breeding. In H. J. Baker, J. R. Lindsey, & S. H. Weisbroth. The Laboratory Rat, 1, 153-168. Academic Press.
8. Barfield, R. J., & Thomas, D. A. (1986). The role of ultrasonic vocalizations in the regulation of reproduction in rats. Annals of the New York Academy of Sciences, 474, 33–43. https://doi.org/10.1111/j.1749-6632.1986.tb27996.x
9. Barnett, K. R., Schilling, C., Greenfeld, C.R., Tomic, D., & Flaws, J. A. (2006). Ovarian follicle development and transgenic mouse models. Human Reproduction Update, 12(5), 537–555. https://doi.org/10.1093/humupd/dml022
10. Bazer, F. W., Wu, G., Spencer, T. E., Johnson, G. A., Burghardt, R. C., & Bayless, K. (2010). Novel pathways for implantation and establishment and maintenance of pregnancy in mammals. Molecular human reproduction, 16(3), 135–152. https://doi.org/10.1093/molehr/gap095
11. Bertolin, K., & Murphy, B. D. (2014). Reproductive tract changes during the mouse estrous cycle. In The guide to investigation of mouse pregnancy. 85–94. Academic Press.
12. Borg, C. L., Wolski, K. M., Gibbs, G. M., & O'Bryan, M. K. (2010). Phenotyping male infertility in the mouse: how to get the most out of a 'non-performer'. Human reproduction update, 16(2), 205–224. https://doi.org/10.1093/humupd/dmp032
13. Bowen, J. A., & Burghardt, R. C. (2000). Cellular mechanisms of implantation in domestic farm animals. Seminars in cell & developmental biology, 11(2), 93–104. https://doi.org/10.1006/scdb.2000.0155
14. Byers, S. L., Wiles, M. V., Dunn, S. L., & Taft, R. A. (2012). Mouse estrous cycle identification tool and images. PloS one, 7(4), e35538. https://doi.org/10.1371/journal.pone.0035538
15. Cai, Y. (2009). Revisiting old vaginal topics: conversion of the Müllerian vagina and origin of the "sinus" vagina. The International journal of developmental biology, 53(7), 925–934. https://doi.org/10.1387/ijdb.082846yc
16. Cason, Z. (2012). The effects of sustained delivery of estrogen and novel neuropeptide Y antagonist on the reproductive tract of ovariectomised rats. The University of Mississippi Medical Center.

17. Champlin, A. K., Dorr, D. L., & Gates, A. H. (1973). Determining the stage of the estrous cycle in the mouse by the appearance of the vagina. Biology of reproduction, 8(4), 491–494. https://doi.org/10.1093/biolreprod/8.4.491

18. Clough, G. (1982). Environmental effects on animals used in biomedical research. Biological reviews of the Cambridge Philosophical Society, 57(3), 487–523. https://doi.org/10.1111/j.1469-185x.1982.tb00705.x

19. Cohen, D. J., Ellerman, D. A., & Cuasnicu, P. S. (2000). Mammalian sperm-egg fusion: Evidence that epididymal protein DE plays a role in mouse gamete fusion. Biology of Reproduction, 63 (2), 462–468. https://doi.org/10.1095/biolreprod63.2.462

20. Cora, M. C., Kooistra, L., & Travlos, G. (2015). Vaginal cytology of the laboratory rat and mouse: review and criteria for the staging of the estrous cycle using stained vaginal smears. Toxicologic pathology, 43(6), 776-793. 10.1177/0192623315570339

21. Cunha, G. R., Sinclair, A., Ricke, W. A., Robboy, S. J., Cao, M., & Baskin, L. S. (2019). Reproductive tract biology: Of mice and men. Differentiation; research in biological diversity, 110, 49–63. https://doi.org/10.1016/j.diff.2019.07.004

22. Da Silva-Buttkus, P., Jayasooriya, G. S., Mora, J. M., Mobberley, M., Ryder, T. A., Baithun, M., Stark, J., Franks, S., & Hardy, K. (2008). Effect of cell shape and packing density on granulosa cell proliferation and formation of multiple layers during early follicle development in the ovary. Journal of cell science, 121(23), 3890–3900. https://doi.org/10.1242/jcs.036400

