Overview of the Reproduction of Laboratory Mice and Rats

Eric Nilsson and Michael K Skinner, Washington State University, Pullman, WA, United States

This is an update of Marcia Brower, Overview of the Reproduction of Laboratory Mice and Rats, Editor(s): Michael K. Skinner, Encyclopedia of Reproduction (Second Edition), Academic Press, 2018, Pages 711–721, ISBN 9780128151457, https://doi.org/10.1016/B978-0-12-809633-8.20619-5.

Abstract

A summary of laboratory rat (Rattus rattus, and R. norvegius) and mouse (Mus musculus) reproduction aimed as a general reference to enable understanding of basic concepts needed to successfully breed rats and mice. The reproductive differences between mice and rats are identified. Topics include identification of gender, stages of estrous, breeding and rearing of offspring. This article includes brief discussions of activities surrounding production of genetically altered animals and impacts of inbreeding. References to more in-depth information are included.

Glossary

Blastocyst The blastocyst is a structure formed in the early development of mammals after fertilization of an ovulated egg. There is an inner cell mass which forms the embryo (and is the source of embryonic stem cells). The outer layer of the blastocyst consists of cells called the trophoblast. This layer surrounds the inner cell mass and a fluid-filled cavity known as the blastocoele. The trophoblast gives rise to the placenta.
Chimerism or chimera (also spelled chimaera) A mouse or rat composed of cells from different zygotes. Animal chimeras are produced by combining cells from more than one fertilized egg into one embryo, which can result in two blood types, tissues containing cells from more than one embryo, gametes derived from donor, host or both embryos, or other variations.
Hemodichorial placenta: simplistic definition Approximately two layers of tissue separating the fetal and maternal blood flow.
Hemotrichorial placenta: simplistic definition Approximately three layers of tissue separating the fetal and maternal blood flow.
Intramuscular Referring to injection of material directly into the muscle layers.
Mosaicism Inclusion of two different sets of genes in one individual.
Pheromones Chemicals produced by an organism that transmit a message to other members of the same species.
Photoperiod The number of hours of light to the number of hours of darkness.
Puberty The onset of sexual maturity and ability to bear young in the female. Puberty occurs before full body size and weight are obtained and is not an ideal time to breed for the first time.
Subcutaneous Referring to injection of material into the space between the skin and underlying tissue layers.
Transgenic animals Strains of animals produced by genetic manipulation – includes" knock-out" animals (genetic material deleted) or "knock-in" animals (genetic material added). These strains are developed and then stabilized by strategic breeding strategies.
Triploidy Chromosome abnormalities in which an extra set of chromosomes is included into the fetus genetic material.
Wild type animals Wild type animals have had no genetic manipulation and may be considered equivalent to "out-bred" or "random source" animals.

Key Points

- Overview of the Reproduction of Laboratory Mice and Rats.

Introduction

This article is to present an overview of laboratory rat (Rattus rattus) and mouse (Mus musculus) reproduction. Mice have 40 chromosomes, while the rat has 42. Rats and mice do not breed naturally with each other and attempts to merge rat and mouse DNA into eggs (to develop chimeric animals) have not been successful. When working with both species, appreciating the similarities and differences between these two species can be very helpful.

Because of the many genetic strains of mice and rats that are available, the reproductive data such as length of gestation, age at puberty or number of pups/litter is variable. The anatomical, physiological and reproductive parameter average numbers are used in this article (Table 1 and 2), but successful breeding is always dependent on knowledge of the reproductive tendencies of the

Table 1 Similarities/differences between rat and mouse reproductive anatomy.

Anatomy	*Rat*	*Mouse*
Mammary glands	Six pairs	Five pairs
Cervix	Two cervices	One cervix
Clitoral glands	Connect to urethra	Variable connection to urethra depending on strain
Ovaries, Oviducts, Uterine Horns	Two	Two
Neonate vaginal anatomy	Vaginal plate	No vaginal plate
Male accessory glands	Seminal vesicles, prostate gland of which the anterior lobe is considered the coagulating gland, bulbourethral (Cowper's) glands, ampullary glands, and preputial glands	The same as the rat

Table 2 General characteristics of reproductive cycles in mice and rats.

	Mouse[a] *(unless marked)*	*Rat*[b] *(unless marked)*
Sexual maturity	45–65 days[c]	50–75 days[c]
Puberty	28–49 days	Variation reported – 50–72 days males Females as early as 35 days[a]
Vaginal Opening (days)	24–28	28–60
Recommended first mating	6–8 wks[c]	65–110 d[d]
First mating wt male (gm)	20–30[d]	300[d]
First mating wt female (gm)	20–30[d]	250[d]
Estrus length	6–8 h	20 h
Post partum Estrus (Hours)	14–24, fertile	3–18 h, fertile
Gestation (d)	19–21[c]	21–23[c]
Duration of pup delivery	2 h[a]	15–30 min[c]
Litter Size (pups)	6–12[c]	3–18[c]
Birth weight	0.75–2.0 gm	5–6 gm
Eyes open (d)	12–14	10–12
Able to hear (d)	10[e]	9[e]
Weaning (d)	21–28	20–21
Eating solid food (d)	14–16[e]	11–14[e]
Breeding lifespan	210–270 days[c]	350–440 days[c]

[a]Jacoby, R.D., Fox, J.G., Davisson, M., 1984. Biology and diseases of mice. In: Fox, J.G., Anderson, L.C., Loew, F.M., Quimby, F.W. (Eds.), Laboratory Animal Medicine, American College of Laboratory Animal Medicine Series, second ed. San Diego, CA: Academic Press, pp. 40–52.
[b]Sharp, P., Vilano, J., 2013. The Laboratory Rat, A volume in the Laboratory Animal Pocket Reference Series, second ed., vol. 12–13. Boca Raton, FL: CRC Press Taylor and Francis Group, pp. 26–29.
[c]May vary with strain.
[d]Harkness, J.E., Wagner, J.E., 1995. The Biology and Medicine of Rabbits and Rodents, fourth ed. Media. PA: Williams and Wilkins.
[e]Hrapkiewicz, K., Medina, L., 2007. Clinical Laboratory Animal Medicine: An Introduction, third ed., vol. 42–44. Ames, IA: Blackwell Publishing, pp. 81–83.

strain of interest. For example, androgenic knock-out male mice show decreased reproductive performance due to anatomical changes in the penis anatomy compared to wild type animals (Yang *et al.*, 2010). Some strains of hairless rats tend to be poor mothers as they produce little to no milk (Sharp and Vilano, 2013). New transgenic lines always need to be monitored carefully for reproductive abilities since the many genes which drive reproductive success can be altered in unexpected ways by targeted genetic alterations.

