McGill

STANDARD OPERATING PROCEDURE #609

RAT BREEDING COLONY MANAGEMENT

1. PURPOSE

This Standard Operating Procedure (SOP) describes guidelines for the management of rat breeding colonies.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff, animal care staff and veterinary care staff.

3. MATERIALS

3.1. Caging and environmental enrichment

3.2. Cage cards

3.3. Ear punch or ear tags

3.4. Breeding records

4. CONSIDERATIONS

4.1. Generally, male laboratory rats will reach sexual maturity at approximately 10-12 weeks of age, although females may have their first estrus as early as 8-9 weeks of age.

4.2. The reproductive lifespan of rats is on average between 12 and 15 months.

4.3. The duration of the estrous cycle is 4–5 days. The cycle is divided into four characteristic phases: proestrus, estrus, metestrus and diestrus. The stage of the estrous cycle can be determined by vaginal cytology.

4.4. Rats are polyestrous and breed year round; ovulation is spontaneous. Mating is usually nocturnal.

4.5. Gestation period: 21-23 days

4.6. Weaning age: 21-23 days

4.7. Pregnancy may be confirmed by gentle abdominal palpation after gestation day 12.

4.8. A fertile postpartum estrus occurs within 48 hours of giving birth and matings at that time are better than 50% successful. Failure to conceive at that time will delay breeding until two to four days after the litter is weaned.

4.9. Fertility and litter size vary by strain. The success rate may also be dependent on the age of the breeders.

5. PROCEDURES

5.1. Breeding cages should be observed daily for newborn animals, litters that are ready for weaning, separating of females, and a check of the animals health and general condition. The maintenance of good breeding records is essential.

5.2. To optimize breeding performance:

5.2.1. Delay breeding until the female is at least 90 days old and approximately 200-275g, depending on strain. Young males should not be used until at least three months of age or until they weigh 275-350g.

5.2.2. Replace breeders:

5.2.2.1. Before their reproductive performance begins to decline. Breeding success decreases if the rats are older than 9 months old.

5.2.2.2. If no litters have been born in 2 months and female is not pregnant (unless strain is known to have low fertility).

5.2.2.3. If several litters have been born but no pups have been weaned.

5.2.2.4. If a significant decrease in litter size is noted, e.g., 1-2 pups born per litter when previously average litter size was 8-9 pups.

5.2.3. Do not replace all breeding animals at the same time. It is best to have breeding animals of various ages in the colony.

5.2.4. Provide adequate environmental enrichment.

5.2.5. Handle breeding cages gently and place in a low-traffic area of the housing room. Avoid handling cages with newborn litters.

5.2.6. If breeding difficulties persists in the colony, consult with a veterinarian as soon as possible, as fertility decreases with age.

5.3. Breeding schemes:

5.3.1. Monogamous pair

5.3.1.1. One male and one female are housed together for mating.

5.3.1.2. The rats are not separated when the female becomes pregnant or delivers the pups.

5.3.1.3. Takes advantage of postpartum estrus and allows the female to become pregnant and nurse at the same time.

5.3.1.4. Litters are born approximately 21 days apart.

5.3.1.5. The 3-week old litter must be weaned prior to the birth of the new litter.

5.3.1.6. For strains that require pups to be weaned later than 21 days of age, female must be separated to avoid postpartum estrus and overcrowding.

5.3.2. Trio breeding

5.3.2.1. One male and two females are housed together for mating.

5.3.2.2. One of the females must be separated when pregnancy is confirmed, before delivery of pups, to avoid overcrowding. One of the lactating females may be left in the same cage with the male.

5.3.2.3. Pups must be weaned at 21 days of age, prior to the birth of new litters.

5.3.2.4. For strains that require pups to be weaned later than 21 days of age, both females must be separated to avoid postpartum estrus and overcrowding.

5.4. Timed matings:

5.4.1. Used when the precise day of mating is required, e.g., when embryos or fetuses of a specific gestational age are required.

5.4.2. Can be accomplished by monitoring the rat's estrous cycle and mating the rats at the predicted time of ovulation or by observing spermatozoa in a vaginal smear following copulation.

5.4.3. One male and up to three females are housed together for mating.

5.4.4. Breeding cages for timed matings should be set up in the late afternoon as rats usually mate during the dark cycle.

5.4.5. Examination of the cellular morphology of vaginal smears:

5.4.5.1. Vaginal smears taken on consecutive days over a period of time can provide detailed information on the estrous cycle. The normal estrous cycle in the rat usually follows a 4-day pattern and the varying characteristics of the cells in the smear allow the days of the cycle to be classified relative to the predicted time of ovulation.