23. De Felici, M. (2016). The formation and migration of primordial germ cells in mouse and man. Results and problems in cell differentiation, 58, 23–46. https://doi.org/10.1007/978-3-319-31973-5_2

24. Deb, K., Reese, J., & Paria, B. C. (2006). Methodologies to study implantation in mice. Methods in molecular medicine, 121, 9–34. https://doi.org/10.1385/1-59259-983-4:007

25. Diaz, F. J., O'Brien, M. J., Wigglesworth, K., & Eppig, J. J. (2006). The preantral granulosa cell to cumulus cell transition in the mouse ovary: development of competence to undergo expansion. Developmental biology, 299(1), 91–104. https://doi.org/10.1016/j.ydbio.2006.07.012

26. Dixon, D., Alison, R., Bach, U., Colman, K., Foley, G. L., Harleman, J. H., Haworth, R., Herbert, R., Heuser, A., Long, G., Mirsky, M., Regan, K., Van Esch, E., Westwood, F. R., Vidal, J., & Yoshida, M. (2014). Nonproliferative and proliferative lesions of the rat and mouse female reproductive system. Journal of toxicologic pathology, 27(3-4 Suppl), 1S–107S. https://doi.org/10.1293/tox.27.1S

27. Dutta, S., & Sengupta, P. (2016). Men and mice: Relating their ages. Life sciences, 152, 244–248. https://doi.org/10.1016/j.lfs.2015.10.025

28. Edrey, Y. H., Hanes, M., Pinto, M., Mele, J., & Buffenstein, R. (2011). Successful aging and sustained good health in the naked mole rat: a long-lived mammalian model for biogerontology and biomedical research. ILAR journal, 52(1), 41–53. https://doi.org/10.1093/ilar.52.1.41

29. Ekambaram, G., Sampath Kumar, S. K., & Joseph, L. D. (2017). Comparative study on the estimation of estrous cycle in mice by visual and vaginal lavage method. Journal of clinical and diagnostic research: JCDR, 11(1), AC05–AC07. https://doi.org/10.7860/JCDR/2017/23977.9148

30. Elmaoğulları, S., Kadan, E., Anadol, E., Gökçeoğlu, A., Çetinkaya, S., Yarım, G. F., Uçaktürk, S. A., & Aycan, Z. (2020). Effects of 5-hydroxymethylfurfural on pubertal development of female wistar rats. Journal of clinical research in pediatric endocrinology, 12(1), 79–85. https://doi.org/10.4274/jcrpe.galenos.2019.2019.0080

31. El-Mestrah, M., Castle, P. E., Borossa, G., & Kan, F. W. (2002). Subcellular distribution of ZP1, ZP2, and ZP3 glycoproteins during folliculogenesis and demonstration of their topographical disposition within the zona matrix of mouse ovarian oocytes. Biology of reproduction, 66(4), 866–876. https://doi.org/10.1095/biolreprod66.4.866

32. Enders A. C. (1965). A comparative study of the fine structure of the trophoblast in several hemochorial placentas. The American journal of anatomy, 116, 29–67. https://doi.org/10.1002/aja.1001160103

33. Enders, A., & Schlafke, S. (1967). A morphological analysis of the early implantation stages in the rat. American Journal of Anatomy 120 (2), 185-225. https://doi.org/10.1002/aja.1001200202

34. Euker, J.S., and Riegle, G.D. (1973). Effects of stress on pregnancy in the rat. Journal of Reproduction and Fertility, 34, 343-346.