Anatomy

Reproductive anatomy of rats and mice is basically similar to all mammals and in-depth discussions are easily found (Jacoby *et al.*, 1984; Kohn and Clifford, 1984; Cunha *et al.*, 2019; Cook, 1965). A few significant points are discussed below (**Table 1**).

Female Rats and Mice

Cervical Anatomy

The literature often describes rats and mice as possessing two cervixes, which is incorrect. Rats have two separate cervixes, one in each uterine horn, which unite at the vagina. The mouse uterine horns unite into an externally undivided uterine body with cranial and caudal internal parts. Internally the uterine body has two lumina separated by a midline septum which are sometimes labeled as paired cervixes. However, the two lumens join into one narrowed, tapered canal which forms the uterine neck or cervix of the mouse which then projects into the vagina (Leppi, 1964; Hamilton, 1947). Technically, the mouse has one cervix while rats have two.

Exocrine Glands

Clitoral exocrine glands are present in both species which secrete sebaceous pheromones, very important to the sex life of mice (apparently not such a big deal for rats!). The clitoris of rats connects directly to the urethra while the mouse clitoris may be completely separate from the urethra or may be partially connected, depending on the strain of the mouse (Yang *et al.*, 2010).

Mammary Tissue

Female rats have six pairs of mammary glands, while mice have five pairs. Mammary gland tissue is extensive, extending from the nipples up along the sides and flanks of the animal towards the back and the sides of the neck. Older rats and mice can develop mammary tumors in unusual areas, such as the flanks or neck.

Male Rats and Mice

Inguinal Canal

The inguinal canals remain open throughout the animal's life and testicles may be retracted into the abdomen. A large fat pad exists which tends to prevent inguinal hernias secondary to castration, however it is recommended to close the inguinal canal when performing castration.

Accessory Glands

Rats and mice possess seminal vesicles, coagulating glands (which may be part of the prostate), prostate glands, ampullary glands and preputial glands. Secretions from the coagulating gland and seminal vesicles make up most of the plug that is deposited after coitus. The preputial and ampullary glands are not found in humans and are a source of pheromones.

Determining Sex

Male Rats and Mice

Male adult animals show distinct scrotums with visible penises. Sometimes the testes may be retracted into the inguinal canal. Hold the animal vertically (head up) to allow the testes to slide into the scrotum and become visible.

Female Rats and Mice

Female mice and rats show a shorter ano – genital distance than males but sometimes the genital opening can be confused with a penis, especially in very young animals. Prominent rows of nipples start to become visible at 8–9 days of age in females of both species.

Pre-Pubertal Animals

To determine sex it is easiest to compare ano-genital distances of newborn animals to each other within a litter. Males tend to have about 1.5–2 X greater distance than females. In male mice there may be a dark pigmentation visible in the pre-scrotal region. Pale undescended testes may be visualized through the abdominal wall of mice. As neonates age, it can become harder to differentiate sex until 8–10 days of age when nipples appear on females. At 18–28 days (weaning), the male penis is more recognizable (**Fig. 1**).

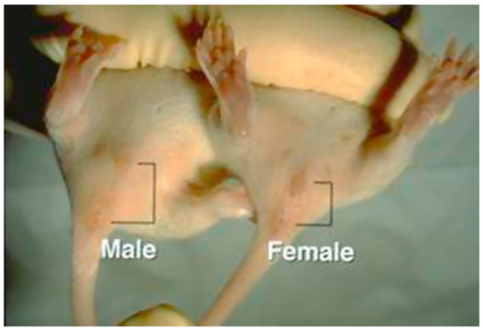

Fig. 1 Sex identification of neonatal mice.

Puberty

Puberty begins as Follicle Stimulating Hormone (FSH) promotes egg, sperm and hormone production leading to the animals becoming capable of reproducing. Age of onset of puberty varies between strains. For example, the C3H/HeOuJ mouse begin successful mating at about 6 weeks old, whereas BALB/cJ mice must be about 8 weeks of age (Silver, 2008). But puberty can begin as early as 30 days and animals of different sex should be separated to prevent uncontrolled mating. It is best to allow the animal to mature before allowing breeding.

Males

Mature sperm has been reported in mice as early as 30–35 days of age (Sharp and Vilano, 2013) with most sources reporting best fertility beginning about 50 days. If a male is still a virgin at 2.5–3 months, he will not plug well (create a vaginal plug after mating) and is less likely to be a prolific breeder (Burds Connor, 2007). In rats, sperm may be present at about 45 days of age, but males are not fertile until 62–65 days, and do not reach maximal sperm production until around 75 days (Otto *et al.*, 2015).

Females

Though some female rats and mice may ovulate as early as 28–30 days of age, most animals first ovulate naturally around 6–8 weeks of age. Both species require the vagina to open before they can be bred. Female rats have a vaginal plate which must degenerate prior to vaginal opening. (For more details, see Kohn and Clifford (1984) or Del Vecchio (1992)). In mice, exposure to adult males or their urine can bring on earlier ovulation; exposure to mature females or their urine may delay the initial ovulations. A female will produce best over her lifetime if she is allowed to obtain most of her body growth before breeding. Once paired with a mate, a litter should be successfully produced in less than 2 months. The number of litters per female mouse can vary per strain – for example, the average number of litters is 2.2 in AKR/J females compared to 4.8 in FVB/N strains (Burds Connor, 2007).