5.4.5.2. Rats should be mated at the end of proestrus or beginning of estrus. Females may display behavior changes during this time such as lordosis (arching of the back and hindleg extension that elevates the rump and head).

5.4.5.3. Vaginal smears must be taken at the same time each day, preferably in the morning, for a minimum of 7 consecutive days.

5.4.5.4. Dry smears:

- Lift the female by the base of her tail.
- Gently insert a cotton-tipped swab moistened with saline approximately 1cm into the vaginal cavity of the rat. Press gently against the vaginal wall and roll slightly before withdrawing.

- Roll swab onto a clean microscope slide.
- Fix the smear quickly to prevent air drying by spraying with an alcohol fixative spray, e.g., Cytospray.
- Stain the slide using a Papanicolaou or Diff-Quick® stain.

5.4.5.1. Wet smears:

- Lift the female by the base of her tail.
- Use a blunt-tipped disposable pipette to flush then aspirate approximately 0.2 ml of saline into the vaginal cavity, repeat 2 times. Place fluid onto a clean microscope slide and cover using a cover slip.

5.4.5.2. Examine smears under a microscope at low power:

- Proestrus: lasts approximately 12 hours and has abundant nucleated non-cornified epithelial cells.
- Estrus: lasts up to 12 hours and is indicated by the presence of large cornified cells in the vaginal smear.
- Metestrus: lasts approximately 21 hours and usually has many neutrophils in the smear and scattered squamous epithelial cells.
- Diestrus: lasts up to 57 hours and there are abundant neutrophils and a few nucleated non-cornified epithelial cells.

5.4.6. Identification of spermatozoa in vaginal smears:

5.4.6.1. Mating can be confirmed by the presence of spermatozoa in the vaginal smear. Spermatozoa will be present for at least 12 hours following copulation.

5.4.6.2. Wet smears:

- Lift the female by the base of her tail.
- Use a blunt-tipped disposable pipette to flush then aspirate approximately 0.2 ml of saline into the vaginal cavity, repeat 2-3 times. Place fluid onto a clean microscope slide and cover using a cover slip.

5.4.6.3. Examine smears under a microscope at low power.

5.5. Weaning:

5.5.1. Weaning refers to removing a pup from its home cage (rather than to the time a pup stops nursing and starts eating exclusively solid food).

5.5.2. Generally, laboratory rats are weaned between 21 and 23 days of age. Most strains are weaned when they are 21 days old.

5.5.3. Weaning age may vary depending on weanling size and maturity, some strains benefit from being weaned later. When pups are to be weaned after 21 days of age, the female must be separated from the male prior to giving birth as to avoid postpartum estrus and overcrowding.

5.5.4. Upon weaning, pups are separated by sex into cages housing a maximum of 4 rats.

5.5.5. A small amount of food may be provided to the weanlings in the bottom of the cage.

5.6. Identification and Recordkeeping:

5.6.1. Identify breeders by ear punching or ear tags.

5.6.2. Identify cages of breeding animals with the appropriate cage card, include the following information:

5.6.2.1. Identification of breeders

5.6.2.2. Strain

5.6.2.3. Mating date

5.6.2.4. Date of birth and expected date of weaning for all litters

5.6.3. Maintain breeding records that include:

5.6.3.1. Date of breeding

5.6.3.2. Date litter is born

5.6.3.3. Litter size

5.6.3.4. Number of pups that have been weaned

5.6.3.5. Gender frequencies

5.6.3.6. Interval between litters

5.6.3.7. Phenotype

5.6.3.8. Number of animals euthanized

6. REFERENCES

6.1. Suckow, M.A., Weisbroth, S.H. & Franklin, C.L. (2005). *The Laboratory Rat, 2nd Edition.* San Diego, CA: Elsevier Academic Press.

6.2. Guide to the Care and Use of Experimental Animals, Vol. 1 (2nd ed). Canadian Council on Animal Care, Canada, 1984: http://ccac.ca/Documents/Standards/Guidelines/Vol2/rats.pdf

6.3. CALAS Québec Workshop # 402 – Vaginal Smears, Method for assessing stages of (rat-mouse) oestrous cycle. April 2010.

SOP REVISION HISTORY

DATE OF MODIFICATION	DETAILS
March 2016	Modification to items 4.6, 4.9, 5.2.6

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How many animals in a standard rat cage?
Combien d'animaux par cage à rat standard?

4 adults < 200g each
4 adultes < 200g chaque

OR
OU

3 adults < 300g each
3 adultes < 300g chaque

OR
OU

2 adults > 300g each
2 adultes > 300g chaque

OR
OU

♀
With litter
Avec portée

Your Veterinarian
Votre Vétérinaire