35. Everett, J. W., & Sawyer, C. H. (1949). A neural timing factor in the mechanism by which progesterone advances ovulation in the cyclic rat. Endocrinology, 45(6), 581–595. https://doi.org/10.1210/endo-45-6-581

36. Filant, J., & Spencer, T. E. (2014). Uterine glands: biological roles in conceptus implantation, uterine receptivity and decidualization. The International journal of developmental biology, 58(2-4), 107–116. https://doi.org/10.1387/ijdb.130344ts

37. Foitzik, T., Hotz, H. G., Eibl, G., & Buhr, H. J. (2000). Experimental models of acute pancreatitis: are they suitable for evaluating therapy?. International journal of colorectal disease, 15(3), 127–135. https://doi.org/10.1007/s003840000216

38. Fransen, J., Verheyen, A., and Marsboom, R. (1993). Morphological study of the corpus luteum in the rat. Journal of small animal practice, 34(5), 232-238. https://doi.org/10.1111/j.1748-5827.1993.tb02673.x

39. Freeman, M. E. (1988). The Ovarian Cycle of the Rat. In E.Knobil & J. D. Neill. The Physiology of Reproduction, 1893-1928. Raven Press.

40. Gan, L., Duan, H., Xu, Q., Tang, Y. Q., Li, J. J., Sun, F. Q., & Wang, S. (2017). Human amniotic mesenchymal stromal cell transplantation improves endometrial regeneration in rodent models of intrauterine adhesions. Cytotherapy, 19(5), 603–616. https://doi.org/10.1016/j.jcyt.2017.02.003

41. Garside, D. A., Charlton, A., & Heath, K. J. (1996). Establishing the timing of implantation in the Harlan Porcellus Dutch and New Zealand White rabbit and the Han Wistar rat. Regulatory toxicology and pharmacology: RTP, 23(1), 69–73. https://doi.org/10.1006/rtph.1996.0010

42. Grive, K. J., & Freiman, R. N. (2015). The developmental origins of the mammalian ovarian reserve. Development, 142(15), 2554–2563. https://doi.org/10.1242/dev.125211

43. Haibet, S. M., & Rabie, F. O. (2009). Histomorphological Study of the Ovaries and Follicles Growth in Adult Female Albino Rats.

44. Hamid, H. Y., & Zakaria, M. Z. A. B. (2013). Reproductive characteristics of the female laboratory rat. African journal of biotechnology, 12(19), 2510-2514.

45. Hamid, H. Y., Zuki, A. B. Z., Yimer, N., Haron, A. W., Noordin, M. M., & Goh, Y. M. (2012). Effects of elevated ambient temperature on embryo implantation in rats. African Journal Biotechnology, 11(24), 6624-6632.

46. Hammond, J. M., Hsu, C. J., Mondschein, J. S., & Canning, S. F. (1988). Paracrine and autocrine functions of growth factors in the ovarian follicle. Journal of Animal Science, 66(2), 21-31.

47. Hardy, D. F. (1970). The effect of constant light on the estrous cycle and behaviour of the female

rat. Physiology Behaviour, 5, 421-425.

48. Harley, V. R., Clarkson, M. J., & Argentaro, A. (2003). The molecular action and regulation of the testis-determining factors, SRY (sex-determining region on the Y chromosome) and SOX9 [SRY-related high-mobility group (HMG) box 9]. Endocrine reviews, 24(4), 466–487. https://doi.org/10.1210/er.2002-0025

49. Heape, W. (1900). The "sexual season" of mammals and the relation of the "pro-oestrum" to menstruation. Journal of cell science, 2(173), 1–70.

50. Hirshfield, A. N., & DeSanti, A. M. (1995). Patterns of ovarian cell proliferation in rats during the embryonic period and the first three weeks postpartum. Biology of reproduction, 53(5), 1208–1221. https://doi.org/10.1095/biolreprod53.5.1208

51. Hofstetter, J., Suckow, M. A., & Hickman, D. L. (2006). Morphophysiology. In The laboratory rat (pp.93-125). Academic Press.

52. Hotchkiss, A. K., & Vandenbergh, J. G. (2005). The anogenital distance index of mice (Mus musculus domesticus): an analysis. Contemporary topics in laboratory animal science, 44(4), 46–48.