Reproductive Cycles

Estrous

Both rats and mice are continuously polyestrus and ovulate spontaneously. The entire cycle of four stages (proestrus, estrus, metestrus, diestrus) is approximately 4–5 days long in both species (Marcondes *et al.*, 2002). Knowing where a female animal is in her cycle enables coordination of pup production or embryo transfer. The stages are identified by characteristic cellular changes in microscopic exams of vaginal smears and are well described (see **Fig. 2**) (Cora *et al.*, 2015). Rats tend to ovulate later in their cycle than mice (**Table 2**).

Cycle regularity is generally stable in adult animals but may be disrupted. Rats exposed to 24 h light tend to develop persistent estrus and cystic ovaries (Sharp and Vilano, 2013) while chronic low light exposure during dark cycles causes earlier vaginal opening and ovarian atrophy (Fox and Laird, 1970). Exposure to estrogenic substances in feeds or plastic housing may affect cycling regularity (Gorence *et al.*, 2019). The cycles become more irregular in older animals. Pheromones play an important role, more in mice than in rats, and can be manipulated for timed breeding.

Timing of Ovulation

Ovulation in mice usually occurs during the dark period about 2–3 h after the onset of estrus (though there may be cycles without ovulation). Rats tend to ovulate during the metestrus period (Sharp and Vilano, 2013) about 8–11 h after the onset of estrus and

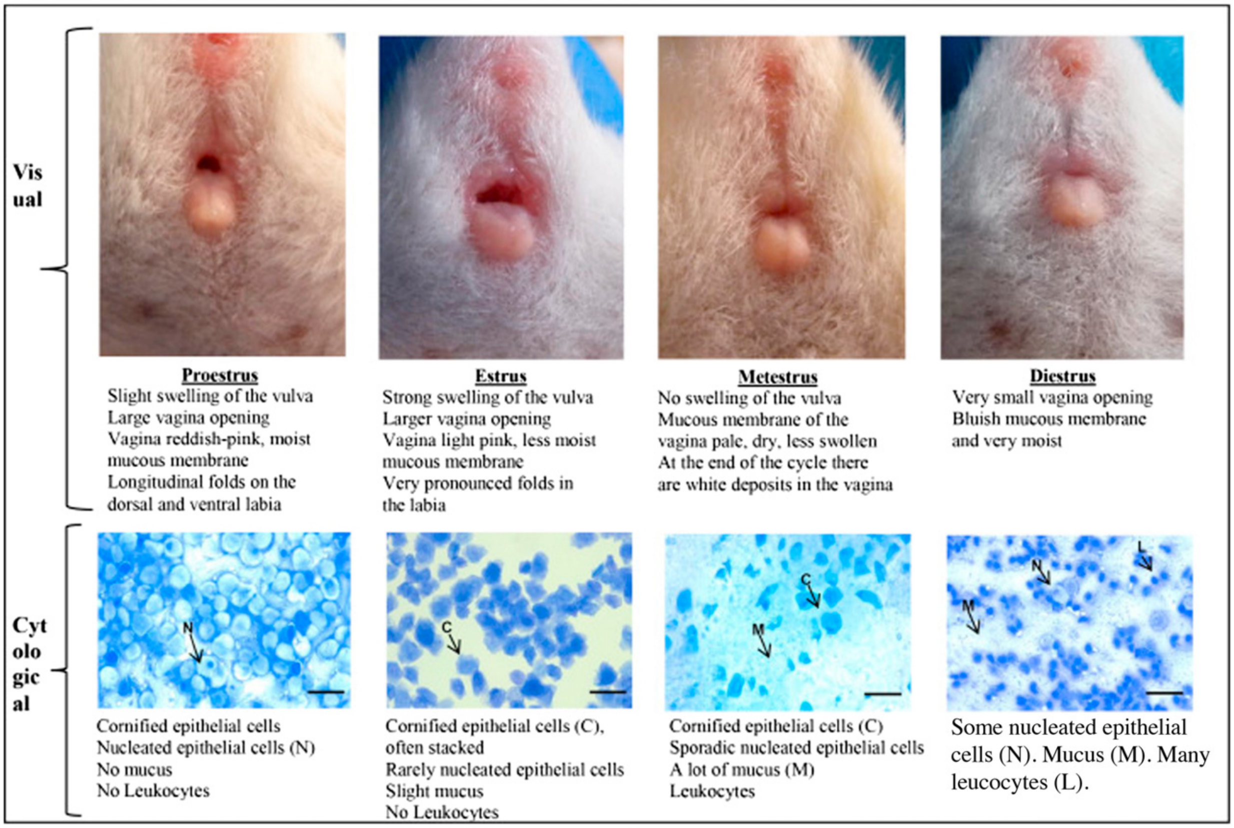

Fig. 2 Characteristics of vaginal smears and external changes. Reproduced from Heykants, M., Mahabir, E., 2016. Estrous cycle staging before mating led to increased efficiency in the production of pseudopregnant recipients without negatively affecting embryo transfer in mice. Theriogenology 85 (5), 813–821.

usually between midnight and 2 am (Peluso, 1992). Ova remain viable about 10–12 h after ovulation (Fox and Laird, 1970). Rats and mice both have fertile post-partum estrus.

Physical Changes Associated With the Cycle

In female mice, the opening of the vaginal orifice becomes more evident, and the vulvar tissue swells during proestrus and estrus. The changes can be visible to the experienced eye (see **Fig. 2**). Female rats in estrus will exhibit characteristic behavior: ear quivering when the back or head are stroked and lordosis (a "sway-back" posture) when the pelvic area is stimulated. Rat vulvar tissue may swell, and the vaginal wall appears dry (due to thickening of the tissues). The rat vaginal wall area is moist during metestrus and diestrus.

Determining Stage of Cycle

Vaginal cytology is used to determine the stage of an animal's cycle (Ajayi and Akhigbe, 2020). Methods to obtain cells for analysis are well described (Bronson *et al.*, 1996; Soleberg, 2004) and multiple videos are available on Youtube (see "Relevant Websites" section).

Vaginal wall cells can be gently collected and then microscopically examined. Presence and proportions of epithelial cells with nuclei, epithelial cells without nuclei ("cornified"), leukocytes and bacteria can be evaluated to identify the cycle stage (see **Fig. 3**).

