Review

Reproductive characteristics of the female laboratory rat

Huda Yahia Hamid[1] and Md Zuki Abu Bakar Zakaria[2]*

[1]Department of Anatomy, Faculty of Veterinary Medicine, University of Khartoum, 13314 Khartoum North, Sudan.
[2]Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.

The laboratory rat is widely used in research studies as a model of mammalian health and disease. This review provides the basic facts about female rat reproduction and highlights the reproductive characteristics which make the rat a preferred animal model for research on reproduction. Rats have short estrous cycle, lasting four to five days. The estrous cycle consists of four stages known as proestrus, estrus, metestrus and diestrus. Phases of the estrous cycle can be detected by observing behavioral changes or examining vaginal cytology. Detection of sperm in vaginal smear is an excellent predictor of pregnancy in rats. Implantation is initiated on day 5 and completed by day 7 of pregnancy. Gestation takes 21 to 23 days from copulation to parturition. Short estrous cycle and gestation period make the rat an ideal animal model for research on reproduction.

Key words: Rat, estrous cycle, pregnancy detection, fertilization, embryonic development, implantation, gestation, weaning.

INTRODUCTION

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". The laboratory rat is widely used in toxicological, nutritional, genetic, behavioral 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. 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. 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.

FEMALE REPRODUCTIVE SYSTEM

The female reproductive system consists of the two ovaries and the female genital tract. The genital tract includes the 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 and Carr, 2001).

*Corresponding author. E-mail: zuki@ vet.upm.edu.my. Tel: 603-94873405. Fax: 603-89471971.

Table 1. Behavior and vaginal cytology with estrous cycle phases (Lohmiller and Swing, 2006).

Cycle phase	Duration (h)	Behavior	Vaginal smear morphology
Proestrus	12	Male acceptance at end of phase	Nucleated epithelial cells
Estrus	12	Lordosis; male acceptance	75% nucleated cells; 25% cornified cells
Metestrus	21	No male acceptance	Many leukocytes with nucleated and cornified cells
Diestrus	57	No male acceptance	Leukocytes

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).

Estrous cycle phases

The estrous cycle in the rat consists of four stages known as proestrus, estrus, metestrus and diestrus. Proestrus lasts approximately 12 h; estrus, 9 to15 h; metestrus, 21 h; and diestrus (the longest phase), over 57 h (Lohmiller and Swing, 2006).

Hormones play critical roles in the estrous cycle. Gonadotrophins, which are secreted by the anterior pituitary, regulate the estrous cycle through luteinizing 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).

Identification of estrous cycle stages

Phases of the estrous cycle can be detected by observing behavioral changes or examining vaginal cytology (Lohmiller and Swing, 2006). The latter method is widely used and considered as 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. Behavior and vaginal smear morphology during the different phases of estrous cycle as well as the duration of each phase are shown in Table 1.

Estrus is defined as the period when the female accepts the male and allows copulation. Many behavioral changes occur during this phase, including increased running activity, lordosis and ear quivering. During estrus, dry vaginal wall 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 and 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 (Marcondes et al., 2002; Lohmiller and Swing, 2006).

Environmental factors affecting the estrous cycle

The estrous cycle in the rat can be affected by various environmental factors, such as temperature, photoperiod, noise, restraint, immobilization, 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 delay of the estrus phase and mating in the rat 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 immobilization 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).

Mating and reproductive behavior

Mating behavior in females is controlled by both estrogen

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

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. On the other hand, detection of sperm in 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.

Fertilization and early embryonic development

Fertilization in mammals takes place in the oviduct. Successful fertilization requires complex spermatozoa-ova interactions. Fertilization involves many sequential steps, beginning with the binding of spermatozoa to the zona pellucida, follows 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 rat oviduct depends on both estradiol and progesterone (Orihuela et al., 1999).

Fertilization steps in mammals are thought to be regulated by proteins located in the acrosome of the spermatozoa. Fertilization in rats is regulated by rat epididymal 37 kDa protein (DE) (Cohen et al., 2000). After fertilization, a single cell embryo (zygote) doubles to two cells, 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 and DeMay, 2004). In rats, fertilization takes place in the morning at 04:00 to 05:00 h. 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 fertilization. The embryo develops to the morula stage on day four and to the blastocyst stage on day five of pregnancy (Agca and Critser, 2006). Pre-implantation rat embryos can be collected from the female reproductive tract and used in basic research, embryo culture studies, genome banking, and establishment of stem cells (Jiang et al., 1999; Agca and Critser, 2006). However, time of embryo collection depends on the embryo stage needed.

Maternal recognition of pregnancy

The establishment of pregnancy requires the presence of a functional corpus luteum (CL) 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. Maternal recognition of pregnancy in rodents involves activation of the non-functional CL of the estrous cycle into the functional CL of pregnancy. This functional CL must be maintained until day 17. The formation and maintenance of CL and 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 CL 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 CL formation and progesterone production (Soares, 2004).

Embryo implantation

Implantation is divided into three stages; apposition, adhesion (attachment), and invasion (Enders and Schlafke, 1967). In rodents, the embryo that enters the uterus attaches to the uterine epithelium immediately. After loss of the zona pellucida, closure of the uterine lumen brings the blastocyst into close apposition to the luminal epithelium (Parr and 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 phagocytized 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 and 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 and Enders, 1975; Bowen and 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, decidualization requires both estrogen and progesterone. These hormones exert their effects on the endometrium via nuclear estrogen (ER) and progesterone (PR) receptors (Wang and 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 (Enders and Schlafke, 1967; Garside et al., 1996; Hamid et al. 2012).

Gestation, parturition and weaning

Gestation in rats takes 21 to 23 days from copulation to parturition. Placentation in rats is discoidal and hemochorial (Enders, 1965; Kaufmann and Burton, 1994). That is, the fetal and maternal tissue attach 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 h depending on the litter size, with an average of 1.5 h (Baker, 1979). Weaning in rats occurs at around 21 days of age. At this age, pups are able to eat and drink.

CONCLUSION

Rats have been used in researches for almost two centuries. Short estrous cycle and gestation period make the rat an ideal animal for research on reproduction. In view of mode of implantation and hemochorial placentation, studies in rats can provide insights into the cellular and molecular basis of human implantation. Therefore, the rat is a good model for the studies of human embryo implantation and early pregnancy disorders.

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