53. Hsueh, A. J., McGee, E. A., Hayashi, M., & Hsu, S. Y. (2000). Hormonal regulation of early follicle development in the rat ovary. Molecular and cellular endocrinology, 163(1-2), 95–100. https://doi.org/10.1016/s0303-7207(99)00245-2

54. Hussen, T. J., Al-Shaeli, S. J. J., Al-Mahna, B.H.R., and Gharban, H.A.J. (2024). Biochemical and histological effects of long-term administration of estrogen on female mice. Advances in Animal and Veterinary Science 12(8), 1563-1572.

55. Jiang, J. Y., Miyoshi, K., Umezu, M., & Sato, E. (1999). Superovulation of immature hypothyroid rdw rats by thyroxine therapy and the development of eggs after in vitro fertilisation. Journal of Reproduction Fertility, 116, 19-24.

56. Johnson, G. A., Burghardt, R. C., Bazer, F. W., & Spencer, T. E. (2003). Osteopontin: roles in implantation and placentation. Biology of reproduction, 69(5), 1458–1471. https://doi.org/10.1095/biolreprod.103.020651

57. Jollie, W.A., & Wynn, R.M. (Eds.). (2013). Biology of the Uterus. Springer Science and Business Media.

58. Kagami, K., Ono, M., Iizuka, T., Matsumoto, T., Hosono, T., Sekizuka-Kagami, N., & Fujiwara, H. (2020). A novel third mesh-like myometrial layer connects the longitudinal and circular muscle fibers-A potential stratum to coordinate uterine contractions. Scientific Reports, 10(1), 8274.

59. Kaufmann, P., & Burton, G. (1994). In E. Knobil & J. D. Neill. The Physiology of Reproduction 2nd ed. (pp.441-484). Raven Press.

60. Kent, G. C., & Carr, R. K. (2001). Comparative Anatomy of the Vertebrates. 9th Ed. McGraw-Hill Companies.

61. Kusmeirczyk, J., Kling, M., Kier, A. B., Milligan, S. M., & Heatley, J. J. (2020). Rats and mice. Exotic animal laboratory diagnosis, 81-112.

62. Laffan, S. B., Posobiec, L. M., Uhl, J. E., & Vidal, J. D. (2018). Species comparison of postnatal development of the female reproductive system. Birth defects research, 110(3), 163-189.

63. Lee, K. Y., & DeMayo, F. J. (2004). Animal models of implantation. Reproduction , 128(6), 679–695. https://doi.org/10.1530/rep.1.00340

64. Lohmiller, J., & Swing, S. P. (2006). Reproduction and Breeding. In M. A. Suckow, S. H. Weisbroth & C. L. Franklin. The Laboratory Rat. 2nd Ed. (pp.147-164). Elsevier Academic Press.

65. Long, G. G. (2002). Apparent mesonephric duct (rete anlage) origin for cysts and proliferative epithelial lesions in the mouse ovary. Toxicologic Pathology, 30(5), 592–598.

66. Lu, K. H., Hopper, B. R., Vargo, T. M., & Yen, S. S. (1979). Chronological changes in sex steroid, gonadotropin and prolactin secretions in aging female rats displaying different reproductive states. Biology of reproduction, 21(1), 193–203. https://doi.org/10.1095/biolreprod21.1.193

67. Maeda, K. I., Ohkura, S., & Tsukamura, H. (2000). Physiology of reproduction. In G. J. Krinke. The Laboratory Rat (pp.145–176). Academic Press.

68. Mara, J. N., Zhou, L. T., Larmore, M., Johnson, B., Ayiku, R., Amargant, F., Pritchard, M. T., & Duncan, F. E. (2020). Ovulation and ovarian wound healing are impaired with advanced reproductive age. Aging, 12(10), 9686–9713. https://doi.org/10.18632/aging.103237

69. Marcondes, F. K., Bianchi, F. J., & Tanno, A. P. (2002). Determination of the estrous cycle phases of rats: some helpful considerations. Brazilian journal of biology Revista brasleira de biologia, 62(4A), 609–614. https://doi.org/10.1590/s1519-69842002000400008

70. Me, F. (1994). The neuroendocrine control of the ovarian cycle of the rat. The physiology of reproduction, 2, 613-658.