As shown by Koto *et al.* (1987), measurement of electrical impedance of the vagina may also be used to determine the stage of the cycle in female rats. The correlation of the changes with hormonal changes is not exactly the same for every strain but is consistent within strains – correlations must be established for each strain for the procedure to be predictive.

Pseudopregnancy

Pseudopregnancy, a condition resembling pregnancy that is marked by a persistent corpus luteum and no pups, is purposefully induced during embryo transfer procedures to produce recipient mice or rats (see below) (Terkel, 1988). Vaginal stimulation of an

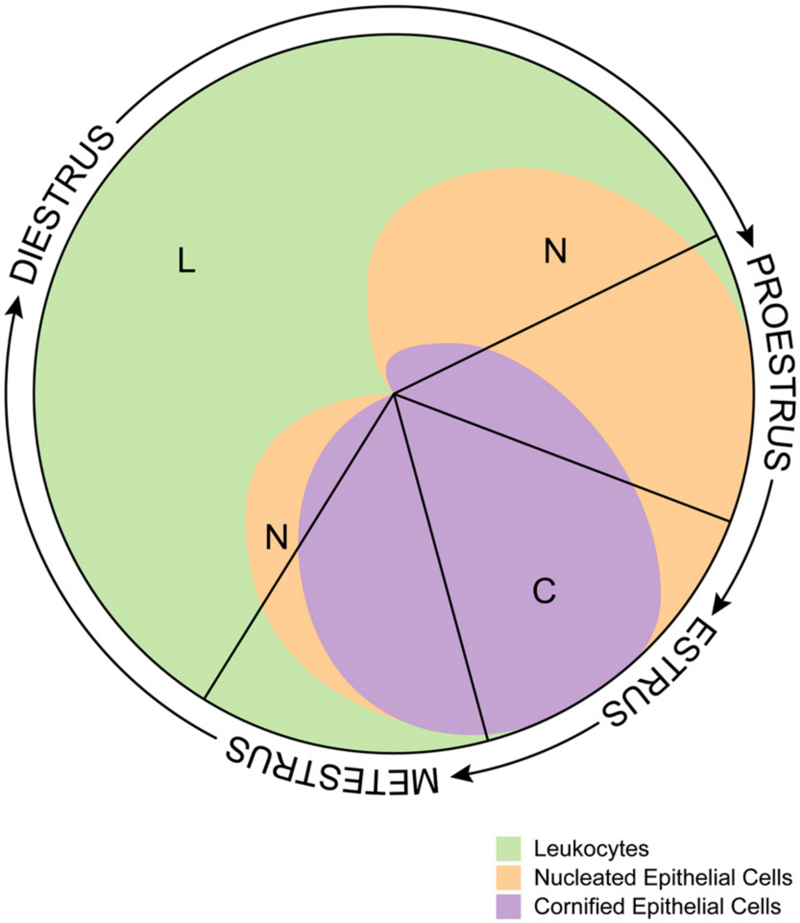

Fig. 3 Mouse estrous cycle identification tool. Reproduced from Byers, S.L., Wiles, M.V., Dunn, S.L., Taf, R.A., 2012. Mouse estrous cycle identification tool and images. PLoS One 7 (4), e35538. Open access article: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0035538 .

animal near or in estrus or mating with an infertile (exhausted, vasectomized or poor breeder) male will induce pseudopregnancy. The condition may also be induced by manipulating pheromones in mice. Pseudopregnancy may last 13 days or longer in some strains of mice and rats.

Breeding

Consistent husbandry of breeding animals and knowledge of the strain's reproductive tendencies are important in any successful breeding system. Consistent photoperiod is crucial to regular cycling (as discussed above). Caloric restriction may cause total cessation of estrous cycles and delay sexual maturity. High ambient temperatures (above 26.6 C) can result in male infertility, causing irreversible degeneration of seminiferous epithelium (Pucak *et al.*, 1977) while cooler temperatures may lead to chilling and loss of pups.

Cycling can be irregular in young females but tends to stabilize at about 50–60 days of age. Aging female rats are considered "reproductively senescent" by about 10–12 months (Sharp and Vilano, 2013). Mice are usually retired from 6 months to one year of age depending on strain (Fox *et al.*, 1984). As animals age, their eggs show higher frequencies of genetic abnormalities such as triploidy and mosaicism in blastocysts recovered after superovulation (Shaver and Martin-DeLeon, 1975; Del Vecchio, 1992).

Copulation and Fertilization

Rats and mice perform coitus during the dark phase of the photoperiod. If the light cycle is reversed, the behavior will reverse as well (Jacoby *et al.*, 1984). Male activity is described by Dewsbury (1970). Again, activity is strain dependent. For example, recovery of libido occurs in about one hour in DBA mice but may take up to four days in B6 mice (Silver, 1995).

Plugging

Mating is confirmed when a visible vaginal plug is observed in the vagina or on the cage floor. Vaginal plugs are easier to see in mice compared to rats. The plug is formed by secretions of the vesicular and coagulating glands of the male following copulation and extends from the vulva to the cervix. Pregnancy is rare if the plug is not observed in mice, but the presence of the plug does not guarantee pregnancy.

When a plug is observed, the next day is considered day #1 or day #0.5 of pregnancy (if the mating was fertile). To observe a plug, the animal is gently lifted by the tail and the vagina inspected. The plug is deeper in the mouse and often the vulvar lips need to be gently spread with a cotton swab or a dull probe to facilitate viewing. Plugs tend to dislodge after 12–24 h so it is best to look for them right away in the morning. The combination of plug observation and vaginal cytology will determine where an animal is in the reproductive cycle.

Fertilization

Fertilization usually occurs in the ampulla or upper portion of the oviduct in mice. The ova can be fertilized up to 10–12 h after ovulation. The blastocyst implants into the endometrium within 4–7 days after post-coitus. For a detailed description of implantation, apposition, attachment and invagination processes, see Lee and DeMayo (2004).

