5.2.2.4. If more than 2 litters have been born but no pups survive to weaning age.

5.2.2.5. 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, nesting material is essential in breeding cages.

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

5.3. 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. Rats can continue to be housed together 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, the male 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, by observing spermatozoa in a vaginal smear following copulation, or by observing vaginal plugs after 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.

4.3heaor tid mati1 (i62 (n)-12.313.1 (he l)-8.9 (at)-17V5n8)-12.3 (e ac)-8 (u7.1 ( )-12.1 (up)1)]Tet-0.007 Tw 0 -1.157 TD (4)-12.3 8.1002 Tw

5.4.6.3.2. To see the plug, lift the female by the base of her tail and examine her vaginal opening for a whitish or cream-colored plug. If necessary, use a cotton-tipped swab or a blunt metal probe to gently open the vagina.

5.4.6.4. If a vaginal plug is found, separate the female. Pregnancy could also be confirmed by abdominal palpation after gestational day 12.

5.4.6.5. If there is no plug, leave the female with the male and check for a copulatory plug each morning. If after 7 days there is no vaginal plug, remove the male.

5.4.6.6. The first day of gestation is considered to be the day the vaginal plug is observed, 0.5 days post-coitum (dpc).

5.4.7. Identification of spermatozoa in vaginal smears:

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

5.4.7.1. Wet smears:

5.4.7.1.1. Lift the female by the base of her tail.

5.4.7.1.2. 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.

5.4.7.2. Examine smears under a microscope at low power.

5.4.7.3. The first day of gestation is considered to be the day spermatozoa is observed, 0.5 days post-coitum (dpc).

5.5. Weaning:

5.5.1.

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. Canadian Council on Animal Care (CCAC) guidelines: Rats, February 2020.

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

6.4. Wong, M. D., van Eede, M. C., Spring, S., Jevtic, S., Boughner, J. C., Lerch, J. P., & Henkelman, R. M. (2015). 4D atlas of the mouse embryo for precise morphological staging. Development (Cambridge, England), 142(20), 3583–3591. https://doi.org/10.1242/dev.125872

2023.06.09