71. Meisel, R. L., & Sachs, B. D. (1994). In E. Knobil & J. D. Neill. The Physiology of Reproduction (pp.3-105). Raven Press.

72. Mercier, O., Perraud, J., & Stadler, J. (1987). A method for routine observation of sexual behaviour in rats. Laboratory Animals, 21, 125-130.

73. Mirsky, M. L., Sivaraman, L., Houle, C., Potter, D. M., Chapin, R. E., & Cappon, G. D. (2011). Histologic and cytologic detection of endocrine and reproductive tract effects of exemestane in female rats treated for up to twenty-eight days. Toxicologic pathology, 39(4), 589–605. https://doi.org/10.1177/0192623311402220

74. Miyabayashi, K., Tokunaga, K., Otake, H., Baba, T., Shima, Y., & Morohashi, K. (2015). Heterogeneity of ovarian theca and interstitial gland cells in mice. PloS one, 10(6),. https://doi.org/10.1371/journal.pone.0128352

75. Mohamed, G. K., Selim, A.A., Abdelhafeez, H. H. & Mohamed, M. B. (2017). Histomorphological developmental studies of the left ovary in the Japanese Quail (Coturnix Coturnix Japonica). MJ Cyto, 1(1), 002.

76. Myers, M., Britt, K. L., Wreford, N. G., Ebling, F. J., & Kerr, J. B. (2004). Methods for quantifying follicular numbers within the mouse ovary. Reproduction (Cambridge, England), 127(5), 569–580. https://doi.org/10.1530/rep.1.00095

77. Namlı Kalem, M., Anadol, E., Kalem, Z., Sezginer, P. Y., Elmas, C., Yılmaz, C., & Bakirarar, B. (2023). A rat study on the PTEN expression in ovarian tissue in PCOS and folliculogenesis. Scientific reports, 13(1), 20774. https://doi.org/10.1038/s41598-023-47809-y

78. Natraj, U., & Richards, J. S. (1993). Hormonal regulation, localization, and functional activity of the progesterone receptor in granulosa cells of rat preovulatory follicles. Endocrinology, 133(2), 761–769. https://doi.org/10.1210/endo.133.2.8344215

79. Nelson, J. F., Felicio, L. S., Randall, P. K., Sims, C., & Finch, C. E. (1982). A longitudinal study of estrous cyclicity in aging C57BL/6J mice: I. Cycle frequency, length and vaginal cytology. Biology of reproduction, 27(2), 327–339. https://doi.org/10.1095/biolreprod27.2.327

80. Nelson, R. J. (1995). An Introduction to Behavioural Endocrinology. Sinauer Associates.

81. Nzalak, J. O. (2010). Anatomical and histochemical studies of the digestive system of the African

giant rat (Cricetomys gambianus-Waterhouse). Department of veterinary anatomy, faculty of veterinary medicine, Ahmadu Bello University, Zaria.

82. Olson, L. K., Tan, Y., Zhao, Y., Aupperlee, M. D., & Haslam, S. Z. (2010). Pubertal exposure to high fat diet causes mouse strain-dependent alterations in mammary gland development and estrogen responsiveness. International journal of obesity (2005), 34(9), 1415–1426. https://doi.org/10.1038/ijo.2010.51

83. Orihuela, P. A., Ortiz, M. E., & Croxatto, H. B. (1999). Sperm migration into and through the oviduct following artificial insemination at different stages of estrous cycle in the rat. Biology of Reproduction, 60(4), 908-913. https://doi.org/10.1095/biolreprod60.4.908

84. Parr, M. B., & Parr, E. (1989). The implantation reaction. In R. M. Wynn, Jollie W. P. Biology of The uterus. Plenum Press.