Implantation

Mice and rats experience delays in implantation. This delay appears to be at least partially controlled by quiescent uterine stromal cells which cause the embryos in the blastocyst state to remain dormant (Lee and DeMayo, 2004). Pregnancies which occur during the post-partum estrus in a lactating female are often extended by delayed implantation 7–16 days beyond the normal 19 or 21 days of gestation (Bronson *et al*., 1996).

Manipulation of Breeding Cycle

Mice, more than rats, are strongly affected by pheromones which are chemical substances secreted from the body to elicit a specific behavioral reaction in the recipient. Mice show three well-documented pheromone-driven responses which are used to help synchronize estrus and breeding.

Lee-Boot effect

A group of females housed together will tend to go into anestrous (or pseudopregnancy) and stop cycling for a period of time (Wölfl *et al*., 2023). Even smelling a male mouse will counter-act this effect, so the animals must be truly removed from male mice.

Whitten effect

Introducing a male mouse to a group of non-cycling females will cause 40%–50% of those females to show estrus within 3 days and a second estrus in about 11 days (Gangrade and Dominic, 1984). This can be used to synchronize ovulation in mice that would be super-ovulated as ova/embryo donators, used as embryo recipient mice or to control breeding intervals to synchronize pup arrival.

Bruce effect

Pheromones from an unfamiliar (different strain) male mouse may cause delayed implantation, pseudopregnancy or abortion in female mice bred within 24 h to 4 days of introduction (depending on strain). This effect is not seen if the new male is the same inbred stain as the female (Silver, 2008). Females pregnant over five days will not be affected by a new male introduced to the cage, so when pairing new couples, it is prudent to introduce the new male after the fifth day of gestation. The male is more likely to accept the pups as his own if they are born while he is in the cage (as opposed to being introduced to a new female after her litter is born).

Some strains of rats may show mild Whitten effects but the literature is divided about this. It is generally agreed that rats are not susceptible to the Bruce effect. Rats, in general, do not seem to be as sensitive to pheromone effects as mice.

Post-Partum Estrus

Post parturition estrus occurs usually within the first 12–24 h after birthing. Approximately 50% of the time the post-partum estrus is fertile. This can make it possible for a new litter to be born to a mother who has not fully weaned her previous one. Optimal pup survival depends on close attention to weaning on-time when the post-partum estrus is used. It is important to have a system to track breeding and expected weaning dates!

Determination of Pregnancy

Rats' gestation period is 21–23 days with conception rates of 85% or better for outbred animals, slightly lower for inbred animals. At about 10 days, experienced palpation of the rat's abdomen can detect fetuses (easier to feel after 12 days). Mammary glands and nipple development become evident at 12–14 days.

Mouse gestation is about 19–21 days. An increased rate of weight gain may be noted during daily weight measurements at about 13 days into gestation. Fetuses may be very gently palpable mid to late gestation. Mammary structures develop beginning about 9 days and are pronounced by day 14. The abdomen is noticeably enlarged by day 14 as well.

Breeding Systems

Monogamous

Monogamous systems (1 male to 1 female) are used in both the rat and mouse species depending on the strain and aggression tendencies of the parents. Monogamous systems require more male animals and more labor to be sure litters are removed prior to the next parturition. Monogamous systems maximize the numbers of litters per female, but litters may be smaller with slower growth rates over the lifetime of the female.

Colony Mating

In a colony mating scheme, one male and 2–6 females may be housed together. The young are removed at weaning. This system is efficient for space and labor, as the post-partum estrus is utilized, however tracking individual female production and specific maternal parentage of offspring can be challenging. Pups will be allowed to nurse off several females, especially if pups are close to the same age. Accurate record keeping of breeding and birth dates enables prompt removal of weanlings prior to the arrival of the next litter.

Polygamous or Harem Mating

In this scheme, one male is housed with 2–6 females but the females are moved to separate cages before parturition. The post-partum estrus is lost as a breeding opportunity; however, females tend to produce larger young with more animals weaned per litter. There are a lowered number of total litters per the life of the female rat and there is an increased labor cost with more individual cages. Some females can become territorial, so it is best practice to place the female animal in the male's cage. Once bred, females will not accept another male until the pregnancy terminates.

Generating and Maintaining of Genetic Lines

Generating and maintaining specific desirable genetic lines of animals is done both by general pet breeders as well as scientists. In the past, spontaneous mutations were recognized and bred – resulting in many inbred strains of interest. Now scientists can genetically engineer animals, where, simply stated, a specific gene can be inserted into or removed from an animal's genome and the effects studied. A very short list of genetic alterations includes animals which are prone to obesity or diabetes, rats that become spontaneously hypertensive, animals that lack specific parts of their immune systems, and animals that develop signs consistent with Parkinson's disease or Alzheimer's disease, animals with genetic modifications that include a genetic "switch" to turn the modification on and off.

Sophisticated techniques of superovulation, genetic manipulation and embryo transfer are used in labs to generate new strains of mice and rats (transgenic and chimeric animals) (Jackson and Abbott, 2000). Once the genetic modification is established, careful breeding for about 20 generations stabilizes the mutation into a recognized strain of animal. Once that strain is stabilized, careful out breeding must occur to decrease inbreeding and maintain hybrid diversity. Breeding to establish and maintain stable genetic lines is a skill!

Superovulation

Superovulation is used to generate embryos for collection and transfer to living recipients or freezing (cryopreservation) for storage of desirable strains. Female animals are chemically induced to ovulate a greater number of eggs than normal at a predictable time. After treatment with hormones, the animals are mated (by a fertile or infertile male depending on desired outcome) to stimulate egg release into the oviduct. Eggs may be surgically collected from the oviduct for in-vitro fertilization (if not already fertilized), development into blastocysts and freezing or implantation (see below). For reviews of technique see Silver (2008) or Luo *et al.* (2011).

Embryo Transfer

Embryo transfer serves at least three purposes: to support a genetic line which has difficulty reproducing, to develop disease free animals or to manipulate genetics. Embryo transfer is performed by transplanting embryos from a donor animal into a pseudopregnant recipient. It is necessary to generate a number of recipients at the same time by bringing multiple females into estrus at the same time.

Most strains of mice can be brought into synchrony relatively easily using the Lee-Boot and Whitten pheromone effects discussed above. Rats are not as susceptible to pheromone manipulation and require chemical synchrony (Rouleau *et al.*, 1993; Borjeson *et al.*, 2014). Not all females will cycle as predicted but usually over 50% can be brought into a state close enough to estrus to be tested with a vasectomized male. For technique review, see Silver (2008).

Parturition

Rats and mice have a hemotrichorial placenta with multiple layers separating the fetal and maternal blood flow (humans have hemodichorial) (Benirschke, 2011). Average litter sizes are 6–12 pups. Nursing is usually allowed only after all the pups are delivered. Rodent mothers become better mothers with age behaviorally but "fetal wastage" (a measure of loss of fetuses as animals age) increases with age due to implantation failure and post-implantation mortality. Stress and strenuous maternal exercise can lead to increased fetal wastage as well (Mottola *et al.*, 1993). Dystocia is rare but may occur. Cannibalism is not frequent and is a sign of maternal stress.

Mice

Mice should be supplied sufficient bedding material to build nests. Nest building behavior is an important external indication of the animal's general health. First litters tend to be the smallest with production in mice improving after the 2nd litter. There are strain differences in gestation length and size of litters produced between strains (for example the average litter size of BALB/CJ mice is about 5, where the average litter size of FVB/N mice is about 9 (Silver, 2008)).

Mother mice will retrieve scattered pups and return them to the nest. She will lay over the top of the pups to nurse. In cages with more than one litter, the young may suckle several lactating dams.

Rats

Many rat strains will build shallow nests beginning about 5 days prior to birth of the litter. About 1.5–4 h prior to the first pup there may be a clear mucoid fluid noted from the vagina. The female will move about the cage, stretching and then lie on the floor with the rear legs extended off the cage floor. As the pups come, she will consume the placenta. The entire process takes approximately 1.5–3.5 h depending on litter size.

Dystocia

Since most births occur during the dark period of the photoperiod, an animal found laboring in the morning should be suspected to be in dystocia. Causes of dystocia may include uterine inertia, malposition of the pups or obstruction of the birth canal. Consult a veterinarian. To treat dystocia, the state of the mother should be assessed. If she is exhausted, dehydrated, quiet or lethargic, the wisdom of trying to treat the dystocia vs surgical delivery of the pups should be considered. The birth canal should be examined and if a pup is found, it should be gently delivered if possible.

If chemical treatment of dystocia is elected, the following actions could be considered. Support the mother with subcutaneous fluids (such as LRS with 2.5% dextrose – about 1 cc per 10 g body weight), extra warmth and perhaps antibiotics if considered necessary. Oxytocin (0.3–4 IU/Kg) may be given intramuscularly or subcutaneously. For mice, one can dilute 20 IU Oxytocin in 1 cc of LRS and give approximately 0.1 cc subcutaneously or IM. Give the animal approximately 30–60 min. If there are no more pups produced, surgical extraction is recommended.

Post-Partum

Pups are altricial (born in an undeveloped state) and require feeding and care from their mothers. The pups are blind, hairless and their ear canals are closed. The larger the litter, the smaller the pups will be. Retina's take about a month to mature; even though the animals' eyes open prior to that, they are not fully visual. Maternal antibodies are transferred across the yolk sac in utero and from colostrum. It is recommended that newly born pups be left undisturbed for the first 2 days if possible, to allow full bonding with the mother; however some early quiet careful observation of pups may be prudent, especially when developing a new genetic strain (Wells, 2016). When changing cages for the first time after parturition, moving the nest and some dirty bedding into the new cage helps smooth the transition.

Rats

The external ear opens at about 2.5–3.5 days and pups appear to be able to hear at about 9 days. Incisors erupt about 6–8 days of age. Pups are generally fully haired by about 7–10 days of age. Eyes open at about 14–17 days of age.

Mice

Pups are called "pinkies". When they are weaned, they are called "hoppers". These terms are used by breeders (and reptile feeders) to identify the size of mouse. Mouse pups are mildly transparent and the white "milk spot" can be observed in their stomachs after they have nursed. They are fully haired by about 10 days of age and their eyes open at about 12 days of age. They can begin eating solid food and drink water at about 14 days of age.

Weaning

Weaning is generally done at about 21–28 days of age. Rats can be weaned as early as 17 days but 20–21 days (40–50 g body weight) is standard. Inbred strains may need more time before weaning. One indication of readiness for weaning is when pups remain very active when the cage cover is removed. If they just sit still, they are still too young and should not be weaned (Burds Connor, 2007). Recently weaned pups should be kept together by sex at least one week to help maintain body temperature.

If mothers are not bred on the post-partum estrus, most females will resume cycling about 2–4 days after weaning. If the post-partum estrus was used, wean the litter before a new litter is produced. Competition between the older animals and the newborns can cause injury to the newborns and the mother may not have enough milk for all the offspring.

Orphans

Wikipedia features a nice article about how to raise an orphan mouse (see "Relevant Websites" section). See that article for more specific details. The best way to raise an orphan less than 10 days old is to try to foster it onto a mother of similar aged pups (within 1–2 days). Rub orphans with scented material from the "natural" nest (perhaps moist dirty bedding, feces and birth fluids if the litter is that young), then mix the new pup(s) in with the original pups in the nest. Without disturbing the cage, recheck in several hours. If the mother has gathered the babies together, she will most likely accept them.

If the pup is over 10 days old, the pup may be able to be hand raised but this is difficult to do successfully. Kitten Milk Replacer can support baby rodents. Baby rats and mice cannot urinate or defecate on their own, so their genitals should be gently stimulated with a soft moist Q-tip or cotton after feeding. Do not allow them to get chilled!

Trouble Shooting Breeding Problems

If there are problems with breeding of strains, it is important to look at the husbandry and environment of the animals as well as the specific animals themselves. Temperatures in the wrong range, presence of vibrations or disturbing ultrasound noise, multiple disturbance of the cages (explosive noise, people opening and closing the cages), absence of nesting materials, dietary fat content (too much or too little) all can have an effect. It may take some investigation to determine the cause of breeding issues. For a checklist of questions to consider, see Perret-Gentil (2015).