85. Pepling, M. (2013). Oocyte development before and during folliculogenesis. Oocyte physiology and development in domestic animals, 1–19. https://doi.org/10.1002/9781118538074.ch1

86. Pepling, M. E. (2006). From primordial germ cell to primordial follicle: mammalian female germ cell development. Genesis, 44(12), 622–632. https://doi.org/10.1002/dvg.20258

87. Picut, C. A., Dixon, D., Simons, M. L., Stump, D. G., Parker, G. A., & Remick, A. K. (2015). Postnatal ovary development in the rat: morphologic study and correlation of morphology to neuroendocrine parameters. Toxicologic pathology, 43(3), 343–353. https://doi.org/10.1177/0192623314544380

88. Polenz, D., Sauer, I. M., Martin, F., Reutzel-Selke, A., Ashraf, M. I., Schirmeier, A., Lippert, S., Führer, K., Pratschke, J., Tullius, S. G., & Moosburner, S. (2024). A new bicornuate model of rat uterus transplantation. Acta obstetricia et gynecologica Scandinavica,. https://doi.org/10.1111/aogs.14857

89. Porto, G.G., Vasconcelos, B.C.D.E., Andrade, E.S.D.S., and Silva-Junior, V.A. (2010). Comparison between human and rat TMJ: anatomic and histopathologic features. Acta cirurgica brasileira, 25, 290-293.

90. Prasad, M. C., Pawar, Y. P., Vadaviya, R. A., Bagal, A. T., & Paneliya, S. M. (2009). Vaginal smear cytology and histomorphology of vagina, uterus and ovaries at different stages of oestrus cycle. Indian Journal of Environmental Toxicology, 19(1), 1-10.

91. Pritchett, K. R., & Taft, R. A. (2007). Reproductive biology of the laboratory mouse. In The mouse in biomedical research (pp.91–121). Academic Press.

92. Psychoyos A. (1986). Uterine receptivity for nidation. Annals of the New York Academy of Sciences, 476, 36–42. https://doi.org/10.1111/j.1749-6632.1986.tb20920.x

93. Rabie, F. O., & Haibat, S. M. (2020). Anatomical and histological study of the uterus in adult female albino rat. Biochemical and Cellular Archives, 20(1).

94. Roelen, B. A. J., & Chuva de Sousa Lopes, S. M. (2022). Stay on the road: from germ cell specification to gonadal colonization in mammals. Philosophical transactions of the Royal Society of London. Series B, Biological sciences, 377(1865), 20210259. https://doi.org/10.1098/rstb.2021.0259

95. Salvetti, N. R., Panzani, C. G., Gimeno, E. J., Neme, L. G., Alfaro, N. S., & Ortega, H. H. (2009). An imbalance between apoptosis and proliferation contributes to follicular persistence in polycystic ovaries in rats. Reproductive biology and endocrinology: RB&E, 7, (68). https://doi.org/10.1186/1477-7827-7-68

96. Sandrock, M., Schulz, A., Merkwitz, C., Schöneberg, T., Spanel-Borowski, K., & Ricken, A. (2009).

Reduction in corpora lutea number in obese melanocortin-4-receptor-deficient mice. Reproductive Biology and Endocrinology, 7(1), 1-9.

97. Schlafke, S., & Enders, A. C. (1975). Cellular basis of interaction between trophoblast and uterus at implantation. Biology of reproduction, 12(1), 41–65. https://doi.org/10.1095/biolreprod12.1.41

98. Scudamore, C. L. (2014). Reproductive system. A Practical Guide to the Histology of the Mouse, 87-107.

99. Sharp, J.L., Zammit, T.G., and Lawson, D.M. (2002). Stress-like responses to common procedures in rats: Effect of the estrous cycle. Contemporary Topics by the American Association for Laboratory Animal Science, 41, 15-22.

100. Simpson, J., & Kelly, J. P. (2011). The impact of environmental enrichment in laboratory rats--behavioural and neurochemical aspects. Behavioural brain research, 222(1), 246–264. https://doi.org/10.1016/j.bbr.2011.04.002

101. Soares M. J. (2004). The prolactin and growth hormone families: pregnancy-specific hormones/cytokines at the maternal-fetal interface. Reproductive biology and endocrinology: RB&E, 2, 51. https://doi.org/10.1186/1477-7827-2-51

102. Sod-Moriah, U. A. (1971). Reproduction in the heat-acclimatised female rat as affected by high ambient temperature. Journal of Reproduction Fertility, 26, 209-218.