Summary

The various reproductive parameters of the rat and mouse models reviewed provide basic information on these most utilized mammalian model systems, with more information gathered over the past half century than with any other models. A limitation of the use of inbred animals, such as nearly all the mice strains and most rat strains, is the inbreeding depression of epigenetics that alters the phenotypic variation in the models (Achrem *et al.*, 2023; Venney *et al.*, 2016; Vergeer *et al.*, 2012). All other non-inbred models provide a normal biological system and molecular control, compared to the inbred lines of rodents. This limitation has not been considered previously due to a lack of understanding of the integration of genetics and epigenetics (Nilsson *et al.*, 2018). This will need to be addressed in the future and limitations on conclusions from the models considered more seriously.

Acknowledgment

Marcia Brower, Boston Scientific Corporation, St. Paul, MN, United States is acknowledged for the previous edition writing of original draft of this chapter.

References

Achrem, M., Stępień, E., Kalinka, A., 2023. Epigenetic changes occurring in plant inbreeding. Int. J. Mol. Sci. 24 (6), 5407.
Ajayi, A.F., Akhigbe, R.E., 2020. Staging of the estrous cycle and induction of estrus in experimental rodents: an update. Fertil. Res. Pract. 6, 5.
Benirschke, K., 2011. Comparative placentation. http://placentation.ucsd.edu/indxfs.html.
Borjeson, T.M., Pang, J., Fox, J.G., García, A., 2014. Administration of luteinizing hormone releasing hormone agonist for synchronization of estrus and generation of pseudopregnancy for embryo transfer in rats. J. Am. Assoc. Lab. Anim. Sci. 53 (3), 232–237.
Bronson, F.H., Dagg, C.P., Snell, G.D., 1996. Reproduction. In: Green, E.L. (Ed.), Biology of the Laboratory Mouse, 2nd Ed. New York: McGraw Hill, pp. 187–204.
Burds Connor, A., 2007. Aurora's Guide to Mouse Colony Management at MIT. Available at: https://ki.mit.edu/files/ki/cfile/sbc/escell/mouseManagement.pdf.
Cook, M.J., 1965. The Anatomy of the Laboratory Mouse. M.R.C. Laboratory Animals Centre Carshalton. Surrey, England: Elsevier, p. 143. https://www.informatics.jax.org/cookbook/index.shtml.
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. Toxicol. Pathol. 43 (6), 776–793.
Cunha, G.R., Sinclair, A., Ricke, W.A., *et al.*, 2019. Reproductive tract biology: Of mice and men. Differentiation 110, 49–63.
Del Vecchio, F.R., 1992. vaginal plate. In: Mohr, U., Dungworth, D.L., Capen, C.C. (Eds.), Pathobiology of the Aging Rat. Washington DC: ILSI Press, pp. 331–349.
Dewsbury, D.A., 1970. Copulatory Behavior. In: Hafez, E.S.E. (Ed.), Reproduction and Breeding Techniques for Laboratory Animals. Philadelphia: Lea and Febiger, pp. 123–136.
Fox, J.G., Anderson, L.C., Loew, F.,M., Quimby, F.W. (Eds.), 1984. Laboratory Animal Medicine, American College of Laboratory Animal Medicine Series, second ed. San Diego, CA: Academic Press.
Fox, R.R., Laird, C.W., 1970. Sexual cycles. In: Hafez, E.S.E. (Ed.), Reproduction and Breeding Techniques for Laboratory Animals. Philadelphia: Lea and Febiger, pp. 107–122.
Gangrade, B.K., Dominic, C.J., 1984. Studies of the male-originating pheromones involved in the Whitten effect and Bruce effect in mice. Biol. Reprod. 31 (1), 89–96.
Gorence, G.J., Pulcastro, H.C., Lawson, C.A., *et al.*, 2019. Chemical contaminants from plastics in the animal environment. J. Am. Assoc. Lab. Anim. Sci. 58 (2), 190–196.
Hamilton, C.E., 1947. The cervix uteri of the rat. Anat. Rec. 97 (1), 47–62.
Jackson, I.J., Abbott, C.M. (Eds.), 2000. Mouse Genetics and Transgenics: A Practical Approach. New York: Oxford University Press.
Jacoby, R.D., Fox, J.G., Davisson, M., 1984. Biology and diseases of mice. In: Fox, J.G., Anderson, L.C., Loew, F.M., Quimby, F.W. (Eds.), Laboratory Animal Medicine, American College of Laboratory Animal Medicine Series, second ed. San Diego, CA: Academic Press, pp. 40–52.
Kohn, D.F., Clifford, C.B., 1984. Biology and diseases of rats. In: Fox, J.G., Anderson, L.C., Loew, F.M., Quimby, F.W. (Eds.), Laboratory Animal Medicine, American College of Laboratory Animal Medicine Series, second ed. San Diego, CA: Academic Press, pp. 127–134.
Koto, M., Miwa, M., Tsuji, K., Okamoto, M., Adachi, J., 1987. Change in the electrical impedance caused by cornification of the epithelial cell layer of the vaginal mucosa in the rat. Jikken Doutsu 36, 151–156.
Lee, Kevin Y., DeMayo, F.J., 2004. Animal models of implantation. Reproduction 128, 679–695. Available at: www.reproduction-online.org/content/128/6/679.full.
Leppi, J.T., 1964. A study of the uterine cervix of the mouse. Anat. Rec 150 (1), 51–66.
Luo, C., Zuñiga, J., Edison, E., *et al.*, 2011. Superovulation strategies for 6 commonly used mouse strains. J. Am. Assoc. Lab. Anim. Sci. 50 (4), 471–478.
Marcondes, F.K., Bianchi, F.J., Tanno, A.P., 2002. Determination of the estrous cycle phases of rats: Some helpful considerations. Braz. J. Biol. 62. 4a(4a) Available at:. https://doi.org/10.1590/S1519-69842002000400008.
Mottola, M.F., Mezzapelli, J., Schachter, C.L., McKenzie, K., 1993. Training effects on maternal and fetal glucose uptake following acute exercise in the rat. Med. Sci. Sports Exerc. 25, 841–856.
Nilsson, E.E., Sadler-Riggleman, I., Skinner, M.K., 2018. Environmentally induced epigenetic transgenerational inheritance of disease. Environ Epigenet 4 (2), dvy016.
Otto, G.M., *et al.*, 2015. Biology and diseases of rats, Laboratory Animal Medicine, third ed. London, England: Academic Press, p. 162. Ch 4.
Peluso, J.J., 1992. Morphologic and physiologic features of the ovary. In: Mohr, U., Dungworth, D.L., Capen, C.C. (Eds.), Pathobiology of the Aging Rat, vol. 1. Washington, DC: ILSI Press, pp. 337–349.
Perret-Gentil, M., 2015. Breeding Troubleshooting Questionnaire. SanAntonio, TX. Available at: http://research.utsa.edu/wp-content/uploads/2015/02/Breeding-Troubleshooting-Questionaire-Updated-2-21-15.pdf.
Pucak, G.J., Lee, C.S., Zaino, A.S., 1977. Effects of prolonged high temperature on testicular development and fertility in the male rat. Lab. Anim. Sci. 27, 76–77.
Rouleau, A.M., Kovacs, P.R., Junz, H.W., Armstrong, D.T., 1993. Decontamination of rat embryos and transfer to specific pathogen – free recipients for the production of a breeding colony. Lab. Anim. Sci. 43, 611–614.
Sharp, P., Vilano, J., 2013. The Laboratory Rat, A Volume in the Laboratory Animal Pocket Reference Series, second ed., 12–13. Boca Raton, FL: CRC Press Taylor and Francis Group, pp. 26–29.
Shaver, E.L., Martin-DeLeon, P.A., 1975. Effects of aging of sperm in the female and male reproductive tracts before fertilization on the chromosome complement of the blastocysts. In: Blandau, R.J. (Ed.), Aging Gametes, Their Biology and Pathology. Washington. Basel: Karger, pp. 151–165.
Silver, L.M., 1995, revised 2008. Mouse Genetics Concepts and Applications. Oxford University Press. Adapted for the Web by Mouse Genome Infomatics Maine. The Jackson Laboratory. Available at: http://www.informatics.jax.org/silver/index.shtml.
Soleberg, P., 2004. Examination of vaginal smears in the rat. Available at: https://norecopa.no/education-training/other-teaching-materials/rat-oestrus.
Terkel, J., 1988. Neuroendocrine processes in the establishment of pregnancy and pseudopregnancy in rats. Psychoneuroendocrinology 13 (1-2), 5–28.
Venney, C.J., Johansson, M.L., Heath, C.D., 2016. Inbreeding effects on gene-specific DNA methylation among tissues of Chinook salmon. Mol. Ecol. 25 (18), 4521–4533.
Vergeer, P., Wagemaker, N.C.A.M., Ouborg, N.J., 2012. Evidence for an epigenetic role in inbreeding depression. Biol. Lett. 8 (5), 798–801.
Wells, S., 2016. Harwell, MRC: It starts at the very beginning – the importance of neonatal assessment. Available at: https://www.nc3rs.org.uk/it-starts-very-beginning-importance-neonate-assessment.
Wölfl, S., Zala, S.M., Penn, D.J., 2023. Male scent but not courtship vocalizations induce estrus in wild female house mice. Physiol Behav. 259, 114053.
Yang, J.H., Menshenina, J., Cunha, G.R., Place, N., Baskin, S.L., 2010. Morphology of mouse external genitalia: Implications for a role of estrogen in sexual dimorphism of the mouse genital tubercle. J. Urol. 184 (4 Suppl), 1604–1609. Available at:. www.ncbi.nlm.nih.gov/pmc/articles/PMC3428050/.

Further Reading

Fox, James G. (Ed.), 2015. Laboratory Animal Medicine, third ed. London, England: Academic Press.
Hafez, E.S.E. (Ed.), 1970. Reproduction and Breeding Techniques for Laboratory Animals. Philadelphia: Lea and Febiger.
Jackson Laboratory Staff. Biology of the Laboratory Mouse, second ed. Blakiston Division of McGraw-Hill.
Pinkert, C.A. (Ed.), 2014. Transgenic Animal Technology A Laboratory Handbook. Boston, MA: Elsevier.
Suckow, M., Weisbroth, S., Franklin, C., 2005. The Laboratory Rat, second ed. Toronto: Academic Press (American College of Laboratory Animal Medicine).

Relevant Websites

https://ki.mit.edu/files/ki/cfile/sbc/escell/mouseManagement.pdf
Burds Connor, A., 2007. Aurora's Guide to Mouse Colony Management at MIT.
http://research.utsa.edu/wp-content/uploads/2015/02/tips_for_successfully_breesing_your_mice.pdf
Good info on mouse breeding, University of Texas, San Antonio.
http://placentation.ucsd.edu/indxfs.html
In depth discussion of placentation of various species, Benirschke, K., Comparative placentation.
http://www.afrma.org/
Mouse Genome Information
Jackson Labs website: http://www.informatics.jax.org. A wealth of information about rodent strains and breeding techniques. Dr. Silver's Mouse Genetics and Applications publication is available here. American Fancy Rat and Mouse Association.
http://research.utsa.edu/research-funding/laboratory-animal-resources-center/training/
Scroll through this site, much rodent information with specific breeding information at the bottom of the page, University of Texas, San Antonio.
https://norecopa.no/education-training/other-teaching-materials/rat-oestrus
Soleberg, P., 2004. Examination of vaginal smears in the rat.
https://www.youtube.com/watch?v=p6CvQtGcl84
Study of Estrous cycle in rats
http://www.med.umich.edu/tamc/tgoutline.html
Transgenic reproduction mice and rats, University of Michigan.
https://www.nc3rs.org.uk
Under 3Rs resource tab scroll down to Hubs and microsites – will find several involving rodents including one focused on Genetically altered mice. National Centre for the Replacement Refinement and Reduction of Animals in Research (NC3Rs].
http://www.wikihow.com/Care-for-Baby-Mice
wikiHow to care for baby mice.