103. Stewart, C. A., & Behringer, R. R. (2012). Mouse oviduct development. Mouse Development: From Oocyte to Stem Cells, 247-262.

104. Tiwari, M., Prasad, S., Tripathi, A., Pandey, A. N., Ali, I., Singh, A. K., Shrivastav, T. G., & Chaube, S. K. (2015). Apoptosis in mammalian oocytes: a review. Apoptosis : an international journal on programmed cell death, 20(8), 1019–1025. https://doi.org/10.1007/s10495-015-1136-y

105. Vidal, J. D. (2019). Pathology of the male and female reproductive system and mammary gland. Toxicologic Pathology for Non-Pathologists, 397–482.

106. Wang, H., & Dey, S. K. (2006). Roadmap to embryo implantation: clues from mouse models. Nature reviews. Genetics, 7(3), 185–199. https://doi.org/10.1038/nrg1808

107. Wang, S., & Larina, I. V. (2021). In vivo dynamic 3D imaging of oocytes and embryos in the mouse oviduct. Cell reports, 36(2), 109382. https://doi.org/10.1016/j.celrep.2021.109382

108. Wimsatt W. A. (1975). Some comparative aspects of implantation. Biology of reproduction, 12(1), 1–40. https://doi.org/10.1095/biolreprod12.1.1

109. Windsor, P. (2018). Abnormalities of development and pregnancy.Veterinary Reproduction and Obstetrics.10th Ed. Elsevier Health Sciences, 168–94.

110. Winkler, I., Tolkachov, A., Lammers, F., Lacour, P., Daugelaite, K., Schneider, N., Koch, M. L., Panten, J., Grünschläger, F., Poth, T., Ávila, B. M., Schneider, A., Haas, S., Odom, D. T., & Gonçalves, Â. (2024). The cycling and aging mouse female reproductive tract at single-cell resolution. Cell, 187(4), 981–998.e25. https://doi.org/10.1016/j.cell.2024.01.021

111. Wulff, C., Wilson, H., Wiegand, S. J., Rudge, J. S., & Fraser, H. M. (2002). Prevention of thecal angiogenesis, antral follicular growth, and ovulation in the primate by treatment with vascular endothelial growth factor Trap R1R2. Endocrinology, 143(7), 2797–2807. https://doi.org/10.1210/endo.143.7.8886

112. Yamanouchi, D., Morgan, S., Kato, K., Lengfeld, J., Zhang, F., & Liu, B. (2010). Effects of caspase inhibitor on angiotensin II-induced abdominal aortic aneurysm in apolipoprotein E-deficient mice. Arteriosclerosis, thrombosis, and vascular biology, 30(4), 702–707.

https://doi.org/10.1161/ATVBAHA.109.200527

113. Yanagimachi, R. (1994). Mammalian fertilisation. In Knobil E. & Neill J. D. The physiology of reproduction (89–317). Raven Press

114. Yoshida, M., Sanbuissyo, A., Hisada, S., Takahashi, M., Ohno, Y., & Nishikawa, A. (2009). Morphological characterization of the ovary under normal cycling in rats and its viewpoints of ovarian toxicity detection. The Journal of toxicological sciences, 34 (1), SP189–SP197. https://doi.org/10.2131/jts.34.s189

115. Young, J. M., & McNeilly, A. S. (2010). Theca: the forgotten cell of the ovarian follicle. Reproduction (Cambridge, England), 140(4), 489–504. https://doi.org/10.1530/REP-10-0094

116. Zakharova, L., Sharova, V., & Izvolskaia, M. (2020). Mechanisms of Reciprocal Regulation of Gonadotropin-Releasing Hormone (GnRH)-Producing and Immune Systems: The Role of GnRH, Cytokines and Their Receptors in Early Ontogenesis in Normal and Pathological Conditions. International journal of molecular sciences, 22(1), 114. https://doi.org/10.3390/ijms22010114